Intramural Activities Volume II

October 1, 1990- September 31, 1991

91 annual report

Division Of

Cancer Treatment

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NATIONAL CANCER INSTITUTE

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SUMMARY REPORT

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DIVISION OF CANCER TREATMENT

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GENERAL ORGANIZATION

The Cancer Therapy Evaluation Program (CTEP) is responsible for the administration and coordination of the majority of the extramural clinical trials supported by DCT. These programs include the activities of the Clinical Cooperative Groups, the Phase I and Phase II new agent development contractors, and the holders of investigator-initiated grants (R01 and P01) relating to cancer treatment. Certain programs in developmental radiotherapy, such as high LET radiation, are administered in the Radiation Research Program. The Phase I development of biologic response modifiers is handled by the Biological Research Modifiers Program.

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and of evaluating them for efficacy and toxicity. It does this by: 1) Coordinating and monitoring the trials of new agents developed by the DCT; 2) Planning with members of the Clinical Investigations Branch (see below overall strategies for new agent studies in specific tumor types; 3) Regulating the distribution of investigational new drugs for which DCT is the sponsor; 4) Maintain close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new agent development proceeds in a coordinated way.

The Clinical Investigations Branch (CIB) is responsible for development and implementation of disease-oriented treatment strategies across the spectrum of human malignancies. In doing so, it provides management and oversight of the clinical cooperative group program. It manages the oncology portfolios of R01 and P01 grants.

The Regulatory Affairs Branch (RAB) monitors the conduct of clinical trials performed in the NCI-supported clinical trials network. It also assures that clinical investigators using experimental agents are in compliance with federal regulations regarding the use of such agents. At the start of the clinical testing of each investigational gent, RAB obtains Investigational New Drug (NDA) exemption authorization from the Food and Drug Administration (FDA) and maintains close communication with FDA in all matters relating to experimental drug studies.

The Biometric Research Branch (BRB) provides statistical consultation to the other branches of CTEP, to the extramural and some intramural activities of other programs in DCT, and to the statistical centers of the clinical cooperative groups. It also carries on research in statistical methodology relating to cancer clinical trials.

The Office of the Associate Director (OAD) integrates the efforts of the Branches.

The process of protocol review is administered within the OAD by a central Protocol and Information Office (PIO) which is also the receipt point at NCI for all protocols entered into the PDQ system. The Program Analysis and Management Office (PAMO) has responsibility for the technical management of CTEP's grants and contracts and carries out analyses, as needed, of certain fiscal and administrative issues of particular interest to the program. The OAD is responsible for overall program supervision and budgetary allocation.

ORGANIZATIONAL AND PROFESSIONAL STAFF CHANGES

Susan Arbuck, M.D., formerly Associate Professor of Medicine, State University of New York at Buffalo, has been recruited as a Clinical Research Scientist in the Developmental Chemotherapy Section, Investigational Drug Branch.

Mr. Alfred Fallavollita, Jr. was appointed to the position of Head, Drug Management and Authorization Section, Investigational Drug Branch.

Mr. Clarence Fortner formerly Head, Drug Management and Authorization Section, Investigational Drug Branch retired from the Public Health Service to join Adria Pharmaceutical Company.

Malcolm Smith, M.D. has been appointed as a Senior Investigator in the Pediatric Section, Clinical Investigations Branch.

Timothy Moore, M.D. resigned as a Medical Officer in the Medicine Section, Clinical Investigations Branch to join Medical Oncology Associates in Pittsburgh, Pennsylvania.

Edward Trimble, M.D. joined the Surgery Section, Clinical Investigations Branch as a Senior Investigator; he was formerly with Memorial Sloan Kettering Cancer Center.

Jan Casadei, Ph.D. formerly a Research Scientist with IGEN, Inc. of Rockville, Maryland was recruited as a Chemist in the Drug Regulatory Affairs Section, Regulatory Affairs Branch.

Ms. Elizabeth Moore, joined the Drug Regulatory Affairs Section, Regulatory Affairs Branch as a Pharmacist; she was formerly with the National Institute of Allergy and Infectious Diseases.

Mr. Michael Montello transferred from the NIH Clinical Center Pharmacy to the Drug Management and Authorization Section, Investigational Drug Branch as a Clinical Research Pharmacist.

HIGHLIGHTS IN PROGRAM DEVELOPMENT

1. REIMBURSEMENT FOR CARE COSTS ASSOCIATED WITH CLINICAL INVESTIGATION

Based upon data generated by a Gallop Survey and from diverse other sources, there is clearly a growing problem in reimbursement which can limit participation in the NCI sponsored therapeutic studies. Especially difficult for high cost procedures like bone marrow transplantation, CTEP has continued to address this situation. Several meetings between various private and public (HCFA) insurers have taken place and a major collaborative effort with Blue Cross/Blue Shield has been initiated. As a demonstration project Blue Cross/Blue Shield is working with investigators to foster definitive trials of ABMT in Breast Cancer patients. This novel and ambitious project is a noteworthy experiment itself. A major conference on ABMT and Breast Cancer was cosponsored by CTEP and a number of insurance companies in an attempt to define this vexing area. Continued efforts are envisioned for the next year.

2. ATTENTIONS AND WOMEN'S HEALTH CARE ISSUES

In light of widespread public and Congressional scrutiny, CTEP carefully reevaluated its commitment to malignancies that affect women. Historically, women and their diseases have been important priorities for CTEP. Data have been gathered and published to indicate that more women than men participate in NCI sponsored therapeutic trials and that breast cancer accounts for the single largest activity area of all diseases. Not content with this record, new data collecting systems have been installed to track our clinical investigators attention to women's (and minority population) concerns. Major new clinical and scientific initiatives are planned for breast, ovarian, and lung cancer.

3. PROMOTING INVESTIGATOR INITIATED CLINICAL TRIALS

Historically, cancer clinical investigation has fared relatively poorly in peer review. In order to address this need for better support, CTEP has worked with DRG (especially the ET-2 Study Section). Collegial efforts to improve both the quantity and the quality of research proposals have been initiated. A special session at ASCO was devoted to the mechanics of submitting higher quality grant applications. Two Special Program Announcements have been issued (one general and one specifically for surgical oncology investigators). A sustained effort to encourage more and better proposals is envisioned.

4. TREATMENT REFERRAL CENTER (TRC)

Several new agents (including Taxol and the camptothecins) appear to have promise for women with refractory ovarian cancer. In order to provide more and better therapeutic options to these patients, CTEP has been working with the Cancer Centers. An integrated network will permit referrals of patients to the Centers for consideration of selected

promising new agents. This new component (the TRC system) will compliment the existing compassionate mechanisms -- including commercially available agents, Group C drugs, compassionate IND drugs and now the Treatment Referral Center.

INVESTIGATOR-INITIATED RESEARCH GRANTS

Coordination and Administration of Investigator-initiated Grants

Investigator-initiated grants (R series grants, program project grants, small business innovative research grants) are administered through the CTEP Program Analysis and Management Office. The purpose of this office is to serve as the contact for extramural investigators for administrative and scientific advice concerning research grants, to integrate relevant research information from all available sources in the development of new research grant concepts, and to disseminate the information contained in the grants to the Disease Coordinators of CIB and the Drug Monitors of IDB.

In FY91, the CTEP managed 154 investigator-initiated research grants with the total cost awarded over $62 million. Grants are assigned to one of three areas within the Program: Clinical Oncology, Surgical Oncology, and Cancer and Nutrition. Clinical Oncology includes clinical research studies designed to improve cancer treatment. Surgical Oncology includes intervention studies in which surgery is the dominant feature to prevent, diagnose, stage, or treat cancer. Cancer and Nutrition concerns nutritional assessment of cancer patients and defining optimal nutritional status and requirements in patients with tumor burden.

Analysis of Investigator-initiated Grant Funding Rates

Table 1 includes information on grant expenditures within CTEP for FY 91 and a breakdown of the number of research grants in the different programs administered by CTEP. In FY91, a total of 86 R01 grants were reviewed by the Division of Research Grants (DRG) Study Sections; nine were awarded for a funding rate of 10%. Six FIRST (R29) applications were reviewed by DRG study sections with one proposal receiving funding for a funding rate of 17%. Twenty eight R01 grants responding to the AIDS-Lymphoma Network Request for Application (RFA) were reviewed by a NCI special review committee; 12 grants were awarded for a funding rate of 42%. Twenty-one P01 grants were reviewed by NCI special review committees; seven of them received funding for a funding rate of 33% The funding rates for grant applications reviewed by DRG study sections were much lower than the funding rates for grant applications reviewed by NCI special review committees. Furthermore, the funding rates of CTEP grants reviewed by DRG study sections were much lower than the average funding rate for all NCI grants reviewed by DRG study sections. This lower funding rate for CTEP grants indicates that clinical research grant applications are having a difficult time competing successfully in DRG study sections. As a consequence, funding of clinical research is heavily dependent on the P01 and RFA mechanisms.

Table 1. GRANT EXPENDITURES FOR FY91 (ESTIMATED)

CANCER THERAPY EVALUATION PROGRAM
DIVISION OF CANCER TREATMENT

TYPE OF GRANT	NUMBER	TOTAL COST COST AWARDED *
Clinical Oncology (CL)		
Research Projects (R01)	60	$ 11,828
Program Projects (P01)	32	41,142
Small Business Innovative Research (SBIR)	7	779
Small Grants (R03)	23	1,480
Merit Awards (R37)	3	560
First Awards (R29)	7	599
Outstanding Investigator Award (R35)	2	$ 793
Subtotal	134	$ 57,181
Surgical Oncology (SO)		
Research Projects (R01)	4	$ 444
Program Projects (P01)	4	3,310
Small Business Innovative Research (SBIR)	2	340
First Awards (R29)	1	87
Subtotal	11	$ 4,181
Nutrition (NT)		
Research Projects (R01)	5	$ 611
First Awards (R29)	3	251
Subtotal	8	$ 862
TOTAL AWARDS	153	$ 62,224

* $ Times 1,000

Program Project Grants

An analysis of the different types of research grants supported by CTEP indicates that program project grants (P01) comprise 70% of the annual expenditures in the research grant pool for CTEP. CTEP manages the largest portfolio of P01 grants both in terms of dollars and numbers of grants within the NCI. It is unusual for a program to have the majority of its research dollars in this category. However, these program projects allow the integration of preclinical research projects with clinical trials projects and have been very successful in translating new basic research advances into the clinic.

The CTEP Grants Program Directors spend a considerable amount of their time and energy in the management of program project grants. During FY 91, the Program Directors attended 20 site visits for the review of program project submissions and performed 2 formal consultations of P01 submissions. During these formal consulting sessions, the applicants bring drafts of their letters of intent, and the Grants Program Director along with other appropriate program staff (Disease Coordinators, Drug Monitors) give scientific as well as logistic advice. In addition to these formal consultations, Program Directors communicate with prospective applicants at meetings and through written and oral communications concerning areas of programmatic interest and the correct format for program project grant proposals.

These P01 grants serve as an important bridge between the preclinical and the clinical sciences. Many basic scientific advances are developed, refined and tested through the P01 grant mechanism and then developed into testable clinical hypotheses. The resultant clinical pilot studies in turn influence the basic science projects so that the desired synergistic effect is achieved. Several successful clinical pilot studies done in these P01 grants have become major studies in the Clinical Trails Cooperative Groups. Thus, the P01 portfolio is an especially important and meaningful activity in CTEP and represents the "cutting edge" of both basic and clinical research.

R01 and R29 (FIRST) Investigator-initiated Research Grants

There has been a general perception by the clinical research community that funding is difficult to obtain for cancer treatment. The CTEP Associate Director and program staff discussed this issue in a special report published in the Journal of the National Cancer Institute (JNCI 83: 838-841, 1991) titled "Poor Funding Rates of Cancer Clinical Research: Intractable Problem or Solvable Challenge?". With the declining funding rate in the research project grants (RPG) pool, CTEP is worried that clinicians will be discouraged from continuing in cancer research. It is difficult for clinical research to compete for funds against basic and preclinical studies reviewed in the DRG study sections.

Clinical investigators have requested that a study section that reviewed only clinical research be formed in the Division of Research Grants. This request has been denied by DRG because of insufficient number of submissions of clinical research grants to justify a new study section and the Experimental Therapeutic 2 study section was originally created for this purpose. In the JNCI special report, program staff encouraged investigators to submit research

grant applications so that enough grants are submitted each round to support a clinical research study section.

In FY 91, the number of research project grants funded increased from the previous fiscal year. On further analysis, this increase is due to two RFAs funded in FY 90; the actual number of unsolicited clinical research proposals declined this fiscal year. CTEP is very concerned with the gradual decrease in research applications that has occurred over the past ten years. CTEP is actively trying to increase the number of research grants and published two new initiatives this year in response to this problem.

FY 91 Research Grant Initiatives

A Program Announcement entitled "Clinical Cancer Therapy Research" was issued to encourage grant applications to conduct clinical therapeutic studies of neoplastic diseases in human subjects. This type of grant solicitation (Program Announcement) is utilized when it is desired to encourage investigator-initiated research projects in areas of special importance to the National Cancer Institute. The Program Announcement encompasses a full range of therapeutic studies and clinical trials employing drugs, biologics, radiation, or surgery. The intent of the announcement is to encourage clinical researchers to translate insights in cancer biology and the development of new agents into innovative cancer therapeutic studies. Over 60 telephone and written inquiries have been received in the past three months since the publication of this Program Announcement. CTEP is presently working with prospective applicants on the preparation of the research proposals for the next submission date.

CTEP also issued a Program Announcement entitled "Surgical Oncology". This Program Announcement encouraged surgeons to participate in surgical oncology research and submit research applications that promote and translate new basic and preclinical research into therapeutic advances. The response to the Surgical Oncology announcement has been encouraging. Over 50 telephone or written inquiries were made and as of June, 1991, 15 applications have been received by the Division of Research Grants.

During the annual meeting of the American Society of Clinical Oncology (ASCO) in Houston, Texas, a workshop was sponsored to promote the above Program Announcements. The CTEP Associate Director chaired the session on "How to Write a Successful Clinical Research Grant". The aim of this session was to offer a didactic explanation of the general organization, process and functions of the grant processes and to point out special problem areas in the writing of clinical research grants. Representatives from program and review made presentations and answered questions. Information on the review process was distributed and the names of CTEP staff that are available to provide assistance were given. Thus, CTEP has made a concerted effort in FY 91 to encourage clinical researchers to submit investigator-initiated research grants so this important area of research can continue to make cancer treatment advances.

In addition to encouraging clinical researchers to submit grant proposals through Program Announcements, CTEP has been active in attracting applicants into specific areas that need development or are ready for clinical study through the issuance of Requests for Applications. These initiatives include

a specific dollar set aside for funding the successful research proposals. The following discusses the four RFAs issued or funded in FY 91:

I. "DCT Small Grants to Stimulate Correlative Laboratory Studies and Innovative Clinical Trials"

A new grant initiative requesting the submission of R03 small grants limited to $50,000 Direct Costs per year for a total of two years funding was issued in FY 90. This RFA had 2 aims: (1) to provide a mechanism for accelerated funding of innovative correlative studies relevant to clinical trials and (2) to stimulate pilot clinical studies with novel laboratory correlations so as to foster the development of interactions between basic science laboratories and clinicians performing clinical trials. Over 170 letters of intent were submitted by potential applicants and a total of 162 applications were received and reviewed. The overwhelming response to this RFA indicated a tremendous need for such a funding mechanism to support clinical research. Initially, $750,000 was set aside in FY 90 to pay approximately twelve grants. CTEP was given an additional $450,000 from STOP CANCER money to pay for R03 grants that involve biological therapies. Four grants were funded using STOP CANCER funds and are noted by * below. During FY 91, $350,000 was added to the set aside to pay five additional grants. As of July, 1991 a total of 23 R03 small grant awards were made. Two grants that received excellent priority scores were awarded funds by the Wendy Will Case Foundation. The 23 NCI awardees and the 2 Wendy Will Case awardees are listed below:

NCI Awardees: * denotes grants funded using STOP CANCER funds

Dr. Camille N. Abboud
1 R03 CA 53352-01
In-Vitro and In-Vivo Effects of GM-CSF on Adult ANLL
University of Rochester Medical Center

Dr. Leonard H. Augenlicht
1 R03 CA 53446-01
Gene Changes and Chemotherapy for Colon Cancer
Montefiore Medical Center

Dr. Jean-Claude Bystryn
1 R03 CA 53468-01
Correla. of Antibody Resp to Melanoma Vaccine with Clin. Outcome
New York University Medical Center

Dr. Charlotte Cunningham-Rundles
1 R03 CA 53341-01
Effects of PEG-IL-2 in Primary Immunodeficiency Disease
Mount Sinai Medical Center

Dr. John F. Ensley
1 R03 CA 53293-01
Clinical Potential of Flow Cytometry in Head & Neck Cancer
Harper-Grace Hospitals

Dr. Bernard Fisher
1 R03 CA 53282-01
Correlative Laboratory Studies and Innovative Clinical Trials
University of Pittsburgh

Dr. Varsha Gandhi
1 R03 CA 53311-01
Biochemical Modulation to Increase Leukemia Response
University of Texas

Dr. Stephen L. Graziano
1 R03 CA 53444-01
Prognostic Factors in Non-Small Cell Lung Cancer
VA Medical Center

Dr. Marc F. Hansen
1 R03 CA 53318-01
Correlation of RBI Expression and Prognosis in Sarcomas
University of Texas

Dr. Daniel F. Hayes
1 R03 CA 53336-01
Circulating c-neu Protein in Breast Cancer Patients
Dana-Farber Cancer Institute

Dr. Edward S. Henderson
1 R03 CA 53503-01
Validation of an In Vitro Drug Sensitivity Assay for CLL
Roswell Park Memorial Institute

Dr. Dorothee M. Herlyn*
1 R03 CA 53411-01
Phase I Clinical Trial With Monoclonal Anti-Idiotype
The Wistar Institute

Dr. Donald W. Kufe
1 R03 CA 53414-01
Clonality in Acute Myeloid Leukemia and Myelodysplasia
Dana-Farber Cancer Institute

Dr. Franco M. Muggia
1 R03 CA 53280-01
Repair of DNA Adducts: Relationship to Platinum Response
University of Southern California

Dr. Gregory H. Reaman*
1 R03 CA 53543-01
Anti-GD3 Monoclonal Antibody Therapy of T-cell ALL
Children's Hospital National

Dr. Neal Rosen
1 R03 CA 53396-01
Gene Expression in Duke's B and C Colorectal Cancer
Lombardi Cancer Research Center

Dr. Brian D. Ross
1 R03 CA 53527-01
Magnetic Resonance Spectroscopy in Clinical Immunotherapy
Huntington Medical Research Institutes

Dr. Lynn M. Schuchter*
1 R03 CA 53357-01
Evaluation of Interleukin-4 Therapeutic and Biologic Effects
University of Pennsylvania

Dr. Robert C. Seeger
1 R03 CA 53329-01
Immunocytology of Marrow Metastases in Neuroblastoma
Childrens Hospital Los Angeles

Dr. R. Graham Smith
1 R03 CA 53284-01
Immunoglobulin Genes as Markers for Residual ALL
The University of Texas

Dr. Paul M. Sondel*
1 R03 CA 53441-01
BRM Monitoring of Pediatric Neuroblastoma/Osteosarcoma
U. W. Clinical Cancer Center

Dr. Charles W. Taylor
1 R03 CA 53372-01
In Vitro Correlations of Suramin Response in Melanoma
The University of Arizona

Dr. Carol A. Westbrook
1 R03 CA 53267-01
Clinical Significance of Molecular Genetic Findings in Colo. Cancer
University of Chicago

Wendy Will Awardees:

Dr. James L. Abbruzzesse
1 R03 CA 53398-01
Human Topo I: An Exploitable Anticancer Drug Target
University of Texas

Dr. Barbara Ann Zehnbauer
1 R03 CA 53365-01
Clonal T. Cell Gene Rearrangements in Pediatric AML
The Johns Hopkins Oncology Center

II. "AIDS-Lymphoma Network"

Adult and pediatric acquired immunodeficiency syndrome (AIDS) patients are surviving longer due to improved retroviral and opportunistic infection treatment and care. As a result, acquired immunodeficiency syndrome associated malignancies have become more prevalent and are a major concern. Lymphoma is one of the malignancies most frequently seen

in AIDS patients. An RFA entitled "AIDS-Lymphoma Network" solicited applications that would bring laboratory research efforts to the clinic to attack the problem of management of AIDS-lymphoma patients. Proposals involving etiology, diagnosis and treatment of this disease were solicited. The AIDS-Lymphoma Network will be composed of those institutions who successfully compete for funding in this RFA to perform new therapeutic AIDS-Lymphoma clinical trials with correlative laboratory studies. This RFA was supported with a set aside of $3 million. Twenty eight grants were reviewed and twelve grants were funded. This RFA is a cross divisional as well as a cross institutional effort involving DCE/NCI, DCBDC/NCI and NIAID. The awardees are listed below:

Dr. Richard F. Ambinder
1 R01 CA 55529-01
EBV as a Tumor Marker in AIDS CNS Lymphoma
The Johns Hopkins University

Dr. John J. Byrnes
1 R01 CA 55506-01
Trial and Correlative Studies of ddI in AIDS Lymphoma
University of Miami Hospital

Dr. Ellen G. Feigal
1 R01 CA 55513-01
Novel Therapeutic Approaches in HIV Associated Lymphomas
Theodore Gildred Cancer Facility

Dr. Richard I. Fisher
1 R01 CA 55509-01
Biology and Treatment of AIDS Lymphomas
Loyola University Medical Center

Dr. Richard J. Ford
1 R01 CA 55526-01
Clinical and Molecular Studies on AIDs Related Lymphomas
University of Texas M.D. Anderson Ca

Dr. Leo I. Gordon
1 R01 CA 55518-01
AIDS Related Lymphomas Clinical and Biologic Studies
Northwestern University

Dr. Henry K. Holland
1 R01 CA 55525-01
Allogeneic Bone Marrow Transplant for HIV-1 Associated Lymphoma
Emory University

Dr. Lawrence D. Kaplan
1 R01 CA 55514-01
Novel Therapeutic Approaches for HIV Associated NHL
San Francisco General Hospital

Dr. Alexandra M. Levine
1 R01 CA 55510-01
HIV Cytokines and Therapeutic Result in AIDS Lymphoma
University of Southern California

Dr. Sharon B. Murphy
1 R01 CA 55507-01
Pediatric AIDS/Lymphoma Network
Children's Memorial Hospital

Dr. David T. Scadden
1 R01 CA 55520-01
Biological Approaches to AIDS Lymphoma
New England Deaconess Hospital

Dr. David J. Straus
1 R01 CA 55531-01
Monoclonal Antibody Treatment of AIDS Related Lymphoma
Memorial Hospital for Cancer

III. "Clinical Treatment and Correlates of Upper GI Carcinoma"

Carcinomas of the organs of the upper GI tract (esophageal, stomach, pancreas) are lethal tumors which rapidly develop resistance to treatment even when chemotherapy, with or without radiation therapy, is effective. Taken collectively, the incidence of these tumors represents a major health hazard to 35,000 patients per year. This RFA encourages applicants to address their research efforts towards the upper GI carcinomas and the development of new clinical therapies. For example, monoclonal antibodies directed against gastrointestinal tumor-specific antigens have been developed, characterized, and applied for diagnostic purposes. The potential for these antibodies to improve clinical management and/or therapy of these diseases needs further investigation. Clinical correlations of oncogenes, growth factors, or markers of drug resistance may prove useful in subsets of patients that would respond to specific treatment therapies. This RFA was issued in December, 1990 with a set aside of $1,500,000 in the FY 92 budget. Twenty-five applications were received in April, 1991. Twenty-two applications were found to be responsive to the RFA and have been scheduled for review.

IV. "New Therapeutic Approaches to the Treatment of Prostate Cancer"

The incidence of prostate cancer continues to increase each year and has now surpassed lung cancer to become the most common carcinoma in males. In recent years, investigators have made promising new advances in understanding the mechanisms of tumor growth and hormonal control in the human prostate cell. Investigators are encouraged to utilize these laboratory advances to develop clinical studies aimed at improving treatment results and clinical outcome. This RFA was issued in May, 1991, with a set aside of $750,000 in the FY 92 budget. As of July, 1991, over fifty telephone or written inquiries had been made regarding this RFA.

New Research Grant Initiatives

I. Cancer Therapy Research in Lung Cancer

A Program Announcement entitled "Cancer Therapy Research in Lung Cancer" was issued to encourage grant applications to conduct clinical therapeutic studies of lung cancer in human subjects. The Program Announcement encompasses a full range of therapeutic studies and clinical trials employing drugs, biologics, radiation, or surgery. The intent of the announcement is to encourage clinical researchers to translate insights in lung cancer biology and the development of new agents into innovative cancer therapeutic studies.

II. Cooperative Agreements for "Clinical Correlative Studies in Solid Tumors"

CTEP supports a program of integrated national networks of clinical investigators and institutions (Clinical Trials Cooperative Groups) for the conduct of large scale, multi-institution clinical trials. The Cooperative Groups have access to tumor specimens from large numbers of patients with solid tumors. NCI is seeking to encourage correlative laboratory studies linked to these large scale clinical trials. An RFA was approved by the Board of Scientific Counselors for clinical correlative studies relevant to cancer treatment or clinical outcome in patients with solid tumors. The solid tumors that will be emphasized include breast, colon, lung, and prostate due to the significant cancer incidence, morbidity and mortality in these tumors. This initiative is jointly sponsored by CTEP and the Cancer Diagnosis Branch, DCBDC and will use the cooperative agreement mechanism so that NCI staff may assist in the coordination of activities Solid Tumor RFA

Minority Research Grant Supplements

CTEP Program Directors have been active in encouraging investigators to apply for minority research supplements to their grants. NCI, through its Comprehensive Minority Biomedical Program, provides support to minority scientists and students in order to influence a great number of minority individuals to develop their research capabilities and pursue independent careers as cancer research investigators. Principal investigators were contacted about the program resulting in six minority research grant supplements.

Small Business Innovative Research Grants

SBIR grants continue to be an important component of the CTEP program. The program has expanded in the past year to include five phase I grants and four phase II grants. In the area of computer software and data management systems, 1 phase I and 2 phase II grants are active. A phase II grant to Cytel Software Consortium supports the development of EAST, a prototype statistical software package for the design and analysis of group sequential clinical trials with time-to-failure endpoints. Civilized Software is continuing the development of CLINSYS, a specialized data base and statistical

analysis system for clinical trials in their phase II proposal. A phase I project proposed by Belmont Research Inc. will develop new software tools that utilize laser graphics to help the clinical investigator perceive significant information in study data sets.

Other phase II grants include a database on questionable cancer therapies that will be useful to insurance companies and may eventually become part of PDQ. Lorad Medical Systems, Inc. is currently developing a prototype instrument that can be used with existing x-ray mammographic equipment to accurately and rapidly place needles into the breast for the purpose of pre-operative localization of non-palpable lesions and for aspiration or core biopsy procedures.

In the areas of developing drugs and machinery useful for the treatment of cancer, a clinical phase I proposal by Matrix Pharmaceutical Inc. will evaluate the utility of an intralesional 5-fluorouracil implant for Kaposi's sarcoma. Another phase I proposal from Fibrogenex, Inc. will determine the efficacy of cellular fibronectin in preventing implantation of metastatic tumor cells at surgical incisions. Quantronix Corporation is involved in a phase I development of a laser-diode-pumped fiber-delivered TM:YLF surgical laser. Cellco Advanced Bioreactors in a phase I study is trying to perfect a hollow fiber bioreactor system to clear metastatic tumor cells from bone marrow cultures in vitro.

Highlights of Investigator-initiated Grants

Several significant discoveries/leads with potentially important clinical applications/implications were made in FY 91 by principal investigators who were supported by grants managed by CTEP. They have been described below:

Dr. Donnall E. Thomas, Fred Hutchinson Cancer Research Center (P01 CA18029) received the Nobel Prize for his research in bone marrow transplantation. He has now retired from his position as the Principal Investigator of this grant and has turned over the grant to Dr. Fred Applebaum. Dr. Philip Greenberg in Project IIId of this grant completed a phase I immunotherapy study using CD8+ cytomegalovirus (CMV)-specific cytotoxic T cells. In this study Dr. Greenberg had to isolate CD8+ CMV-specific CTL clones from CMV seropositive donors, grow these cells in vitro to high concentrations and administer them to patients who underwent bone marrow transplantation for leukemia. The results of this trial indicate that (1) the administration of > 2.2 x 109 CD8+ CMV-specific CTLs is safe and nontoxic; (2) the immunity for CMV was specifically transferred and can be detected following an infusion of 3.3 x 10 cells/m^2, and (3) this transferred immunity persists at least 1 week. Potential application of this adoptive immunotherapy technique in cancer as well as AIDS is very great.

Dr. William E. Evans, St. Jude Children's Research Hospital (R37 CA36401) successfully extended his MERIT award. He continues his studies on the characterization of hepatic drug metabolism and elimination in children with or without cancer and the determination of impact of hepatic drug metabolism and elimination on treatment of childhood cancer. He reported that children with acute lymphocytic leukemia after achieving a complete remission had a faster clearance of teniposide. The degree of hematologic toxicity correlates

better with systemic exposure (area under curve) to unbound than total teniposide. This knowledge is being translated into different dosing guidelines for teniposide depending on whether patients are in remission or have relapsed. The methodologies developed in cancer patients have been adopted for other pediatric disease populations by these and other investigators (e.g. cystic fibrosis). His phenotypic and genetic polymorphic drug metabolism studies have lead to the preliminary finding of a significantly lower prevalence of the debrisoquin-oxidation poor metabolizer phenotype and a higher prevalence of fast acetylators in American black children compared to white children. In the MERIT extension Dr. Evans will continue (1) the studies of the molecular mechanisms underlying age-related and racial differences in drug metabolism phenotypes for two important genetic polymorphisms, N-acetylation and debrisoquin-oxidation; (2) the studies of the hepatic clearance of teniposide and etoposide, two important anticancer drugs that are extensively metabolized by the liver and for which they have discovered major treatment-induced changes in the systemic clearance among children with cancer.

Dr. Robert B. Diasio, University of Alabama at Birmingham (R37 CA40530) continues to make important advances in understanding the biochemical and pharmacologic mechanisms of fluoropyrimidines with particular emphasis on the role of fluoropyrimidine catabolism in humans. His research on fluoropyrimidine catabolism includes the purification to homogeneity of dihydropyrimidines dehydrogenase (DPD), the major, rate-limiting enzyme of pyrimidine catabolism. Dr. Diasio has identified patients experiencing fluorouracil toxicity as being partially or completely deficient in DPD activity. Clinical studies are planned in the coming grant period to screen for individuals who are partially or totally deficient in DPD in a large cooperative group study (CALGB).

Dr. Emil Frei, Dana Farber Cancer Institute (POI CA38493)
The overall objective of this program project grant is to integrate basic and clinical science to provide original and optimal high-dose combination chemotherapy with curative intent to patients with metastatic breast cancer. In their clinical investigations led by Dr. Karen Antman, a recent phase II study combining alkylating agents in the autologous marrow setting in patients with breast cancer indicated a high complete remission rate with 40% of the complete responders continuing in complete remission with a lead follow-up time of 3 years from transplant. Long-term survival has also been achieved inpatients with lymphoma and small cell lung cancer. The hematopoietic supportive care program utilizing GMCSF to increase peripheral blood stem cells has substantially shortened the post-transplantation period of myelosuppression at risk and the duration of hospitalization.

Dr. Beverly Teicher in Project 3 is studying the dose, schedule, combination, resistance, cross-resistance, and biochemical mechanisms of alkylating agents in vitro as well as in preclinical in vivo models. She has quantified the therapeutic effect of a number of modulators including fluosol, etanidazole, novobiocin, BSO, pentoxifylline, and lonidamine. Certain modulators are synergistic in combination such as fluosol plus etanidazole or novobiocin plus lonidamine. These combinations may be integrated into the design of new clinical trials.

Dr. Lawrence Baker, Wayne State University (PO1 CA46560)
Dr. Baker's program project supports the evaluation of new drugs for treatment of solid tumors through all stages of development from in vitro screening to clinical trials. In the last thirty-two months, this group has tested 16,895 agents from the Kodak/Sterling inventory in a disk-diffusion assay. Eight hundred thirty agents were found to be selective and subjected to in vivo analysis in a mouse model. Sixty-six agents were found to be at least modestly active of which twenty-nine were analogs of positive compounds. These were then subjected to further screening with several agents entering clinical trials. A DNA binder PD115934 is entering clinical trials as well as a solid tumor selective ellipticine analog (Datelliptium). Two additional ellipticine analogs could become clinical candidates.

Dr. William McGuire, University of Texas Health Science Center (PO1 CA30195) The goal of this program project is to facilitate the transfer of basic science information from the laboratory to help deal with clinical problems in breast cancer. In project 1, a number of new prognostic factors have been analyzed and correlated. Dr. McGuire has established correlations between DNA ploidy and S-phase fraction and the clinical outcome of patients with node-negative breast cancer. Studies on the Her-2/neu oncogene have found that overexpression of the protein is a strong predictor of disease-free and overall survival for patients with node-negative breast cancer. Analysis of the in-situ and the invasive components of tumors revealed a high rate of overexpression in purely in-situ tumors (56%) which decreases to 21% in mixed in-situ and invasive components with a further decrease to 11% in purely invasive tumors. These findings suggest that over expression of HER-2/neu may be a very early event in the development of certain types of breast carcinoma. Other markers that have been analyzed for correlation with prognosis is an estrogen-induced lysosomal protease cathepsin D, a surface glycoprotein 323/A3, an estrogen inducible polypeptide pS2, and a nuclear antigen expressed in dividing cells called Ki-67.

Dr. Richard O'Reilley, Memorial Hospital (PO1 CA33050) reported the development of a murine xenogeneic transplantation model to evaluate ADA gene transfer and expression in target cells capable of long-term T lymphopoiesis. This transplant model is based on co-transplantation of human and SCID mouse bone marrow cells into lethally irradiated normal Balb c mice. This strategy appears to result in human B and T lymphocyte chimerism that is quantitatively and qualitatively different from previous models involving immunodeficient mice. This model will be useful for analyzing potential gene therapy strategies such as development of retroviral-mediated ADA gene transfer targeted at primitive hematopoietic cells capable of persistently generating T cell progeny. This work was performed by Dr. Eli Gilboa in Project 4 of the PO1 grant.

Dr. Mortimer M. Bortin, Medical College of Wisconsin (PO1CA40053) received funding to support two registries, the International Bone Marrow Transplant Registry (IBMTR) and the North American Autologous Bone Marrow Transplant Registry (NAABMTR). The IBMTR was established in 1972 and the NAABMTR was initiated in late 1989. The IBMTR is an unique resource. Data for more than 10,000 recipients of allogeneic and syngeneic transplants are stored in its data base. Currently 192 transplant teams in the USA and abroad contribute detailed information regarding their consecutive transplant cases to the IBMTR on uniform reporting forms. More than 1500 cases are accrued annually by the

IBMTR. The IBMTR in has served as the information resource for the following groups/entities: Library of Congress, NCI, NHLBI, NIAID, State Governors' Offices, Congressional OTA, FDA, PHS, Congressional Offices, Medicare, Alta Health Strategies, American Red Cross, Aplastic Anemia Foundation, Cancer Information Service, Center for Consumer Health Care Information, Health Care Advisory Board, Leukemia Society of America, Lion's Club International, French Atomic Energy Commission, Sickle Cell Center, National Marrow Donor Program, various insurance companies, news wire services, newspapers, marketing/consulting firms, pharmaceutical companies, hospitals, physicians and patients. The IBMTR through the statistical center can conduct analyses which require large patient numbers that no single institution can accomplish in a short period of time. One particular area where the IBMTR and the NAABMTR can make a major contribution is the performance of prospective studies on long term effects of bone marrow transplantation and the development of secondary malignancies. Another area that the registries can contribute is technology assessment, especially in the evaluation of transplant regimens, and comparison of transplant with alternative treatments. In the case of autologous transplant the value of this procedure in solid tumors, and the role of marrow purging can be evaluated.

Dr. Ronald Levy, Stanford University, (PO1CA34233) developed methodology to search for cell surface molecules that are critical to growth control in large cell lymphomas. They isolated panels of monoclonal antibodies which can shut off the growth of large cell lymphoma cell lines in vitro. To date, 36 antiproliferative MAbs have been isolated and have been submitted to NCI New Drug Screening Program for inclusion for study as potential new agents. Thirteen of these antibodies reacted with molecules previously known to be important in the regulation of cell growth. These include the transferrin receptor, Class I MHC, Class II MHC and immunoglobulin. However, 23 of these clones react with novel cell surface components. Dr. Levy's group has managed to characterize 8 of these clones. Among the eight clones, five clones of the MAbs were directed against a molecule of 110,000 molecular weight and one each directed against molecules of 55,000, 28,000, and 26,000 respectively. The antibody against the 26,000 dalton cell surface protein binds to a diverse group of human cell lines including hematolymphoid, neuroectodermal and mesenchymal inducing aggregation by most of these cells. However, it induces an antiproliferative effect in only a subset of these cells. This 26 kd molecule is non-covalently associated on the cell surface with a molecule named Leu-13. Antibody against Leu-13 has been shown to induce aggregation of cells and antiproliferative effects. Furthermore, this 26 kd protein shows strong homology with the CD37 B associated antigen and the ME491 melanoma associated antigen. The gene for this 26 kd protein has been mapped to the short arm of chromosome 11. The importance of this study is that Dr. Levy has identified a new family of molecules that are likely to play a significant role in transmembrane signaling and cell activation.

Dr. Albert Deisseroth, M.D. Anderson Cancer Center, (PO1CA49639) reported that Dr. M. Talpaz in Project 1A of this PO1 grant has found that the stromal monolayer cultures from CML patients in blast phase constitutively express IL-1 and TGF beta. It is conceivable that such over-production further suppresses the growth of the normal hematopoietic progenitors and that the blast crisis progenitors are resistant to inhibition by such external growth negative regulatory factors.

William N. Hait, Yale University School of Medicine (PO1 CA 08341)
This past year has seen the development of a potentially important approach to the modulation of 5-fluorouracil (5FU) therapy of colon cancer. In project 1, Dr. Handschumacher had established that Brequinar, a potent inhibitor of dihydroorotate, causes a selective but evanescent effect on pyrimidine nucleotide pools of most normal tissues. In a colon tumor model, however, much more prolonged affects are seen even with very low doses of Brequinar. It has been established that delayed administration of 5FU after Brequinar can achieve a major increase on antitumor activity and not exacerbate toxicity. They have received clinical supplies of Brequinar and expect to extend these studies to man. Data from these experiments will be used to guide subsequent therapy in these and other patients when disease is not longer resectable.

In Project 2, Pharmacological Studies of Drugs for Altering Multidrug Resistance, Dr. Hait has transfected two leukemia cells lines with a multidrug resistance (MDR) expression vector. Transfectants were cross-resistant to vinblastine, and doxorubicin and resistance was reversed by cyclosporin a and trans-flupenthixol. A potentially important finding was cross-resistance to hydroxyurea, a drug often used in an attempt to treat the blast crisis of chronic myelogenous leukemia. He has also studied the effect of phosphorylation of phosphoglycoprotein by protein kinase C on drug efflux. These studies have revealed that efflux is increased by agents that activate the enzyme (e.g., phorbol esters) or decrease the activity of serine phosphatases (e.g., okadaic acid).

Dr. Victor Ling, Ontario Cancer Institute (RO1 CA 37130) is one of the leaders in the field of multidrug resistance and was a recipient of the Kettering Award this year. In the past year, Dr. Ling has mapped the epitope binding sites of monoclonal antibodies developed against P-glycoprotein. These monoclonal antibodies are used as specific probes to monitor the expression of different P-glycoprotein isoforms in normal and malignant tissues. His studies have shown that specific P-glycoprotein isoforms are expressed in specialized cells in a tissue and that a single isoform tends to predominate. This finding has implications for understanding the role of P-glycoprotein function in connection with cancer chemotherapy.

A second focus was the investigation of P-glycoprotein expression in clinical samples. In a longitudinal study of childhood soft tissue sarcoma of 30 patients, P-glycoprotein expression was highly correlated with relapse-free survival. Nine patients were positive for P-glycoprotein and all relapsed after a clinical response. Twenty of 21 patients were consistently negative for P-glycoprotein. They all responded clinically and only one has relapsed thus demonstrating that detectable P-glycoprotein appears to be an important adverse prognostic factor.

Administrative Accomplishments

Report on Rare Disease and Condition Research Activities Sponsored by the NIH:

The Grants Program Director acting for the Associate Director, CTEP, DCT served as the NCI representative/coordinator to the NIH Rare Disease and Condition Committee. The NCI Rare Disease Coordinator was responsible for writing the NCI portion of the FY90 annual report of the rare disease and

condition research activities sponsored by the NIH. The Associate Director also participated as a speaker at a Symposium on Rare Diseases.

Organ Systems Program:

The Grants Program Director acting for the Associate Director, CTEP, DCT continues to serve as the Division representative to the NCI Organ Systems Program. The Organ Systems Program represents NCI's effort to promote interactions across the various divisions of the NCI and to foster research in the extramural community. The Organ Systems Program sponsors a series of conferences and workshops based on a specific organ site or disease site. CTEP program directors, disease coordinators and drug monitors have participated in the planning and organization of these meetings. Some of them were invited speakers at these meetings. During FY90 conferences on breast and prostate cancers were held; workshops on levamisole and black-white differences in myeloma were performed and finally an ovarian cancer working group was formed. In FY91, conferences in colorectal and lung cancers were held; workshops on bladder and ovarian cancers were sponsored.

P01 Working Group

The Grants Program Director acting for the Division served on a P01 Working Group of NCI. The Working Group wrote an issues/options paper on alternate funding mechanisms for P01/U10 studies. As a result of this report, the Executive Committee has decided to issue a Program Announcement soliciting "interactive R01 grants" as a pilot experiment in two areas of research.

STAFF PUBLICATIONS:

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Hamilton JM, Sznol M, Friedman MA: 5-Fluorouracil Plus Levamisole: Effective adjuvant treatment for colon cancer. In: Hellman S, DeVita VT, Rosenberg S, eds. Important Advances in Oncology. Philadelphia: J.B. Lippincott Publishing Company, 1990;115-30.

Nerenstone S, Friedman M, Ihde, DC: Primary Liver Cancer. In: Moossa AR, Schimpff SC, Robson MC, eds. Comprehensive Textbook of Oncology, Baltimore: Williams and Wilkins, 1991;948-57.

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BIOMETRIC RESEARCH BRANCH

1. STATISTICAL PLANNING AND MONITORING OF CTEP SPONSORED CLINICAL TRIALS

The Biometric Research Branch (BRB) collaborates in the development of clinical trials to evaluate new chemotherapeutic and biological agents. The BRB reviews all Cancer Treatment Evaluation Program (CTEP) sponsored clinical trials to ensure that they are planned, conducted and reported in a sound and efficient manner. BRB staff interact with extramural investigators and cooperative groups to achieve clinical trial designs that are mutually satisfactory to the NCI and to the extramural organization. The BRB also represents the NCI on data monitoring committees and in decisions for early termination or expansion of CTEP sponsored clinical trials. This activity has grown substantially in the past year and BRB statisticians are involved in all early closure evaluations. Both design and interim monitoring activities often involve extensive simulation studies and data analyses. BRB staff collaborate on the development of drug development plans, such as for Interleukin-3, topotecan, anthrapryazoles, and taxol including the specification of study designs, endpoints and sample sizes. BRB staff participate in national disease oriented strategy meetings to develop study designs for new generations of clinical trials in a particular type of cancer. BRB staff perform interim analyses of contract supported clinical trials and evaluate reports of promising therapeutic regimes for the planning of possible future clinical trials.

The BRB serves as liaison to extramural statistical centers. BRB staff visit centers, review data management and monitoring procedures and organize national meetings in order to improve statistical and data management practices. The BRB organizes regular meetings of the cooperative group statisticians and collaborated with the group statisticians in the development of a 5 year plan for statistical coordinating centers.

2. PRECLINICAL DRUG DISCOVERY

a. Methods for detection of differential cytotoxicity, related to histology, have been developed for the in vitro human tumor cell line assay in collaboration with Drs. K. Paull and R. Shoemaker of the Developmental Therapeutics Program (DTP). The cell line assay is designed to test the in-vitro toxicity of approximately 20,000 potential anticancer agents per year against a panel of 60 human tumor cell lines subdivided into 8 histologic subpanels. A primary goal is the identification of agents with marked differential cytotoxicity related to histology. Detection and analysis tools, both graphical and statistical, have been developed and implemented on the DTP VAX computer. Explanatory presentations have been given at the annual Anticancer Drug Discovery and Development Conference (1990, Wayne State University) and the annual joint MRC-EORTC-NCI Conference (1990, Bethesda). An explanatory manuscript has been published and a second is in press. Additional work is ongoing, in particular, to

explore whether the use of parametric techniques may improve on the detection capabilities of the non-parametric techniques now being used.

b. A statistical comparison of two different in vitro assays (MTT vs SRB) was completed with Drs. Paull and Shoemaker to demonstrate the equivalence of the more practical SRB assay with the previously used MTT assay. The analysis involved several different types of comparisons, based either on the calculated IC50 values or based directly on the dose response curve of cell inhibition levels. It was based on data from 197 compounds tested against the cell line panel with both the MTT and SRB assays. It also included the analyses of the reproducibility of the 2 assays. A presentation was made at the annual AACR conference and a manuscript has been published.

c. New methods are being developed for measuring the cytotoxicity of compounds tested in the in-vitro human tumor cell line assay. Currently used methods measure cytotoxicity as a linear function of reduction of cell number compared to control. The new methods measure cytotoxicity as a function of reduction of cell growth compared to control, and also incorporate attempts to account for the potential relationship between control growth rate and compound growth rate reduction to avoid systematically biasing the measure of cytotoxicity in favor of or against the more rapidly growing cell lines in the screen.

d. Statistical methods of quality control are being developed for the in vitro cell line screen. These methods are designed to detect outlier values in the assay results in real time, as well as to detect changes over time in the growth rates and sensitivities of the cell lines.

e. In collaboration with Dr. M. Alley (and others) of DTP, an analysis was made of the ability to measure cell line growth and drug sensitivity in soft agar with colorimetric analysis, as opposed to using more time-consuming image analysis. It was determined that colorimetric analysis could be beneficially substituted for image analysis in preliminary drug sensitivity assays. A manuscript has been published.

f. Drs. L. Hodes and K. Paull of DTP developed methods of measuring differential cytotoxicity in the in vitro screen using concepts derived from information theory. Related methods were developed to measure the similarity between drugs with respect to patterns of cell-line growth inhibition across the entire tumor panel. BRB staff contributed to the revision of a manuscript, which has been submitted for publication. Work is ongoing to test, apply and extend these methods.

g. In collaboration with Dr. R. Shoemaker and others of DTP, an analysis of the human tumor cell lines is being conducted to attempt to correlate the degree of expression of the multi-drug resistant (MDR) gene with the degree to which the cell line exhibits the MDR phenomenon in in vitro testing (by demonstrating particular resistance to drugs which have been associated with the MDR phenomenon in the clinic). Further analyses of the cell lines are planned, to correlate other genetic characteristics with behavior in the in vitro assay.

3. COMPARATIVE STUDIES TO EVALUATE MAGNETIC RESONANCE IMAGING (MRI)

The BRB has collaborated with the Diagnostic Imaging Branch of the Radiation Research Program and Dr. H. Hricak of UCSF Medical School in the conduct of a prospective multi-institutional evaluation of MRI in the diagnosis of uterine neoplasms.

The BRB participated in the following ways:

a. Primary statistician in the design, supervision, and analysis of the protocols.

b. Supervision of the data management contract.

c. Preliminary analyses were prepared for the NIH Consensus Conference on MRI and the final analysis is completed and has been submitted for publication.

4. THE RELATIONSHIP OF RESPONSE AND SURVIVAL IN ADVANCED EPITHELIAL OVARIAN CANCER

Despite the improvement in response rate, survival is still disappointedly low in the advanced form of epithelial ovarian cancer. Among the several hypotheses proposed to explain this discrepancy, certainly one of the most important is that there is no strong relationship between treatment effect of the first line therapy and survival in this disease. Given the widespread use of response for the measurement of the efficacy of cytotoxic regimens and for clinical decision making, we have quantified the relationship between response and survival by retrospectively analyzing data from all published randomized clinical trials since 1975 using a meta-analytic approach. An "errors in variable" statistical model was developed to analyze the association between endpoints while avoiding the biases of the usual comparison of survivals between responders and non-responders. A bootstrap based method was used to obtain confidence intervals for the model parameters. A manuscript has been submitted for publication describing the results.

5. RELATIONSHIP OF RECURRENCE TO SURVIVAL IN LARGE BOWEL CANCER

Survival is the primary endpoint of many major adjuvant clinical trials of large bowel cancer. Disease free survival would be more "efficient" if it were truly a surrogate. We have evaluated the relationship between these endpoints using individual patient data from multi-institution clinical trials with surgery only control arms and adequate follow-up performed by the cooperative groups. Although disease free survival was not found to be a valid surrogate for survival in the currently accepted statistical sense of Prentice, a conservative method of analysis based on disease free survival has been developed. Two manuscripts describing this work are in preparation.

6. EVALUATION OF SURAMIN FOR THE TREATMENT OF STAGE D2 CARCINOMA OF THE PROSTATE

BRB has collaborated with Dr. M. Christian to organize extramural clinical trials to confirm the promising results by the NCI-COP for the use of suramin in the treatment of patients with stage D2 carcinoma of the prostate who have failed hormone treatment. Patients with measurable disease are being enrolled in a phase II study. A randomized study for patients with non-measurable disease using survival and "quality-of-life" endpoints was closed early due to poor accrual. The suramin dose is determined by blood levels and BRB has collaborated with Dr. C. Myers on evaluating assay calibration at the participating institutions. BRB has analyzed several phase II trials of suramin and correlated baseline variables with response. We are currently collecting all suramin administration and blood level data from all CTEP sponsored phase I trials. We will develop a population pharmacokinetic model and perform pharmacodynamic analyses such as attempting to predict serious neurotoxicity.

7. NEW APPROACHES TO PHASE II TRIALS IN HORMONE REFRACTORY STAGE D2 CARCINOMA OF THE PROSTATE

New approaches are needed for the clinical screening of drugs in patients with hormone refractory stage D2 carcinoma of the prostate. Although the incidence of the disease is great and there are few active drugs, there is also a very limited clinical drug development program. This is because few patients have clinically measurable disease and hence evaluation of drug activity is problematic. In collaboration with the Investigational Drug Branch and the Clinical Investigations Branch, we are designing a new clinical development plan for phase II trials in advanced prostate cancer. The plan is based on a two stage approach in which the endpoint for the first stage clinical screening is prostate specific antigen (PSA) defined response. Activity criteria are being developed based on our analysis of sequential PSA values on CTEP sponsored suramin trials and based on analyses of the Memorial Sloan-Kettering (MSKI)database. Drugs which demonstrate sufficient activity based on PSA response will be tested in patients with measurable disease. The new plan is still under active development but initial

clinical trials using this approach have been approved at MSKI and we have solicited ECOG's interest in participation.

8. THE DESIGN OF PHASE II TRIALS IN SMALL CELL LUNG CANCER (SCLC)

The conventionally targeted response rate of 20% is not appropriate for previously treated SCLC patients -- known active first line agents have lower response rates in this population. This has led to various suggestions in the literature including:

a. lowering the target response to 10%

b. using relapsed patients who had complete responses to conventional chemotherapy,

c. using a window of opportunity design with patients having no prior chemotherapy,

d. limiting the trial to the elderly or poor prognosis patients with no prior chemotherapy. These approaches are critiqued and recommendations given in a paper submitted for publication in collaboration with Dr. T. Moore.

9. MONITORING EPIPODOPHYLLOTOXIN TRIALS FOR THE OCCURRENCE OF SECONDARY AML

Patients on two treatment regimens used at St. Jude Children's Hospital, both involving use of high-dose teniposide weekly or twice weekly, appear to exhibit higher rates of secondary acute myeloid leukemia than do patients who received lower cumulative doses of epipodophyllotoxin, or equivalent cumulative doses administered every other week. However, the relationship between epipodophyllotoxin cumulative dose and/or schedule and possible increased risk of secondary AML is still unclear. BRB staff is involved in a coordinated effort of CTEP, the Children's Cancer Study Group, the Intergroup Rhabdomyosarcoma Study, and the Pediatric Oncology Group to further define this relationship and monitor the potential risk of secondary AML associated with use of epipodophyllotoxins.

10. STATISTICAL ASPECTS OF MEASURING AND COMPARING QUALITY OF LIFE (QOL) ENDPOINTS

The importance of quality life endpoints for certain types of cancer clinical trials is becoming more evident. To help integrate these endpoints into the trials being planned, DCT and DCPC organized a joint conference for the summer of 1990. Investigators who have experience developing quality of life instruments, as well as using them in clinical trials participated. Additionally, representatives from the cooperative groups and pharmaceutical companies attended. BRB staff co-chaired the statistical working group of this conference.

Many of the issues concerning quality of life endpoints are statistical in nature, e.g. (1) how does one ameliorate multiple comparison problems in dealing with high dimensional QOL data, (2) how does one deal with the censoring of QOL data due to death and disease progression.,(3) how should QOL data be incorporated with standard trial outcomes to recommend particular treatment arms, (4) what are the trade-offs between more extensive time coverage vs more extensive questionnaires, (5) what is the role of baseline QOL measurements, and (6) what is the role of QOL variables as prognostic variables. These issues will be addressed by the BRB staff and other statisticians at the conference.

CTEP is especially concerned that protocols that incorporate QOL endpoints are well designed. Guidelines have been developed with BRB staff and other members of CTEP that should be helpful. They have been submitted for publication.

11. INTERGROUP STUDIES

Phase III clinical trials involving two or more cooperative groups are of increasing importance to the national clinical trials program sponsored by the NCI. There are currently over 50 such studies. The growth of intergroup studies represents a recognition of the need for larger sample sizes in many clinical trials and a need for groups to collaborate in exploiting the most promising therapeutic opportunities. In the past, intergroup studies have been developed and conducted in an informal manner. Many participants have been frustrated by lack of adequate quality control mechanisms, opportunities for input in study design and inadequate monitoring procedures.

Improving and facilitating the conduct of intergroup studies is an important priority of CTEP. The BRB has taken the lead in this effort by developing guidelines for the conduct of intergroup studies, by organizing and funding a second national workshop on intergroup data management, by developing guidelines for data monitoring committees in intergroup studies and by beginning to critically review the data collection plans for intergroup studies.

The Chief of BRB or his designee is now a non-voting member of all intergroup data safety monitoring committees and this has increased the study monitoring workload for BRB. The BRB is also very active in fostering intergroup studies by demonstrating the need for substantial numbers of patients. Based on such a presentation, the pediatric groups recently agreed to continue doing intergroup studies in Ewing's sarcoma. The BRB is participating in the planning of a meeting later this year to facilitate the organization and conduct of pediatric intergroup studies.

12. NATIONAL CLINICAL TRIALS OF EARLY OVARIAN CANCER

a. BRB staff has served as primary statistician for clinical trials of the staging and treatment of early ovarian cancer. Final analyses of the therapeutic questions have been performed and a manuscript has been published. The results indicated that adjuvant chemotherapy is not appropriate for patients with very early stage disease (FIGO stages Iai and Ibi). Post-surgical delivery of chromic phosphate (P32) was as effective as chemotherapy for those patients with slightly more advanced disease (FIGO stages Ic, Iaii, Ibii, IIa, IIb).

b. A series of ancillary papers is in preparation concerning further results from the early ovarian clinical trials. Efficacy and toxicity of P32 treatment has been analyzed and a manuscript is in press. Analyses restricted to stage II patients and to low malignant potential patients are both completed and the manuscript relating to the former has been submitted for publication.

13. COLLABORATIVE RESEARCH WITH THE LUNG CANCER STUDY GROUP

BRB staff has served as primary statistician for the following clinical trials:

1. A protocol comparing CAP+RT vs RT in patients with residual non-small cell lung cancer has been completed and demonstrated a modest survival advantage (and a greater time to recurrence advantage) for the CAP+RT treatment. Two papers have been published with Dr. T. Lad.

2. A protocol comparing CAP vs no treatment in patients with T_1N_1 or T_2N_0 NSCLC has been completed and analyzed and a paper has been submitted for publication with Dr. R. Feld.

3. A protocol comparing lobectomy vs limited resection in T_1N_0 NSCLC patients has completed accrual and follow-up and a manuscript is being prepared with Dr. R. Ginsberg.

4. Analysis of the incidence of second primaries and recurrence among T_1N_0 patients, across several protocols, has been completed and a paper has been published with Dr. P. Thomas. Additional analyses based on further follow-up are being conducted for a second manuscript.

14. A MATHEMATICAL MODEL FOR SELECTING DRUG COMBINATIONS BASED ON DOSE INTENSITY

Most success of cancer chemotherapy have required the use of combinations of cytotoxic drugs. This has been true of childhood acute leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, testicular cancer and more recently for the surgical adjuvant treatment of breast cancer and colorectal cancer. The reasons for the necessity of combinations

are less clear. In the chemotherapy of bacterial infections, multi-drug regimens are often employed either to cover the spectrum of possible pathogens before culture results are obtained or to more effectively treat resistent mutants that arise. For the treatment of human neoplasms, there are additional reasons why combinations of drugs may be preferred to single agents. Two common rationale are the exploitation of biochemical synergism and the use of non-overlapping dose limiting toxicity.

Combining drugs with antitumor activity and non-overlapping host toxicity is a popular strategy. There is substantial evidence in experimental tumor systems of steep dose-response curves. The evidence for the steepness is less compelling in human tumors but the question has rarely been addressed prospectively. Many of the successes of combination chemotherapy could be attributed to achieving a higher equivalent cytotoxic dose by combining drugs with primarily non-overlapping toxicity. Consequently it seems worthwhile to examine a methodology for selecting combinations for study on this basis. The development of chemoprotectors such as hematopoietic growth factors provides additional incentive for attempting to exploit dose intensity as a possible route to reducing cancer mortality. It also provides an incentive for the development of tools which offer reasonable guidance about which combinations to develop in the presence of chemoprotectors. We have developed an approach for designing dose intense combination regimens which attempt to exploit non-overlapping toxicities.

We have developed a mathematical model for selecting cytotoxic drugs and dosages for a combination regimen based on the single agent anti-tumor activities of the drugs and their organ specific maximum tolerated doses. The regimen defined maximizes an approximate measure of anti-tumor effect subject to constraints on combined toxicity. This approach does not assume that maximally dose-intense regimens are clinically appropriate in all situations. Whether the identified regimen is superior to standard treatment should be determined by prospective randomized clinical trials. Determining which drugs to combine and in what proportions to combine them offers combinatorially huge numbers of possibilities. The method developed offers one approach to identifying combinations worthy of evaluation in prospective trials. A manuscript describing this work has been published in JNCI. A second manuscript is in press and a third, applying this model to metastatic breast cancer, has been submitted for publication. Invited presentations have been given at two scientific meetings.

15. DOSE INTENSITY ANALYSIS OF OSTEOSARCOMA TREATMENT

In collaboration with Dr. M. Smith, we have performed a retrospective analysis of results for the neoadjuvant treatment of children with non-metastatic osteosarcoma. The endpoint used for analysis was tumor response defined as greater than 90% tumor necrosis. Tumor response was related to dose intensities of doxorubicin, ifosfamide, VP-16, methotrexate and the BCD combination using regression analysis methods. Doxorubicin was identified as having the steepest dose intensity versus

response relationship evaluable for this data. A manuscript describing these results has been accepted for publication.

16. DOSE INTENSITY ANALYSIS OF OVARIAN CANCER TREATMENT

The BRB has performed two separate retrospective analyses of the effect of dose intensity (DI) on the outcome of treatment for patients with advanced ovarian cancer who have not previously been treated with chemotherapy. The first analysis has been performed in collaboration with Drs. L. Levin and W. Hryniuk of the Ontario Cancer Research Foundation. It is based on a more extensive database than used for their previous publication. The second analysis, to be published separately, was performed in collaboration with Dr. V. Torri of the Mario Negri Institute in Milan, Italy. It is based on an improved methodologic approach in which the importance of dose intensity is assessed without comparing treatment arms of different clinical trials. Only randomized clinical trials are included. Two manuscripts are in preparation.

17. DESIGN OF PROSPECTIVE CLINICAL TRIALS TO EVALUATE DOSE INTENSITY

In collaboration with Dr. W. Hryniuk, we have evaluated designs for prospective evaluation the effect of dose intensity on outcome. Issues such as number of dosage groups, sample size, role of pharmacokinetics measurements have been considered. A manuscript is in preparation. The approach developed has been used to design a dose intensity study of taxol in ovarian cancer and will be used for early characterization of clinical dose response for other active drugs.

18. INTERNATIONAL WORKSHOP ON DATA MONITORING COMMITTEES

Most of the large randomized clinical trials sponsored by the National Institutes of Health are reviewed periodically by a committee charged to ensure that the trial is being conducted in a safe, appropriate and ethical manner. These committees have been variously called Data Monitoring Committees, Data and Safety Monitoring Boards, Policy and Data Monitoring Boards, etc. While there has been much published discussion of statistical designs allowing for sequential evaluation of accumulating data, there is virtually no literature on the operational aspects of data monitoring, especially that performed by such committees. When one considers the enormous impact that the recommendations of such committees have, the lack of public discussion of the workings of these committees is surprising.

Data monitoring committees are being increasingly used, not just in NIH-sponsored trials, but in trials sponsored by the pharmaceutical industry and other organizations as well. Preliminary discussions with trial organizers from a variety of settings indicate that many different models are being used. A workshop focusing on the operational aspect of

data monitoring of clinical trials is extremely timely. The workshop is being organized and sponsored by the Biometric Research Branch NCI and collaborators Dr. S. Ellenberg of the AIDS Program, NIAID, and Drs. S. Yusuf and N. Geller of the NHLBI. There will be participation by NIH staff, NIH extramural investigators, representatives of the pharmaceutical industry and trial organizers outside of the United States.

The workshop will review the experience in various trials sponsored by the different institutes (NHLBI, NIAID, NEI, NICHD, NINDS, NIDDK, NIA, and NCI), industry and various non-US organizations (MRC, EORTC, ISIS etc.). A number of issues including qualitative aspects of the selection and operation of data monitoring committees, certain complex scientific issues (eg, multiple endpoints, subsets, changing protocols, planning new trials when data are masked, approach to confirmatory trials), ethical, and regulatory issues will be considered.

The entire proceedings including discussion will be published in Statistics in Medicine.

19. DESIGN CONSIDERATIONS FOR AIDS CLINICAL TRIALS

The AIDS epidemic has created new challenges for the process of developing effective new drugs. In collaboration with leading statisticians at NIH, Harvard and in England, we have re-evaluated many aspects of the drug development process and made recommendations for speeding drug evaluation. The recommendations include combining some of the conventional phases of drug evaluation, relaxing entry criteria, carefully examining the need for masking treatments, answering more than one question in a single trial and permitting patients to enter more than one trial at once. A manuscript describing these proposals has been published in the New England Journal of Medicine. The Chief of BRB has also made presentations to the AIDS statistical working group on the use of selection theory designs.

20. RANDOMIZED CLINICAL TRIALS WITH CLINICIAN-PREFERRED TREATMENT

A new design for a randomized clinical trial has been developed in which clinicians are able to choose for each patient the treatment they believe is most appropriate for that patient. A treatment is randomly assigned to the patient but the patient is treated by a physician who favors that assignment. This design may have application when conventional randomized designs are rejected by the participants because (1) clinicians believe strongly for some patient that one treatment is better than another, but (2) they disagree on some of these same patients about which is the better treatment. A trial has been begun at the University of Pacific Orthodontic Clinic (San Francisco) using this design. A manuscript, written in collaboration with Dr. S. Baumrind of UCSF, has been published in the Lancet describing this trial design.

21. DESIGN OF DOSE ESCALATION SCHEMES IN PHASE I STUDIES

Simulations have been conducted as part of an ongoing project to develop more efficient dose escalation schemes for phase I studies (to define the maximum tolerated dose) and to characterize the statistical properties of these designs. In collaboration with Dr. M. Christian, we are developing a proposal for a new phase I design to be piloted in cases where blood level directed escalation is not feasible. The new approach may permit within patient escalation before any dose limiting toxicity is observed. A retrospective analysis of the phase I database is being done to support the new design.

We have been also concerned about the adequacy of traditional phase I designs for dose escalation of combinations in the presence of hematopoietic growth factors. The maximum tolerated doses defined in such studies are often used directly in phase III trials. Several protocol designs are also being investigated in collaboration with Dr L. Miller for dose escalation of chemotherapy in the presence of CSF's and IL3. A plan for the early randomized evaluation of IL3 in small studies has been developed and is being implemented.

22. BAYESIAN MODEL FOR EVALUATING WHETHER TREATMENT DIFFERENCES VARY AMONG SUBSETS

One of the most difficult and important aspects of interpreting major comparative clinical trials is the evaluation of whether relative treatment efficacy varies substantially among subsets of patients defined with regard to baseline characteristics. Conventional statistical procedures for evaluating such "treatment by subset interactions" are notoriously conservative when the number of subsets is large. We have developed a new statistical approach to this problem. We use the Bayesian notion of a-priori exchangeability of interactions and a non-informative prior for the unknown variance component. Consequently, the result of the analysis is not subjective and does not require the elicitation of prior beliefs. The method is easily applied to the results of proportional hazards or logistic models and we have developed a computationally efficient algorithm for calculating posterior distributions of interaction terms and subset specific treatment effects utilizing decomposition methods. We have re-analyzed the rectal cancer adjuvant clinical trial (R1) of the National Surgical Adjuvant Breast and Bowel Cancer Project and a clinical evaluation of treatments for advanced colorectal cancer conducted by the North Central Cancer Treatment Group using this method. Two manuscripts are in press.

23. QUALITATIVE TREATMENT BY PROGNOSTIC FACTOR INTERACTIONS

Qualitative interactions are said to occur in a clinical trial when one treatment is superior for some subset of patients, while for another subset of treatments the other treatment is superior for other subsets. While some methodology had existed to test this hypothesis, these

methods were appropriate only in situations where the subsets were non-overlapping. We have developed more general methods to allow one to simultaneously examine the data for qualitative interaction with respect to each of several prognostic factors while still using all the information available. This permits one to detect qualitative interactions with smaller sample sizes than the method previously available. A manuscript has been accepted for publication describing these results.

24. PLANNING OF MULTI-TREATMENT CLINICAL TRIALS

Clinical trials with more than two treatment arms often require a more complex analysis strategy than do two-arm trials. For example, a recent CTEP sponsored clinical trial NSABP B21, involves randomization of patients with occult breast cancer primaries to receive either breast irradiation, tamoxifen or both. The treatment of choice will be tamoxifen alone if it is better than XRT alone and no worse than the combination. Similarly for breast irradiation alone. The combination is the treatment of choice if it is better than both single modality regimens. Traditional methods for planning clinical trials do not take into account such compound decision criteria. We have developed statistical methods for the planning (including sample size determination) and analysis of multiple arm clinical trials where the treatments are partially ordered according to a secondary criteria such as toxicity. Our methods provide high probability for selecting the most appropriate treatment. The methods can be used for normal, binomial and censored data. We have performed sample size calculations that account for decision strategies for such clinical trials. These results have been used for the planning of other studies such as the NCCTG 88-24-53 four arm evaluation of thoracic irradiation and chemotherapy for patients with stage 2-3a non-small cell lung cancer and the renal cancer comparison of low dose IL-2 versus high dose IL-2 versus high dose IL-2 plus IFN-α. A manuscript has been submitted for publication.

25. SELECTING THE BEST DOSE WHEN A DOSE-RESPONSE RELATION EXISTS

Many studies involve comparing 2 or more doses of the same drug. The common statistical approach to identify the optimum therapeutic dose is to employ response surface methodology. This methodology typically requires the specification of a dose response model and the use of more dose levels than can be handled reasonably in a multicenter clinical trial. We developed an alternative procedure that uses fewer dose levels and does not require specification of a dose response function. We compute sample sizes required to properly use our technique under a number of situations, for both continuous and dichotomous data. We also suggest clinical situations in which our technique is applicable. A manuscript has been submitted describing these results.

26. MODEL SELECTION IN STEPWISE REGRESSION

Stepwise regression analysis is one of the most commonly used methods of data analysis in statistics. The commonly used methods for deciding when to terminate the stepwise procedure are ad hoc, however. In collaboration with Drs. P. Thall of the M.D. Anderson Tumor Institute and D. Greer of George Washington University, we have evaluated the use of cross-validation as model selection criteria in stepwise regression. The NCI's Cray XMP supercomputer was used for this evaluation. A manuscript has been submitted for publication.

27. GROUP C/TREATMENT IND AND TREATMENT REFERRAL CENTER PROTOCOLS

In order to make effective drugs available to the oncologic community as early as possible, the CTEP has utilized the Group C and Treatment IND categories of the Food and Drug Administration. In order to obtain information on the effectiveness and toxicity of these drugs when used outside of research protocols, data are collected for these patients. The extent of data collection varies substantially by drug. The BRB has statistical responsibility for these protocols:

R88-0001: Treatment of patients with refractory germ cell carcinoma with cisplatin, etoposide (or vinblastine), ifosfamide and mesna.

I88-0015: Pentostatin in patients with active hairy cell leukemia previously treated with alpha-interferon.

I88-0016: VM-26 in combination with Ara-C for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia.

Adjuvant Chemotherapy with a semustine (methyl CCNU) containing regime for patients with resectable adenocarcinoma of the colon.

BRB staffing is also involved in the design and conduct of trials conducted under the new Treatment Referral Center mechanism. In particular, trials in advanced ovarian cancer using taxol and other experimental agents are being developed and monitored.

28. DEVELOPMENT OF A MASTER PROTOCOL FOR COMPARATIVE RADIOLOCALIZATION OF MONOCLONAL ANTIBODIES DIRECTED TO THE TAG-72 ANTIGEN

BRB staff participated in the development of this master protocol for TAG-72 localization in colorectal cancer patients. The primary objective of the study is to compare tumor/normal tissue ratios of various monoclonal antibodies directed to the TAG-72 antigen. Two monoclonal antibodies will be compared at a time by injecting patients with both, one labeled with I-125 and the other with I-131. Review of these studies and their results is ongoing.

29. TIAZOFURIN TOXICITY

In collaboration with Dr. J. Grem of the Clinical Oncology Program (COP), clinical toxicity experience with Tiazofurin in the phase I studies sponsored by CTEP was investigated. This review indicated that such toxicity was infrequent and not dose-dependent. A manuscript has been published.

30. MODULATION OF CYTOSINE ARABINOSIDE

In collaboration with Dr. J. Grem of COP, modulation of the activity of cytosine arabinoside by 3-deazauridine or cyclopentenyl cytosine, in a murine leukemia model, was analyzed. It was found that the modest increase in activity was compromised by increased toxicity, and therefore, maximally tolerable combined agent doses were no more effective than maximally tolerable doses of cytosine arabinoside alone. A manuscript has been published.

31. PILOT STUDY OF IL-2+LAK+IFN-ALPHA 2A IN RENAL CELL CARCINOMA AND MELANOMA

Analysis of a pilot study of IL-2+LAK+IFN-Alpha 2a in renal cell carcinoma and metastatic melanoma has been completed with Dr. M. Sznol of the Investigational Drug Branch, CTEP, and has been submitted for publication. Response rates observed were comparable to those expected from IL-2 or IFN alone.

32. TRIAL OF THYMOSIN IN NON-SMALL CELL LUNG CANCER

BRB staff is involved in a review of the results of a trial of thymosin 1 in non-small cell lung cancer, conducted by the RTOG.

33. EPIDEMIOLOGIC ANALYSIS USING COMPLEX SURVEY DATA

Large national health surveys offer the potential of examining relationships between risk factors and the development of cancer. For example, a recent paper (Stevens et al., NEJM 319 (1988), pp 1047-52) suggested low total iron-binding capacity was a risk factor for developing cancer. There is a controversy about whether the complex sampling designs used for these surveys must be taken into account when doing the analysis. BRB staff have collaborated with Dr. B. Graubard of DCPC in addressing this issue, both theoretically and by deriving a set of practical recommendations. Two additional papers are in press and one is in preparation. An invited presentation will be given describing some of this work at the Joint Statistical meetings in August 1991.

34. APPLICATIONS OF CRUDE INCIDENCE CURVES

In competing risks problems, crude incidence curves measure the time to certain types of events, in the presence of other events. As opposed to cause-specific curves, they do not try to pretend that the other types of events cannot happen. A survey paper has been submitted for publication in collaboration with Dr. F. Dorey of UCLA describing when one should consider using crude incidence curves, the methodology for using them, and some interesting applications. For example, one application involves radical prostatectomy for localized prostate cancer; the event of interest is recurrence while other causes of death are competing. Another example concerns the cardiotoxicity of different doses of mitoxantrone; death is considered a competing cause here.

35. SURVEY EFFECTS IN LONGITUDINAL STUDIES

Survey effects in longitudinal studies are unexplained increases or decreases in the observed value for all individuals measured at a particular time point. In a study with a single group of subjects, they can lead to biased estimates of the mean slope as well as an increased variability. In a study with a concurrent control group, however, a standard analysis can be used without problems. A manuscript written in collaboration with Dr. D. Roe of the University of Arizona, which describes these results has been submitted for publication.

36. LONGITUDINAL ANALYSIS OF THE DEVELOPMENT OF THE HUMAN JAWS

Describing the growth and development of the human jaws offers some interesting statistical challenges. Using metallic implants, allows one to separate remodeling effects from displacement of the bones themselves. A paper, written in collaboration with Dr. S. Baumrind at UCSF, describing the transverse widening of the jaws in children has been published, and another is in preparation.

37. STATISTICAL INFERENCE WITH CURTAILED HYPOTHESIS TESTS

A very small trial (n=5) was designed to test formally that the observed incidence of severe leukopenia in a trial administering G-CSF concurrently with chemotherapy was higher than the incidence based on historical data without G-CSF. A paper is in preparation describing the results of this trial (with Dr. L. Miller and investigators from the University of Pennsylvania).

This trial was actually curtailed at n=3. There are surprising statistical properties of p-values and confidence intervals from such a curtailed test. These are being described in a paper in preparation with K. Yu, NICHD.

38. INTERIM SAMPLE SIZE ADJUSTMENTS FOR NONCOMPLIANCE

BRB is collaborating with the Radiotherapy Oncology Group Statistical Center in studying the effect of adjusting sample size according to a preliminary estimate of noncompliance rate. A simulation study has been carried out to investigate the type I and type II errors after the adjustments and theoretical investigation is being planned.

39. USE OF SURROGATE ENDPOINT DATA IN THE ANALYSIS OF THE PRIMARY ENDPOINT

In collaboration with Dr. L. Freedman of the Division of Cancer Prevention and Control, NCI, and Dr. E. Slud of the U.Md., Mathematics Department, BRB staff is exploring the extent to which use of surrogate endpoint data can be used to reduce the variance of estimates of treatment effect on the primary endpoint, or, alternatively, increase the power of treatment comparisons involving the primary endpoint.

40. ADAPTIVE MONITORING AND ACCRUAL TERMINATION OF PHASE III TRIALS

In collaboration with Dr. E. Slud of the U.Md., Mathematics Department, BRB staff is exploring the possibility of adaptively tailoring trial sample size and the sequential monitoring scheme, based upon the results of the initial monitoring analyses. Methods have been developed to calculate the successive alpha levels to be used in monitoring and in the final treatment comparison, so as to maintain the desired type I error for the trial as a whole. Particular adaptive strategies are being explored through simulations to determine their usefulness with respect to increasing the power and/or the efficiency of the trial.

41. TYPE ONE ERROR AFTER READJUSTING THE SAMPLE SIZE

Some clinical trials are carried out in two stages. At the first stage, a small randomized study is carried out to estimate the means and variances of two treatments. The sample size requirement is determined by the first stage result. Then a second stage randomized study is done according to the calculated sample size. If at the end of the study, data from both stages are analyzed together, the type I error may not have the nominal value. The upper bound of the type I error in this situation is being investigated.

42. STATISTICAL METHODS FOR POPULATION GENETICS

A new method of estimating population allele frequencies for the ABO blood group system has been developed in collaboration with Dr. Naylor and E. Russek-Cohen (U.Md.). The efficiency of the new approach was contrasted with pre-existing approaches through computer simulations.

43. OTHER ACTIVITIES

The members of the BRB provide consulting services for all programs of the Division of Cancer Treatment and, on occasion, other NCI divisions and institutes. During the past year this has included intramural clinical trials of the Clinical Oncology Program, planning extramural projects with the Radiation Research Program, as well as consultations with investigators of the Developmental Therapeutics Program and Cancer Therapy Evaluation Program. The BRB also participates in training of biostatisticians and physicians in clinical trials methodology. During the past year we hosted two individuals for such training, an Italian physician investigator and an Israeli statistician. The BRB has conducted a five session course in clinical trials methodology for Medicine Branch fellows. We have given invited presentations of a didactic nature for the Statistical Society of Canada, the Biometric Society and the AIDS Statistical Working Group. An invited chapter of meta-analysis was written for the Principles and Practice of Oncology Update Series for mailing to all U.S. oncologists. A letter to JNCI was published discussing common statistical fallacies in the evaluation of the role of diet rat carcinogenesis The Chief of the BRB served on the NIH Panel dealing with early dissemination of the results of clinical trials. A computer program was developed and distributed internationally, on request, for the planning of phase II clinical trials using Simon's optimal two stage design.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06308-20BRB

PERIOD COVERED
October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Biometric Research Branch

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard Simon, Ph.D., Chief Biometric Research Branch,CTEP,DCT,NCI
Others:
Lawrence V. Rubinstein, Ph.D., Statistician, BRB, CTEP, DCT, NCI
Tar Timothy Chen, Ph.D., Statistician, BRB, CTEP, DCT, NCI
Edward L. Korn, Ph.D., Statistician, BRB, CTEP, DCT, NCI
Antonis Koutsoukos, Ph.D., Visiting Fellow, BRB,CTEP,DCT,NCI

COOPERATING UNITS *(if any)* Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Division of Cancer Prevention and Control, NCI; George Washington University; M.D. Anderson Tumor Institute

LAB/BRANCH
Biometric Research Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biometric Research Branch (BRB) is the statistical component for scientific planning and monitoring of the national and international research program of the Division of Cancer Treatment. The branch provides statistical leadership for all extramural activities of the division. The branch is also responsible for statistical consultation and collaboration with the intramural activities of the Biological Response Modifier Program and the Developmental Therapeutics Program.

The Biometric Research Branch performs statistical planning and evaluation of all Division of Cancer Treatment supported therapeutic clinical trials. The branch performs scientific monitoring and analysis of extramural trials. Primary statistical direction is provided by the branch for the conduct of selected national and international studies of therapeutic interventions, prognostic factors, pre-clinical screening and diagnostic imaging. The branch performs evaluations of therapeutic interventions based upon syntheses of results from multiple studies.

The Biometric Research Branch conducts research on experimental designs, biometric method and biomathematical approaches for the development and efficient evaluation of improved cancer treatments.

CLINICAL INVESTIGATIONS BRANCH

OVERVIEW

The Clinical Investigations Branch (CIB) is responsible for coordinating administrative and scientific aspects of the Clinical Trials Cooperative Group Program, and for advising other Cancer Therapy Evaluation Program (CTEP) Branches and National Cancer Institute staff on disease-oriented issues related to clinical trials supported by the Division of Cancer Treatment (DCT). The Clinical Trials Cooperative Group Program is the principal component of the DCT which conducts extramural clinical trials, providing a mechanism for the continuous generation of therapeutic and ancillary studies whose intent is to improve the survival and quality of life of the nation's cancer patients. The CIB assists in creating a strategic framework for the Group Program's efforts by identifying and articulating research questions, encouraging relevant laboratory/clinical correlative studies, and promoting intergroup studies as appropriate to maximize timeliness and accuracy of clinical trials. Its administrative activities include the development of the the Program's funding plan, the reallocation of resources as appropriate to optimize the Program's efficiency, the development of Guidelines for Program participants, and advising the Cancer Clinical Investigations Review Committee regarding Program goals.

I. ORGANIZATION

The Clinical Investigations Branch (CIB) consists of three Sections under the direction of Richard S. Ungerleider, M.D., as follows:

SECTION	STAFF
Medicine	Bruce D. Cheson, M.D. (Head) F. Andrew Dorr, M.D. J. Michael Hamilton, M.D. Timothy Moore, M.D. John F. Brennan, M.D.
Pediatrics	Richard S. Ungerleider, M.D. (Acting Head) Malcolm A. Smith , M.D., Ph. D.
Surgery	Richard S. Ungerleider, M.D. (Acting Head) Edward Trimble, M.D.

In addition, a gynecologic surgeon, Edward Trimble, M.D., has been recruited for the Surgery Section, and will serve as Senior Investigator as of July 15, 1991. Timothy Moore, M.D. will leave CIB at that time.

Secretarial support is provided by Christine Beachley and Bernadette Greenfield, with the part-time assistance of Stephen Sudler.

Individual physicians within CIB are responsible for maintaining information on current developments and evolving opportunities within specific diseases and modalities, as follows:

DISEASE	STAFF
Brain	Hamilton
Breast	Dorr
Endocrine	Brennan
Gastrointestinal	Hamilton
Genitourinary	Dorr
Gynecologic	Moore
Head and Neck	Moore
HIV-associated cancer	Cheson
Leukemia (adult)	Cheson
Leukemia (child)	Smith
Lung	Brennan
Lymphoma (adult)	Cheson
Lymphoma (child)	Smith
Melanoma	Brennan
Myeloma	Cheson
Sarcoma (adult)	Brennan
Sarcoma (child)	Smith

MODALITY	STAFF
Bone marrow transplant	Cheson
Infectious disease	Cheson
Quality of life	Moore
Radiation	Hamilton
Surgery	Trimble
Minority accrual	Hamilton

II. COORDINATION AND ADMINISTRATION OF THE COOPERATIVE GROUP PROGRAM

The CIB monitors, coordinates and advises the Cooperative Groups regarding their scientific agendas, and provides administrative support for their activities. This effort is required to optimize the productivity of the cooperative agreement assistance mechanism (U10), through which the NCI provides funds for definitive, multi-institutional trials and the pilot and developmental studies which precede them. Approximately $60 million is devoted annually to these activities.

The CIB advises and directs the Groups in allocating limited financial, investigator and patient resources. During the past year, Group-related administrative activities have included: supervision of the selection of the third and fourth generations of High Priority Trials by the Cooperative Group Chairmen and the DCT Board of Scientific Counselors; development of a Minority Accrual Initiative, and the initial distribution of funds for that purpose; the development of a Request for Applications for the conduct of Phase I studies in pediatrics, to be supported by funds from the Research Project Grant pool (U01 mechanism); encouragement of the Groups to serve as a resource for the entire NCI and in doing so, seek funding from Divisions other than DCT; facilitating the identification of support for the establishment of a tumor specimen bank for gynecologic malignancies; promoting and overseeing the initial phases of development of a 5-Year Strategic Plan for the Groups, which has subsequently been claimed by the Group Chairmen as a document to be

developed independent of NCI staff; and supervision of responses to a request for application for funds from the NIH Office of Research in Women's Health to enhance the enrollment of women in clinical trials. These activities were in addition to the more routine activities of devising and implementing a funding plan for successfully recompeting Groups and institutions, using available funds which represented a fraction of the amount recommended by peer review, and advising the Groups on routine financial and regulatory matters.

In terms of research activities, staff responsibilities include critical review and participation in the development of protocols conducted by the Groups, with particular attention to the importance and timeliness of the study question, the soundness of its rationale, the adequacy of the design to answer the study question, its feasibility relative to patient and financial resources, and its attention to patient safety and regulatory issues. The CIB and Group leadership act jointly to identify and prioritize clinical research questions of mutual interest. There is regular interaction between CIB staff and Group investigators in order to promote medically valuable clinical trials and to avoid duplicative or unreliable studies. The CIB promotes clinical trials that are sufficiently large to be reliable, and are completed in the briefest posssible time; hence the encouragement of intergroup studies when appropriate. An intergroup study is deemed appropriate when a study by an individual Group would require an inordinately long time for completion, and/or might accrue too few patients to permit statistically valid conclusions. CIB staff organize strategy meetings in selected cancers in order to help establish an overview of current therapeutic issues whose resolution might be facilitated through collaborative clinical trials. Representatives of the Groups, as well as other interested investigators, participate in these meetings in which a consensus regarding the objectives and design of optimal trials is developed. The likelihood of duplicative efforts is thereby diminished and the probability of intergroup collaboration is thereby enhanced.

Since it is not feasible to conduct strategy sessions for all disease areas and issues addressed by the Groups, CIB is increasingly emphasizing Concept Review, an evaluation by CTEP Senior Investigators of the essence of a major Phase III study proposal while still in an early stage of evolution, rather than attempting to modify a protocol at the final stages of development. A brief document outlining the scientific rationale, objectives, eligibility, treatment schema and statistical considerations is sent by the investigators to CIB, which, in concert with other CTEP staff, provides relevant criticism in return. During the past year (7-1-90 to 6-30-91) 31 concepts were reviewed, of which 8 are currently in review as protocols.

The formal Protocol Review process is a major analytic activity of CIB staff. Prior to activation by the Groups, all protocols using NCI-sponsored investigational agents or involving more than 100 patients undergo review by CTEP staff for safety and scientific issues. CTEP staff critique these protocols and request changes when appropriate. A written consensus review is provided the investigators which outlines required and/or recommended changes in the protocol document. During the past year, 431 protocols were reviewed by the Protocol Review Committtee. Finally, but with ever-increasing emphasis, the CIB promotes relevant laboratory-clinical correlative investigations which might prove scientifically productive. Information concerning the best correlative studies comes not only from Group pilot

activities, but also from information gained form the R01/P01 grant portfolio which CTEP manages.

The following is a list of the Cooperative Group organizations that were functioning with NCI support in FY91 and the CIB staff member responsible for scientific liaison with that organization.

Group	CIB Liaison
Brain Tumor Cooperative Group (BTCG)	Hamilton
Cancer and Acute Leukemia Group B (CALGB)	Cheson
Children's Cancer Study Group (CCSG)	Smith
Eastern Cooperative Oncology Group (ECOG)	Dorr
European Organization for Research on Treatment for Cancer (EORTC)	Cheson
Gynecologic Oncology Group (GOG)	Moore, Trimble
Intergroup Rhabdomyosarcoma Study (IRS)	Smith
National Surgical Adjuvant Breast and Bowel Project (NSABP)	Dorr
National Wilms' Tumor Study Group (NWTS)	Smith
North Central Cancer Treatment Group (NCCTG)	Hamilton
Pediatric Oncology Group (POG)	Smith
Quality Assurance Review Center (QARC)	Hamilton
Radiation Therapy Oncology Group (RTOG)	Hamilton
Southwest Oncology Group (SWOG)	Cheson

COOPERATIVE GROUP OUTREACH PROGRAM

The Cooperative Group Outreach Program (CGOP) has been under CIB supervision since 1987, when it was transferred from the Division of Cancer Prevention and Control. This Program allocates money to participating Groups for the purposes of increasing access to clinical trials among community patients and physicians. The current peer-reviewed participants in the CGOP are ECOG, SWOG, CCSG, CALGB and NSABP. In 1990, these Groups accrued a total of 3727 patients to their clinical trials through this Program.

III. SCOPE OF GROUP ACTIVITIES

ACCRUAL

In 1990, the Clinical Trials Cooperative Group Program involved 4630 investigators in roughly 1300 institutions, hospitals or practices, and accrued 23,767 patients to 504 therapeutic studies, with most of these patients entering Phase III trials (Table A). Virtually every type of malignancy is being studied in this collaborative enterprise (Table B). Phase II/III estimates of activity and definitive tests of efficacy are the central components of the effort to reduce cancer mortality. Patient accession by disease is indicated by Figure A. Accrual to therapeutic clinical studies by each Group is displayed in Figure B. In addition, over 15,000 patients were entered on Group non-therapeutic/laboratory correlative studies using clinical trials patients/samples.

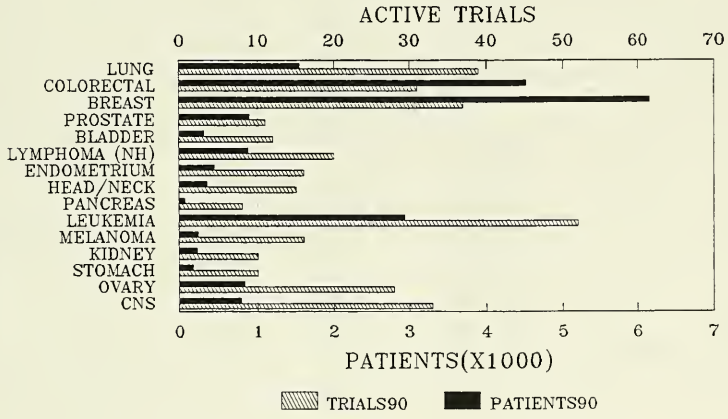
TABLE A

NCI CLINICAL COOPERATIVE GROUPS
ACCRUAL SUMMARY
CALENDAR YEAR 1990

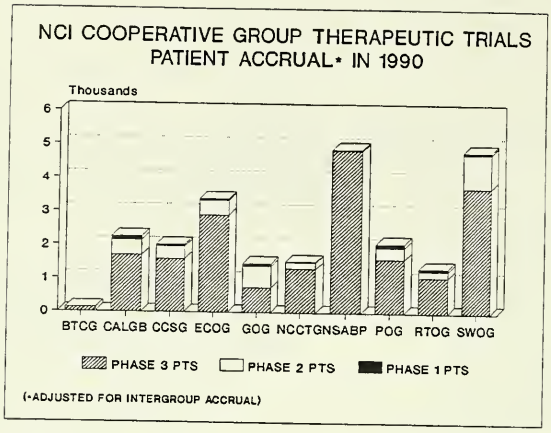
	PATIENT ENTRIES	OPEN STUDIES
PHASE I	559	65
PHASE II	3,661	252
PHASE III	19,547	187
NON-THERAPEUTIC/CORREL.	15,186	138
EORTC (1990) (EST)	6,100	215

FIGURE A

COOPERATIVE GROUP TREATMENT TRIALS
PROTOCOL ACTIVITY BY DISEASE/SITE - 1990
(PHASE II & III STUDIES)



(IN ORDER OF DECREASING INCIDENCE)



To put this into a national perspective, the following table compares the impact of the most common cancers with the group program's clinical research effort.

TABLE B

COOPERATIVE CLINICAL GROUP STUDIES IN SELECTED DISEASE AREAS - PROTOCOLS ACTIVE IN 1990 ACCRUAL TO PHASE II AND PHASE III STUDIES

ORGAN	NEW CASES IN 1990 (ACS DATA)	DEATHS IN 1990	RATIO DEATHS TO NEW CASES	STUDIES OPEN TO ACCRUAL	TOTAL ACCRUAL 1990	% ONTO GROUP STUDIES
Lung	157,000	142,000	0.90	39	1,553	1.0
Colon/Rectum	155,000	60,900	0.39	31	4,513	2.9
Breast	150,900	44,300	0.29	37	6,160	3.5
Prostate	106,000	30,000	0.28	11	894	0.7
Bladder	49,000	9,700	0.20	12	317	0.6
Lymphoma, Non-H	35,600	18,200	0.51	20	875	2.4
Endometrium	33,000	4,000	0.12	16	449	1.4
Oral (HN)	30,500	8,350	0.27	15	355	1.2
Leukemia	27,800	18,100	0.65	52	2,937	10.5
Melanoma	27,600	8,800	0.32	16	243	0.8
Kidney	24,000	10,300	0.43	10	232	0.9
Pancreas	28,100	25,000	0.89	8	71	0.3
Stomach	23,200	13,700	0.59	10	96	0.4
Ovary	20,500	12,400	0.60	28	827	4.0
Brain/CNS	15,600	11,100	0.71	33	788	4.7
Liver	14,600	11,900	0.82	4	110	0.7
Cervix	13,500	6,000	0.44	26	566	4.4
Myeloma	11,800	8,900	0.75	10	374	3.0
Esophagus	10,600	9,500	0.90	4	82	0.8
Lymphoma, Hodgkin's	7,400	1,600	0.22	15	290	3.9
TOTAL	941,700	454,750		397	21,732	

HIGH PRIORITY CLINICAL TRIALS

The NCI established the High Priority Clinical Trials Program in 1988 to stimulate accrual to certain cancer treatment trials and to generate greater awareness and enthusiasm for clinical trials by the general public and health care workers. The trials were selected for this program based on their potential to increase the survival rate for a number of common cancers or for their ability to answer questions of special biological significance. The accelerated patient accrual was intended to speed the resolution of underlying medical questions and to bring successful new cancer treatment procedures to the cancer patient more quickly.

It was recently established, for example, in an intergroup clinical trial of colon cancer that treatment with levamisole in combination with 5-fluorouracil (5-FU) markedly improves patients' survival. As a result, a recent NIH Consensus Development Conference has recommended that this therapy be considered for all Dukes' C colon cancer patients. Other studies suggest that the combination of 5-FU and leucovorin is beneficial to patients. Further development of the adjuvant treatment of colon cancer is the goal of (INT-0089) one of the current High-Priority Clinical Trials. This study will evaluate the effectiveness of levamisole plus 5-FU plus leucovorin compared to 5-FU/levamisole or 5-FU/leucovorin.

Efforts to increase accrual to designated High Priority Clinical Trials are progressing along two parallel tracks:

a. The Office of Cancer Communications (OCC) is coordinating assessment and information campaigns for the lay and professional communities. The general public is being educated about clinical trials via print and electronic media, including two newly available videotapes on the subject of participation in clinical trials. The various Cancer Information Services are also being targeted for OCC attention. The aim of this effort is to stimulate lay enthusiasm for volunteering for protocol studies.

b. The multidisease, adult Cooperative Groups have expanded their clinical bases. More than 4000 American Society of Clinical Oncology (ASCO) member physicians were contacted and hundreds responded to the invitation to participate in the High Priority Trials. After screening, about 157 practices or institutions (new and/or currently unfunded) were identified as promising resources. The Groups submitted detailed proposals to enhance accrual to the selected trials and received supplementation of their awards in FY88, 89 and 90 to provide financial reimbursement for the costs of accruing additional patients.

As of May 1991, three series (1988,1989 and 1990) of High Priority Trials had been designated by NCI:

Series I High Priority Trials - Name and NCI Identification Number

- Adjuvant Chemotherapy and Radiation Therapy for Rectal Cancer (NCCTG-864751)
- Adjuvant Chemotherapy for Bladder Cancer (INT-0080)
- Adjuvant Chemotherapy with and without Radiation Therapy for Rectal Cancer (NSABP-R02)
- Comparison of Chemotherapy for Non-Hodgkin's Lymphoma (INT-0067)
- Adjuvant Chemotherapy Following Surgery for Colon Cancer (NSABP-C-03). CLOSED

Series II High Priority Trials - Name and NCI Identification Number

- Chemotherapy Before and After Surgery for Breast Cancer (NSABP-B-18)
- Adjuvant Chemotherapy with and without Tamoxifen for Breast Cancer (INT-0102)
- Chemotherapy with Two Forms of Radiation Therapy for Small Cell Lung Cancer (INT-0096)
- Adjuvant Chemotherapy for Colon Cancer (INT-0089)
- Radiation Therapy and Chemotherapy for Non-Small Cell Lung Cancer (RTOG-8808)
- Comparison of Treatments for Early-Stage Breast Cancer (NSABP-B-21)

Series III High Priority Trials - Name and NCI Identification Number

- Post Remission Treatment of Adult Acute Non Lymphocytic Leukemia comparing Autologous Bone Marrow Transplantation with intensive Chemotherapy (EST-3489)
- Adjuvant Therapy of Rectal Cancer (INT-0114)
- Adjuvant Chemotherapy Following Surgery for Colon Cancer (NCCTG-894651)

In June, 1991 an additional seven trials (Series IV) were selected by the Cooperative Group Chairmen and endorsed by the DCT Board of Scientific Counselors, to replace trials which are anticipated to complete their accrual within the next year. These are:

- Comparison of high dose chemotherapy including autologous bone marrow reinfusion versus standard dose chemotherapy for high risk non-metastatic breast cancer patients (CALGB-9082)

- Comparison of conventional adjuvant therapy vs high dose chemotherapy and autologous bone marrow transplant following conventional adjuvant therapy for high risk non-metastatic breast cancer (INT-0121)

- Lumpectomy and breast irradiation with and without tamoxifen for noninvasive intraductal carcinoma of the breast (NSABP B-24)

- Post-operative adjuvant interferon in resected high-risk melanoma (EST 1690, CALGB-9190)

- Thoracic radiotherapy with and without chemotherapy for completely resected non-small cell lung cancer (INT-0115)

- Hormonal therapy versus observation in patients with advanced prostate cancer (EST-3886, SWOG-8793)

- Adjuvant chemoradiation versus observation after gastric resection for adenocarcinoma (INT-0116)

RESULTS OF THE HIGH PRIORITY CLINICAL TRIAL INITITATIVE

Accrual to the High Priority Trials continues to accelerate, and the rate of entry of new patients onto these studies is well beyond the average rate of accrual to other large NCI-supported Phase III treatment studies. The nine active High Priority Trials now are accruing patients about four times the average rate of the other large Phase III trials.

Four of the five Series I trials have succeeded in accruing patients more rapidly than initially anticipated. The National Surgical Adjuvant Breast and Bowel Project (NSABP) Study C-03 reached its accrual goal and closed in April, 1989. The North Central Cancer Treatment Group (NCCTG) rectal study (864751) closed in September, 1990 having accrued 680 patients in half the time originally projected. The NSABP rectal study (NSABP-R02) and the lymphoma intergroup (INT-0067) trial are entering patients somewhat more quickly than planned, and will complete accrual on or ahead of schedule. The SWOG Bladder intergroup study is an unprecedented effort involving genitourinary surgeons (INT-0080), and accrual is progressing at a reduced rate; an extended accrual period will be required.

Although the Series II trials are mostly very large studies, their planned accrual periods are all less than 3.3 years. Accruing beyond their projected rates are the Eastern Cooperative Oncology Group small cell lung cancer study (INT-0096) and intergroup node-negative breast cancer study (INT-0102). These studies will complete accrual in less than the projected time period and are expected to close about one year early. The Radiation Therapy Oncology Group lung intergroup study (RTOG-8808), which opened in January 1989, also is accruing patients quickly and is expected to close earlier than originally projected.

The NSABP breast study B-18 addresses an important question in adjuvant therapy and is accruing at nearly the projected rate, while the NSABP breast study B-21 (Occult Stage I Disease) deals with a unique patient population and

is entering patients more slowly than planned. The further study of levamisole in colon cancer (INT-0089) is accruing patients very rapidly, and should be completed on schedule.

The third series of High Priority Trials was initiated in June, 1990. The NCCTG study of levamisole as adjuvant treatment for resectable colon cancer has already accrued over half of the patients which will be required for the study. The intergroup study of levamisole as adjuvant therapy for rectal carcinoma opened in August, 1990 but is already accruing patients beyond the rate anticipated. The chemotherapy/bone marrow transplant intergroup study of myeloid leukemia which opened in February, 1990 is still accruing patients somewhat below hoped-for rates.

These studies satisfy the intent of the High Priority Trials Program, which is to hasten the resolution of important medical questions in disease settings where therapeutic benefit and new scientific knowledge can be anticipated.

The following tables compare accrual targets with accrual reached at the indicated time points for the initial three series of High Priority Trials.

ACCRUAL TO HIGH PRIORITY TRIALS
SERIES I

STUDY	ACTIVATION DATE	ACCRUAL TARGET	1/88	1/89	1/90	1/91
Lymphoma (INT 0067)	04/86	1,000	219	481	755	1027
Bladder (INT 0080)	08/87	298	5	35	96	130
Rectal (NCCTG 86-47-51)	06/87	450	26	87	403	483 (CLOSED)
Colon (NSABP C-03)	08/87	855	114	830	1,081 (CLOSED)	
Rectal (NSABP R-02)	08/87	750	27	191	373	572

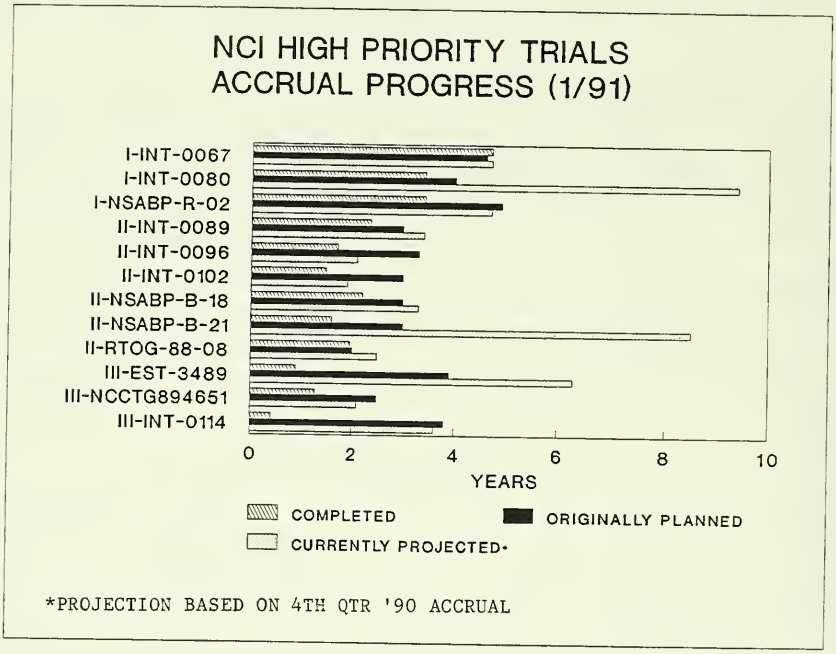
ACCRUAL TO HIGH PRIORITY TRIALS
SERIES II

STUDY	ACTIVA-TION DATE	ACCRUAL TARGET	7/88	1/89	7/89	1/90	7/90	1/91
Colon Cancer INT-0089	08/88	2,700	30	113	382	659	923	1765
Lung (small cell) INT-0096	04/89	250	-	-	3	34	65	186
Breast (node-) INT-0102	07/89	2,600	-	-	-	233	542	1766
Breast NSABP-B18	11/88	1,275	-	40	211	390	483	763
Breast NSABP-B21	06/89	1,350	-	-	5	41	59	162
Lung (non-small cell) RTOG-88-08	01/89	360	-	-	16	90	132	263

ACCRUAL TO HIGH PRIORITY TRIALS
SERIES III

STUDY	ACTIVA-TION DATE	ACCRUAL TARGET	7/89	1/90	7/90	1/91
Leukemia (EST-3489)	2/90	560	-	-	18	61
Rectal Cancer	8/90	1378	-	-	-	106
Colon Cancer	9/89	800	-	63	153	451

FIGURE C



INITIATIVE FOR INCREASING MINORITY ACCRUALS TO COOPERATIVE GROUP TRIALS

CTEP has initiated a program to increase participation of individuals from minority ethnic groups in clinical trials. Among the major racial/ethnic groups, blacks have the highest incidence rate for all cancers combined, followed by Native Hawaiians and then whites. Blacks also experience the highest overall cancer mortality rates, followed by Native Hawaiians and whites (U.S. mortality data classify Hispanics as whites). Blacks and Native Americans experience the least favorable survival rates.

In order to extend the benefits of participation in clinical trials to all segments of the population, additional funds have been made available to Clinical Trials Cooperative Groups by the National Cancer Institute to cover the costs associated with accruing these medically disadvantaged patients. Minority patients are defined as Black Americans; Hispanic Americans of Mexican, Puerto Rican, Cuban or Central American origins; and native Americans, including American Indians, native Hawaiians and Alaskan natives. Current estimates of minority participation in Cooperative Group Clinical trials are shown in the following table:

ESTIMATE OF CURRENT MINORITY ACCRUAL TO COOPERATIVE GROUP TRIALS

GROUP	% GROUP ACCRUAL WITH RACIAL DATA	TOTAL*	ACCRUAL MINORITY	% MINORITY
BTCG	65	98	5	5 %
CALGB	100	2177	247	11 %
CCSG	100	1641	404	25 %
ECOG	63	1788	203	11 %
GOG	14	171	23	13 %
NCCTG	100	1845	90	4 %
NSABP	100	2627	286	11 %
POG	85	1890	528	28 %
RTOG	43	710	205	29 %
SWOG	100	4717	526	11 %
TOTALS		17,664	2517	14.6%

* TOTAL NUMBER OF PATIENTS WITH RACIAL DATA; 1989 DATA

STRATEGY MEETINGS

Strategy meetings help provide an overview and prioritize national efforts in selected disease sites. Expert oncologists from the Cooperative Groups and Cancer Centers meet at the National Cancer Institute or elsewhere to review ongoing clinical experiments and identify short-term priorities for research. CTEP staff have found that the conduct of strategy/planning meetings at the annual ASCO meetings is a valuable activity at considerably reduced cost to the Government, taking advantage of the presence of a number of investigators at the ASCO Meeting. The status of current studies is reviewed, follow-up trials are planned, and drug development or toxicity issues are discussed. Below is a schedule of the meetings and attendance at ASCO, May 1991. Those marked with an asterisk involved Cooperative Group/CIB activities.

MEETING	INVESTIGATORS ATTENDING
Interleukin-2 Working Group	20
Breast Tumor Bank	15*
ECOG Lymphoma Committee	9*
HMBA (Investigational Agent)	10
GI Club	39*
(INT-0089 Monitoring Comm.)	8
(INT-0114 Monitoring Comm.)	12
Ewing's Sarcoma/PNET	25*
Intergroup Melanoma	20*
Intergroup APL	10*
ECOG Myeloma Committee	7*
Brain Tumor Club	22*
Epipodophyllotoxin/Secondary-AML	30*
ECOG GI Committee	12*
Adjuvant Retinoid	6
	225

The format of strategy meetings is to review the ongoing Cooperative Group clinical trials (with current estimates of accrual and projections of when studies would be completed) with discussion devoted to strategies for the next generation of clinical trials. Where appropriate, intergroup efforts are encouraged in order to achieve greater economy and statistical power. These meetings result in considerable exchange of information. The following strategy meetings were held in FY 90:

1. **BREAST CANCER**

TOPIC: High dose chemotherapy for breast cancer

DATE: October 11, 1990

COORDINATOR: F. Andrew Dorr, M.D.

There has been a striking increase in the use of high dose chemotherapy with stem cell support (either bone marrow or peripheral blood) for the treatment of women with either metastatic or high risk non-metastatic breast cancer. Nevertheless, the optimal regimen, the clinical indications, and the benefit of this treatment relative to standard therapies are not completely clear. Moreover, third party reimbursement remains a problem for many patients offered this therapy. This meeting resulted in three cooperative group protocols which will characterize the role of high dose chemotherapy in the treatment of women with high-risk, primary breast cancer and those with metastatic disease. Participants included representatives from the adult cooperative groups and major cancer centers.

2. **SMALL CELL LUNG CANCER**

TOPIC: Small cell lung cancer clinical trials

DATE: March 11, 1991

COORDINATOR: John Brennan, M.D.

Representatives from the Cooperative Groups and other large, independent institutions were invited to the meeting to discuss a variety of issues in small cell lung cancer.

Possible areas for future studies in limited and extensive stage disease were discussed.

Limited stage disease:

1. the determination of the optimal treatment schedule for radiotherapy administration

2. late, consolidation treatment with high dose chemotherapy for patients who achieve a complete response to initial therapy

3. the addition of biologic response modifier treatment for patients who achieve a complete response to initial therapy

4. the incorporation of CSF's into protocols that employ combination chemotherapy and concurrent radiotherapy, in order to reduce the toxicity of treatment

Extensive stage disease:

1. a comparison of treatment with cisplatin and intravenous etoposide, vs. carboplatin and intravenous etoposide, vs. oral etoposide

2. the addition of thoracic and cranial radiotherapy to treatment for patients who achieve a complete response to initial therapy

Potential studies of the effects of prophylactic cranial irradiation (e.g., neurologic sequelae), and issues in the study design for the development of new drugs were also discussed.

3. **ALL TRANS-RETINOIC ACID CLINICAL DEVELOPMENT**

TOPIC: All trans-retinoic acid as a potential cellular differentiating agent.

DATE: March 25, 1991

COORDINATOR: Bruce D. Cheson, M.D.

Impressive results have been reported by investigators from China and France with ATRA in acute promyelocytic leukemia with complete remissions in 80% of relapsed patients. Moreover, there is evidence that the drug works as a cellular differentiating agent, presumably through a retinoic acid binding receptor located at the site of the t15;17 breakpoint, wherein is located the myl-oncogene. A meeting was chaired by Drs. Cheson and Parkinson to discuss potential clinical trials in other malignancies, and to identify basic science correlative studies to make use of the clinical material to provide additional insight into the biology of retinoids in human malignancies. Other tumors to be considered include cutaneous T-
cell lymphoma, head and neck cancer, myelodysplastic syndromes, testicular cancer, and neuroblastoma.

4. **EWING'S FAMILY OF TUMORS**

TOPIC : Peripheral Neuroectodermal Tumor (PNET)/Ewing's Sarcoma (ES) Clinical Trials:

DATE: February 11, 1991 and May 19, 1991

COORDINATOR: Malcolm A. Smith, M.D., Ph.D.

The Pediatric Section, CIB organized a strategy meeting to discuss the appropriate treatment setting for patients with PNET. The impetus for this meeting was the proposal by the Intergroup Rhabdomyosarcoma Study (IRS) committee to treat children with PNET on the IRS-IV study, and the concern of some POG and CCSG members (especially Bone Tumor Committee members) that these patients should be treated on an ES protocol because of the close similarities of these tumor types. The initial meeting was in February, 1991 with a follow-up meeting at ASCO in May, 1991. In attendance were clinicians and pathologists representing IRS, POG, and CCSG, as well as CTEP staff. The overall conclusions from these meetings included:

(a) PNET and ES are closely related tumors of neuroectodermal origin and can be distinguished from the great majority of rhabdomyosarcoma cases by available diagnostic methods;

(b) the group of tumors classified as "extra-osseous Ewing's sarcoma" (EOE) is diverse, includes some tumors which might now be classified as PNET, and has been difficult to diagnose using histologic methods;

(c) advances in diagnosis and classification of pediatric neuroectodermal tumors include new monoclonal antibodies (e.g., HBA-71 and 12E7 which appear to rather specifically recognize PNET/ES tumors); and molecular

biology methods to detect chromosomal aberrations and altered gene expression;

(d) children with PNET will not be treated on the IRS-IV study and will be treated on the next ES protocol, scheduled to open in 1992; and

(e) the next ES protocol will likely address the ability of dose intensification achieved by cytokine use to improve outcome and will be an intergroup study in order to assure adequate accrual in a timely fashion.

5. **OSTEOGENIC SARCOMA**

TOPIC: Muramyl Tripeptide Phosphatidylethanolamine (MTP-PE) treatment for Osteosarcoma

DATE: April 8, 1991

COORDINATOR: Malcolm A. Smith, M.D., Ph.D.

The purpose of this meeting was to discuss with CCSG investigators and Ciba-Geigy representatives the use of MTP-PE in the next CCSG osteosarcoma protocol. Concerns discussed at the meeting included the limited pre-clinical experience with MTP-PE and concurrent cytotoxic therapy, lack of clinical experience in children with MTP-PE and concurrent chemotherapy, and the possibility that concurrent administration of MTP-PE and chemotherapy might jeopardize the proposed factorial design and interfere with the ability of the study to answer an important chemotherapy question. The following strategy to address these concerns was developed as a result of this meeting and follow-up correspondence:

(a) additional pre-clinical studies will address chemotherapy/MTP-PE interactions.

(b) clinical experience combining MTP-PE with chemotherapy will be obtained at several institutions prior to opening of the CCSG study.

(c) MTP-PE use in the CCSG osteosarcoma study will be restricted to the period after surgical resection of the primary tumor, allowing histologic response of the tumor to be evaluated without the confounding variable of MTP-PE administration.

6. **ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

TOPIC: Epipodophyllotoxin and Secondary Acute Myeloblastic Leukemia (AML)

DATE: May 20, 1991

COORDINATOR: Malcolm A. Smith, M.D., Ph.D.

The Pediatric Section, CIB organized a meeting of pediatric investigators from CCSG and POG to discuss their collective experience regarding the incidence of AML developing following therapy with epipodophyllotoxins. St. Jude

investigators provided an update of the outcome of patients treated on their two most recently completed ALL protocols (Total X and Total XI) which indicated an increased incidence of AML for two treatment regimens, one each from Total X and XI. Interpretation of the data by St. Jude investigators indicated that there was a statistically significant association between the schedule of teniposide treatment and the development of AML. Other pediatric oncologists at the meeting described lower rates of AML development using treatment protocols which lacked epipodophyllotoxins. As a result of this meeting, the following steps have been taken (or are in progress) by CTEP staff:

(a) a letter has been sent from CTEP to investigators using teniposide informing them of the possible increased risk of AML developing following treatment with teniposide. The letter advises investigators to amend consent documents to indicate this possible risk, and reminds investigators of their responsibilities to report second malignancies as adverse drug reactions.

(b) a letter has been drafted by CTEP to send to principal investigators of protocols using etoposide to inform them of these new findings and to remind them that consent documents should reflect the risk of possible secondary AML, and that second malignancies occurring in Phase III trials should be reported to CTEP as severe adverse events occurring with commercial agents.

(c) a plan is being prepared by CTEP staff to assess the risk of AML developing following different cumulative doses of etoposide. In order to study this relationship, data will be collected and integrated from specific CTEP-sponsored Phase III trials which use etoposide.

STRATEGY SESSIONS PLANNED FOR 1992

1. Gastric Cancer
2. Prostate Cancer
3. Adult Acute Lymphoblastic Leukemia
4. Non-Hodgkin's Lymphoma in HIV-positive patients
5. Metastatic colon Cancer
6. Brain Tumors

CLINICAL UPDATE ON ADJUVANT THERAPY OF RECTAL CANCER

J. Michael Hamilton, M.D. oversaw the drafting and distribution of the NCI Clinical Update on Adjuvant Therapy of Rectal Cancer, which was sent to more than 100,000 physicians in March, 1991. That Update summarized the results of an NCI-supported clinical trial which demonstrated an improved survival after combination adjuvant chemotherapy and irradiation compared to treatment with adjuvant irradiation alone. The trial from the North Central Cancer Treatment

Group, which enrolled about 200 patients and was reported in the March 14, 1991 edition of the New England Journal of Medicine, demonstrated a 36% reduction in distant metastases and a 29% reduction in patient deaths after combined modality treatment. The Update was intended to draw physicians' attention to that report and included a summary of related trials and a recommendation for therapy for individuals who cannot participate in clinical trials. The Update was mailed to primary care physicians (American Academy of Family Physicians and American College of Physicians), oncologists (ASCO, ASTRO, and SSO) as well as other sub-specialist surgeons and gastroenterologists.

LABORATORY-CLINICAL CORRELATIONS

Over the past few years, the Clinical Trials Cooperative Groups have become increasingly interested in integrating important laboratory science into clinical trials. There are 445 active protocols in the CIB hypothesis data base as of April 24, 1991; 133 of these (30% of all studies, 35% of treatment studies) have laboratory correlates. Of this I33, 2 (1.5%) are phase I/II, 30 (23%) phase II, 3 (2%) phase II/III and 35 (26%) phase III. The remaining 63 (47%) are non-therapeutic protocols. Some examples are:

1. Both CALGB and SWOG are evaluating in vitro assays of mdr and correlating results with response to chemotherapy +/- reversing agents (e.g. R-verapamil, cyclosporine) in AML, CML in blast crisis, NHL, and multiple myeloma.

2. CALGB is evaluating the clinical implications of molecular subtypes of the Philadelphia chromosome in CML.

3. CALGB and SWOG are evaluating protooncogene expression in adult AML.

4. CALGB is evaluating the clinical relevance of acetylator phenotype in patients treated with amonafide therapy.

5. CALGB investigators are evaluating the clinical significance of bcl-2 rearrangements in NHL using pcr technology.

6. POG is conducting a comprehensive genetic analysis of pediatric brain tumors.

7. A large intergroup trial for previously untreated patients with CLL has recently been activated to compare chlorambucil with fludarabine with the combination of the two agents. Companion studies will examine the biology and immunology of this disorder.

8. Intergroup studies in breast cancer have evaluated and are continuing to incorporate analysis of a number of potential prognostic factors in adjuvant trials including Her-2 oncogene expression as well as flow cytometry and cathepsin D, S-phase fraction, haptoglobin-related protein, and estrogen receptor determination by immunocytochemistry.

9. Pediatric neuroblastoma studies are currently stratifying patients on the basis of n-myc amplification and DNA ploidy, and pediatric A.L.L. patients are being stratified on the basis of DNA ploidy.

10. The Southwest Oncology Group has recently established a lymphoma repository with which to store samples to analyze as new probes and immunologic markers are developed. They are prospectively exploring the prognostic importance of Ki-67, a marker of cell proliferation, on large numbers of patients.

11. The Gynecologic Oncology Group is prospectively evaluating the role of tumor markers such as CA-125 in ovarian cancer.

12. SWOG is planning to evaluate specimens from patients with ovarian cancer for the multi-drug-resistance phenotype and glutathione-S-transferase to develop approaches to circumvent acquired drug resistance.

13. CALGB has established a series of companion studies to the colon adjuvant trial which will assess the importance of molecular genetic changes, laminin binding proteins, and the clinical significance of tumor progression genes. ECOG and NCCTG are evaluating the prognostic implications of ploidy and proliferative activity in patients with primary colorectal carcinoma.

14. The Cooperative Groups are conducting a number of studies in urologic malignancies including flow cytometric analysis of bladder cancer, prostate cancer, and testis cancer which SWOG will compare with quantitative fluorescence imaging. ECOG is evaluating EGF receptors in superficial transitional cell bladder cancer as a prognostic factor and potential therapeutic target. SWOG is also evaluating prostate-specific antigen as a marker for recurrence of early stage disease.

15. RTOG is currently evaluating whether flow cytometry can be used as an early predictor of recurrence for patients with bladder cancer, as a potential predictor of disease-free and overall survival for patients with anal cancer, and as a predictor of response in non-small cell lung cancer patients. The RTOG is also attempting to compare flow cytometry results with computer aided image analysis (CAIA) as a predictor of proliferative activity in prostate patients with paraffin fixed tissues.

IV. SPECIFIC PROGRAM ACCOMPLISHMENTS

The following are selected highlights of the current program and specific plans for the future.

PEDIATRIC MALIGNANCIES:

Accomplishments

The Intergroup Rhabdomyosarcoma Study III (IRS) reported improved survival and bladder preservation among rhabdomyosarcoma patients with bladder/prostate primary tumors (Proc ASCO 10:318,1991). Prior IRS studies reported 70% 3-year survival with only 20-25% retention rate for functional bladders among survivors at 3 years following diagnosis. On the IRS-III study, patients with bladder/prostate primaries had 90% 3-year survival, and 60% of patients retained functional bladders at 3 years post-diagnosis. The major differences

between IRS-II and IRS-III were intensified chemotherapy and radiotherapy for IRS-III patients.

The final report of the Intergroup Ewing's Sarcoma Study II (IESS) (J Clin Oncol 8:1514;1990) demonstrated a significant benefit for Ewing's sarcoma patients with non-pelvic, localized tumors for a high-dose intermittent method (treatment 1) compared to a moderate-dose continuous method (treatment 2) (73% versus 56% disease-free survival at 5 years). The most likely explanation for the improved outcome of treatment 1 is the higher doxorubicin dose intensity of that arm during the first 9 months of therapy (J Clin Oncol 9:889-891;1991). Thus, the IESS-II confirms the importance in Ewing's sarcoma of doxorubicin for positive outcome which was first demonstrated in IESS-I, and represents a significant improvement in outcome for children with localized Ewing's sarcoma.

CCSG investigators reported results of a study (CCG-503) comparing the benefit of daunomycin when added to a four drug regimen (cyclophosphamide-vincristine-methotrexate-prednisone) for patients with disseminated non-lymphoblastic lymphoma without bone marrow or CNS disease (Proc ASCO 10:289;1991). Overall event-free survival at 4 years was 61% and did not differ for patients with small, non-cleaved cell histology compared to large cell histology. The most important finding of the study was that daunomycin increased acute toxicity but did not prolong EFS or survival. The lack of daunomycin benefit is important in suggesting that future trial designs should attempt to improve outcome by emphasizing intensification of agents other than anthracyclines.

POG investigators extended their previous work on defining pre-therapy prognostic factors for children with B-cell progenitor ALL (Proc ASCO 10:234;1991). Using a combination of DNA index (DI) and clinical features (age and WBC), three distinct groups with widely different event-free survival rates were identified: Stage I (DI > 1.16) with EFS 97.6%; Stage II (DI < 1.16 with age < 11 year, WBC < 50 x 10^9/l) with EFS 85.2%; and Stage III (DI < 1.16 with age $\geq$ 11 years and/or WBC $\geq$ 50 x 10^9/l) with EFS 53.5%. Identification of distinctive prognostic groups is critical to designing risk-directed treatments which maximize effectiveness and minimize morbidity.

Significant advances in predicting outcome for children with neuroblastoma were reported by both POG and CCSG investigators. CCSG investigators reported that immunocytologic analysis of bone marrow aspirates is more sensitive than conventional analysis in detecting tumor cells (N Engl J Med 324: 219-26;1991). Immunocytologic detection of bone marrow metastases predicted poor outcome for patients > 1 year with Stage II and III disease; for patients < 1 year with disseminated disease (Stage IV), outcome was poor if the marrow contained more than 0.02% tumor cells. POG investigators reported on the combined use of tumor cell ploidy and N-myc gene amplification to predict outcome (J Clin Oncol 9:581-591;1991). Infants (< 12 months) with hyperdiploidy had > 90% disease-free survival, while diploidy invariably predicted early failure. N-myc gene amplification was associated with diploidy and predicted a high likelihood of early treatment failure. For children younger than 2 years with disseminated disease, tumor cell ploidy and N-myc gene copy number provided complementary prognostic information.

Pediatric Future Plans:

CCSG is planning a Phase III osteosarcoma protocol which will use a factorial design to ask both a chemotherapy and a biologic therapy question. The chemotherapy questions are: (a) does the doxorubicin/ifosfamide combination have greater activity than the doxorubicin/cisplatin combination as measured by tumor necrosis noted at surgical resection of the primary tumor? and (b) does the addition of the doxorubicin/ifosfamide combination to a regimen combining doxorubicin/cisplatin and high-dose methotrexate improve event-free survival? A pilot study (Proc ASCO 10:310;1991) noted excellent tumor responses to the doxorubicin/ifosfamide combination given with high-dose methotrexate prior to surgery. The biologic question is whether muramyl tripeptide phosphatidylethanolamine (MTP-PE) given for approximately 4 months following tumor resection (and concurrently with chemotherapy) increases event-free survival. Pre-clinical studies suggest benefit for MTP-PE in preventing the development of osteosarcoma pulmonary metastases.

The current intergroup Phase III trial for Ewing's sarcoma (INT-0091) should close in 1992, and plans for a successor intergroup study to open in 1992 are in progress. The next study will differ from the current study by allowing patients with soft tissue peripheral neuroectodermal tumor (PNET) to enter. The probable therapeutic question for the successor study will be whether increasing dose intensity improves outcome for patients with Ewing's sarcoma and PNET. A POG pilot study is determining whether G-CSF allows escalation of ifosfamide in the ifosfamide/etoposide combination, and a CCSG pilot study is planned which will use G-CSF with dose intensive vincristine/doxorubicin/ cyclophosphamide and ifosfamide/etoposide.

Important pediatric phase I trials will be opening in the coming year, including phase I studies of taxol and piroxantrone. POG has just initiated a phase I study of taxol for children with refractory solid tumors, and CCSG will study taxol in children with refractory leukemias. Given the significant efficacy of taxol seen in adult patients with refractory malignancies and given the novel mechanism of action of this agent (and the related semi-synthetic agent taxotere), it is important that clinical investigations in children be initiated. CCSG investigators are planning a phase I investigation of piroxantrone. This anthrapyrazole is an anthracycline derivative with significantly decreased ability to induce free radical formation compared to doxorubicin, and consequently decreased cardiotoxicity as assessed in pre-clinical models. Given the importance of anthracyclines to positive outcome for pediatric solid tumors such as osteosarcoma and Ewing's sarcoma, and given the significant incidence of cardiotoxicity with available anthracyclines, it is quite important to investigate new agents which may maintain the efficacy of the anthracycline family without their cardiotoxicity.

PEDIATRIC RESEARCH PROGRAM INITIATIVES

The Division of Cancer Treatment Board of Scientific Counselors approved an RFA for "Phase I Trials of New Cytotoxic and Biologic Agents in Children". The need for the RFA was based on biologic differences between adult and children oncology patients both in the behavior of their tumors and in their tolerance of chemotherapeutic agents. Additionally, pediatric Phase I trials have become increasingly difficult to perform in a timely and comprehensive fashion because of limitations in patient numbers and increases in the ancillary studies required to support informative Phase I trials. The anticipated release of the RFA is Fall, 1991, and two awards of 4 years duration ($375,000 per year) are anticipated in 1992.

GU CANCER

Testis Cancer

Accomplishments

At the American Society of Clinical Oncology, the Eastern Cooperative Oncology Group presented the results of their trial which compared the 3-drug combination bleomycin, etoposide and cisplatin (BEP) with the 2-drug combination, etoposide and cisplatin (EP), each given for 3 cycles in patients with good or moderate risk germ cell cancer. The purpose of the study was to evaluate whether bleomycin could be deleted from the treatment of these patients in order to decrease toxicity while maintaining the high cure rate. The study was stopped at an interim analysis in December, 1989 when it appeared that patients receiving EP might be at greater risk of disease recurrence. Further follow up of that study confirmed this finding, thus indicating the need to include bleomycin, at least when chemotherapy is given for only 3 courses.

Another study continues to compare EP given for 4 cycles with etoposide + carboplatin given for 4 cycles in patients with good risk germ cell cancer. The purpose of this study is also toxicity reduction as carboplatin is less neurotoxic than cisplatin.

In advanced or poor risk germ cell cancer, an intergroup study by ECOG and SWOG is comparing standard BEP therapy with a 3-drug regimen which employs ifosfamide instead of bleomycin (VIP). This study should complete accrual in the coming year. For patients with recurrent, refractory germ cell tumors, a pilot study is evaluating the ability of high-dose chemotherapy with autologous bone marrow support to produce long-term disease-free survival in a setting where standard dose therapy is minimally effective.

For patients with chemotherapy-refractory disease, trials evaluating new agents continue to have an important role. A trial evaluating 5-azacytidine has recently closed demonstrating its inactivity.

Future Plans:

In patients with good risk disease, toxicity reduction continues to be an area of interest but there are no immediate plans to commence any randomized trials in the next fiscal year. Pilot studies will evaluate the ability of various retinoids to induce differentiation of germ cell tumor elements. If promising, a retinoid may be added to standard BEP therapy to attempt to improve on the 90% cure rate already possible.

In patients with poor risk disease, a randomized trial of high-dose chemotherapy with autologous bone marrow rescue may be conducted pending the results of the current pilot studies.

For patients with recurrent, refractory disease, new agent trials will be evaluating all-trans retinoic acid, fenretinide and taxol as these drugs become available in the coming year.

Prostate Cancer

Accomplishments

Accrual continues on a number of important trials in early stage prostate cancer including the comparison of radical prostatectomy versus radiation therapy in the treatment of Stages A and B prostate cancer, adjuvant hormonal therapy (medical or surgical castration) for patients with Stages B, C and D1 disease, and adjuvant radiation therapy in patients with Stage C disease who have positive surgical margins following radical prostatectomy. All of these trials are accruing well with the exception of the comparison of radiation and surgery in patients with Stages A and B disease.

The past year has seen extremely rapid accrual to a study comparing orchiectomy + flutamide with orchiectomy + placebo in patients with newly diagnosed metastatic prostate cancer. This should be the final evaluation in the international debate about the role of complete androgen blockade in advanced prostate cancer. A worldwide overview of trials evaluating complete androgen blockade is underway; this trial will have a significant impact on the conclusions of that overview.

Priority has been given to the evaluation of promising new agents in patients with prostate cancer. One such agent, taxol, has been administered to 10 patients with measurable prostate cancer in an Eastern Cooperative Oncology Group Study. At this time 5 patients have been evaluated for response with one patient having responded. Thus it is too early to know if this drug will have meaningful activity in prostate cancer. Other drugs studied but found to be ineffective include Iproplatin, Didemnin B, and Ifosfamide.

A trial designed to evaluate Strontium89's ability to palliate patients with hormone refractory prostate cancer is underway in the Radiation Therapy Oncology Group.

Another new agent, suramin has had interesting anti-tumor activity but with significant toxicity and a cumbersome schedule of administration. This compound appears to function by perturbing cancer cell growth factors.

Current trials are exploring alternative, more practical schedules of administration. A comparison of suramin with other new agents is planned once the optimal schedule of suramin administration has been finalized.

Future Plans:

A trial to evaluate antiandrogen therapy as an adjuvant following prostatectomy for Stages A and B disease is being considered. The advantage to antiandrogen therapy is that it is potency sparing in significant fraction of patients and thus would be more likely to be acceptable than impotency-producing therapy. An additional consideration is the use of fenretinide as an adjuvant therapy. This synthetic retinoic acid analog has induced differentiation in preclinical prostate cancer models. Its use as an adjuvant depends in part on studies, described below, to be done in the next several months in patients with metastatic prosate cancer.

Trials evaluating fenretinide and other retinoid compounds are being considered for hormone refractory prostate cancer. These agents appear to be able to induce growth inhibiting peptides, induce differentiation of prostate cancer cells and to initiate programmed cell death.

Bladder Cancer

Accomplishments

Accrual continues on several randomized studies including a comparison of BCG and mitomycin in superficial bladder cancer and the evaluation of preoperative chemotherapy in locally advanced bladder cancer. The latter is a high-priority trial which has been disappointingly slow in accrual but remains a critically important study. A study of somewhat similar design is being conducted by the Radiation Therapy Oncology Group in which the primary treatment of the bladder tumor is radiation therapy rather than cystectomy as is the case in the high priority trial.

A new agent that has been identified as being active in bladder cancer is gallium nitrate administered by continuous infusion. A combination study of cisplatin plus 5-fluorouracil is being evaluated by SWOG.

Future Plans:

New approaches to the study of superfical bladder cancer include the evaluation of photodynamic therapy, the use of oral bropirimine and the combination of intravesical alpha interferon and BCG.

In metastatic bladder cancer, a trial to evaluate dose intense chemotherapy relative to standard dose chemotherapy is currently being planned, pending the results of a Phase I study to identify the MTD of the dose-intense regimen. The comparative trial would be an international collaboration among the US cooperative groups, the National Cancer Institute of Canada and possibly Australian investigators.

New agents to be evaluated for the treatment of patients with metastatic bladder cancer include taxol, topotecan and piroxantrone.

Renal Cell Cancer

Accomplishments

In early stage renal cell cancer, the most important ongoing trial is an ECOG study in which patients with no evidence of disease are randomized to receive interferon alpha for one year or no adjuvant therapy following resection of the primary tumor. This study was started in 1986 but should complete accrual in late 1991 or early 1992. This is the first and only study of its kind worldwide and if a treatment impact is demonstrated will significantly alter the standard approach to patients with resectable renal cell cancer.

For patients with metastatic renal cell cancer, an ongoing SWOG trial is administering alpha interferon to all patients and randomizing them to nephrectomy or no nephrectomy to evaluate whether removal of bulk primary tumor increases the frequency of response to therapy and prolongs survival.

The role of IL-2 in the treatment of renal cell cancer has continued to be evaluated in multiple trials. It appears to induce a durable response in a small, but real, fraction of patients. The FDA is to review these data later this year for possible licensing.

Phase II trials continue to evaluate new cytotoxic therapies such as merbarone, piroxantrone, amonafide, didemnin B, and echinomycin. None of these have thus far been particulary active in renal cell cancer.

Future Plans

Several studies have suggested that the combination of IL-2 and interferon alpha produces a higher response rate than either alone. This will be the subject of a randomized trial in ECOG in which patients with metastatic disease will be randomized to IL-2 alone, IL-2 + interferon, or interferon alone.

MELANOMA

Accomplishments

Clinical research in malignant melanoma continues to be very active. Studies of new agents such as amonafide, crisnatol mesylate, echinomycin, piritrexim, and suramin have been conducted. Many biologic response modifiers have been tested alone, or in combination with other biologic agents or standard chemotherapeutic drugs. These agents include the interleukins, interferons, levamisole, LAK cells, tumor vaccines, and hormonal agents such as tamoxifen and megestrol acetate. An important phase III trial to evaluate the benefit of postoperative alpha-interferon in patients with completely resected, high risk melanoma was closed (EST-1684). Final results of many of these trials have not yet been published.

Future Plans

New work that has begun or is being further developed includes trials of the chemotherapeutic agents edatrexate, merbarone, and thioguanine, as well as monoclonal antibodies (e.g., R24, 14.G2A, and others), and recently developed

interleukins. An interesting, large scale phase III trial has opened (EST-3690). It compares four postoperative treatments for patients with completely resected, high risk melanomas: dacarbazine alone, vs. dacarbazine and alpha-interferon, vs. dacarbazine and tamoxifen, vs. dacarbazine, alpha-interferon, and tamoxifen. The study will confirm or refute the results of EST-1684, and will look for improved postoperative treatment regimens.

GYNECOLOGIC MALIGNANCIES

Ovarian cancer

Accomplishments

The GOG has initiated an important phase III trial comparing cisplatin + taxol to cisplatin + cyclophosphamide in women with suboptimally debulked stage III-IV ovarian cancer. This is the first test of taxol, a promising investigational agent having a novel mechanism of action, in ovarian cancer patients who have not received prior chemotherapy. Demonstration of taxol's efficacy in this setting could impact substantially on the ability to affect the natural history of this disease.

The population of patients with advanced optimally debulked stage III disease is the focus of a phase III intergroup effort evaluating intraperitoneal cisplatin with systemic cyclophosphamide, versus intravenous cisplatin and cyclophosphamide. This is a very important intergroup trial (SWOG, GOG) that tests the theoretical advantage of regional therapy in small volume ovarian cancer. It has recently been modified in order to provide sufficient power to detect a treatment difference in the subgroup of women whose residual disease is not greater than 0.5 cm.

Patients who have no evidence of residual disease after second look laparotomy are being enrolled in two separate studies of adjuvant intraperitoneal therapy. SWOG randomizes patients to alpha interferon versus observation, while the GOG is looking at P^{32}.

A larger number of women with ovarian cancer fail standard induction treatment, or recur after responding to cisplatin based regimens. Several approaches to salvage therapy are being developed. For patients with minimal residual disease the GOG is systematically evaluating drugs administered via an intraperitoneal route. For women with larger amounts of disease, both the GOG and SWOG are studying intravenously administered drugs having novel mechanism of action in a phase II setting. This approach will be used to investigate topotecan, a promising drug which through its inhibition of topoisomerase I provides a unique way to attack a cancer cell.

Future Plans

The GOG and SWOG are in the process of planning a protocol to replace the current intra-peritoneal (IP) study in women with optimally debulked stage III disease. It appears that this will compare the sequence of a dose intense platinum-based regimen followed by a taxol-platinum combination , with at least one of the drugs given IP. The conventional arm will consist of intravenous cisplatin, or carboplatin, in combination with cyclophosphamide.

Cervical Cancer

Accomplishments

The GOG has demonstrated a 34% objective response rate with Dibromodulcitol in patients with advanced disease. This forms the basis for a recently initiated phase III trial comparing cisplatin + Dibromodulcitol to cisplatin + ifosphamide, to cisplatin alone, which is considered the standard of care by most oncologists.

In women with advanced disease the SWOG is investigating retinoids, both cis-retinoic acid and trans-retinoic acid, either alone or in combination with alpha-interferon, in phase II trials. This is based upon provocative data from the use of these agents in other squamous cell malignancies such as head and neck cancer.

Endometrial Cancer

Accomplishments

The GOG is rapidly accruing patients having advanced disease into a trial comparing the combination of cisplatin + doxorubicin to single agent doxorubicin. Demonstration of the combination's superiority not only would prove the worth of combination chemotherapy in this disease, but it would also validate the importance of a dose intensity hypothesis in this clinical setting.

HEAD AND NECK CANCER

Accomplishments

The Head and Neck Intergroup trial comparing postoperative radiation with postoperative radiation plus cisplatin and 5-FU in the adjuvant setting closed to accrual one year ago. When the data matures it will help to define what role, if any, is played by adjuvant cytotoxic therapy in this disease.

The RTOG has been testing a radiation sensitizer, SR-2528, in patients having locally advanced disease. This larger phase III study is expected to close by the fall of 1991. Data from this study is crucial in assessing the efficacy of chemo-sensitization of radiation therapy.

Future Plans

Several pilot studies have recently indicated that head and neck cancer might be sensitive to multiple daily fractions of radiation therapy. The RTOG plans to test this hypothesis in a four arm randomized study comparing various altered-fractionation regimens to standard-daily RT. This study will be conducted in patients with locally advanced disease, and should commence within the next year.

RTOG plans to initiate a laryngeal preservation study in order to confirm the results of a recently reported VA Cooperative group study in which a substantial number of patients with advanced laryngeal cancer were spared laryngectomy.

ECOG plans to initiate a phase III trial comparing standard RT to a combined chemo-RT-surgery regimen. When piloted in a few ECOG institutions this aggressive approach proved to have substantial toxicity. However the relatively long disease-free survival, and the high proportion of patients converted to being resectable, has led to considerable enthusiasm for this regimen, particularly among the otolaryngologists of the group.

SARCOMA

Accomplishments

During the last year, studies of new chemotherapeutic agents (i.e., fotemustine, and menogaril), as well as new combinations of established agents, have been completed. New ways of giving treatments, such as hyperfractionation of radiotherapy, or intraperitoneal administration of cisplatin were attempted. Various agents alone, or in combination, have been tested in the treatment of HIV-related Kaposi's sarcoma, including AZT and alpha-interferon, AZT and beta-interferon, and cimetidine. Studies of the new agents fazarabine, piroxantrone, and trimetrexate were begun during the past year. A variety of chemotherapy regimens have been developed for trials in advanced or high risk sarcomas.

BREAST CANCER

Accomplishments

Adjuvant Therapy of Breast Cancer

Ongoing trials in the adjuvant setting include evaluation of: a) dose intensification (NSABP B-18, INT-0108, CALGB-9082, INT-0121); b) chemoendocrine therapy (INT-0100, INT-0101, INT-0102, NSABP B-20 and B-23); c) different combination chemotherapy regimens (INT-0101, INT-0102, NSABP B-20 and B-23); d) sequencing of therapy (NSABP B-18 pre-op vs post-op chemotherapy); e) the role of adjuvant tamoxifen in patients with DCIS (NSABP B-24) and occult, invasive cancers (NSABP B-21); and f) the value of combined endocrine therapy in the elderly (NCCTG 89-32-51). Two of the dose intensity studies are evaluating very high-dose chemotherapy with autologous bone marrow support (CALGB-9082 and INT-0121).

One recent adjuvant study, CALGB 8541, completed accrual at the end of 1990. This study was the first to prospectively evaluate dose intensity in the adjuvant therapy of breast cancer. Preliminary reports of the study show that patients receiving higher dose-intense chemotherapy experience, on average, superior disease-free survival compared to those with standard or lower dose therapy.

Ongoing trials for patients with metastatic disease include Phase II studies evaluating the antitumor efficacy of piroxantrone, taxol and taxol + adriamycin among others. Some other important pilots are evaluating dose intensification of several different cytotoxic combinations up to and including doses which require autologous bone marrow support and/or peripheral blood progenitor cell support. Phase III studies are comparing: a) new agents versus standard chemotherapy (CALGB 8641, NCCTG 87-32-52); b) different cytotoxic combinations (EST 3186, SWOG 8697); c) different endocrine therapies

(SWOG 8692, SWOG 8312, NCCTG 89-32-53) and d) chemotherapy versus chemoendocrine therapy (SWOG 8621, EST 3186).

Future Plans

A pilot study evaluating the combination of tamoxifen + fenretinide will be done in anticipation of studying 4-HPR alone and in combination with tamoxifen in patients with DCIS. This may be done as an independent study or added to the current NSABP B-24 study of tamoxifen vs placebo in patients with DCIS.

A study comparing standard chemotherapy to standard chemotherapy followed by high-dose consolidation chemotherapy will be conducted by ECOG and SWOG. This study will have 3 different treatment groups: 1) those receiving only standard doses of chemotherapy, 2) those receiving standard therapy plus escalated doses which do not require autologous bone marrow support and 3) those receiving standard therapy plus high-dose chemotherapy which requires autolgous bone marrow support for reconstitution of normal hematopoietic function.

The CALGB is considering a study in which women $\geq$ 75 years of age will be randomized to undergo axillary node dissection or not. Axillary node dissection is a standard part of breast cancer surgery but carries some morbidity. The primary utility of the node dissection is defining prognosis. Since the adjuvant therapy of women $\geq$ 75 years of age is most commonly tamoxifen alone regardless of the presence or absence of tumor in the axillary lymph nodes, the necessity of continuing the node dissection is questionable. Thus this study would be done to be certain that the axillary node dissection could be eliminated without compromising the long-term disease outcomes (disease-free and overall survival).

The past year has seen extensive planning towards the conduct of a study using tamoxifen to prevent breast cancer and perhaps to lower cardiovascular morbidity by decreasing serum cholesterol. This study, to be conducted by the National Surgical Adjuvant Breast Project (NSABP), should begin to accrue volunteers in the last quarter of 1991.

Primary breast cancer is diagnosed in over 100,000 women each year in the United States. Following removal of their breast tumors and resection of the regional axillary lymph nodes, patients are at varying risks of recurrence depending on particular characteristics of their tumor. For patients with node negative breast cancer, five clinical trials are currently active (Table 1). All of these trials were opened in the 1989 fiscal year and continue to accrue patients during 1990, with one study (NSABP B-19) about to complete the accrual phase of the study. A replacement study is now being planned and will be instituted before the end of the 1990 fiscal year. These studies have accrued approximately 3,000 women this year. For patients with node positive breast cancer, there are six currently active trials testing the most important concepts in adjuvant therapy for breast cancer (Table 2). One of these trials (CALGB 8541) began in 1985 and will be completed later this year. All of the remaining adjuvant therapy trials began in fiscal year 1989 and have been successfully and rapidly entering patients during the current year.

Two studies which were closed to accrual in recent years were analyzed this year and have demonstrated improved survival for different subgroups of patients treated with combination chemotherapy and tamoxifen. In EST-5181, premenopausal women with node positive breast cancer were randomized to one of two chemotherapy combinations. At the completion of chemotherapy, there were again randomized to either tamoxifen for five year or no further therapy. A recent interim analysis has found that tamoxifen in addition to chemotherapy prolongs the survival of patients with estrogen receptor positive tumors. The recommendations for this group of patients has been that chemotherapy alone represents the standard of care. An ongoing intergroup study is designed such that this finding can be confirmed in the near future. Another study, NSABP B-16, was designed to compare standard tamoxifen therapy for postmenopausal women with hormone responsive, node positive breast cancer with tamoxifen plus combination chemotherapy. Thus study, reported in June, 1990, in the Journal of Clinical Oncology has found that treatment with adriamycin plus cyclophosphamide in addition to tamoxifen enhances the chance for survival compared to tamoxifen alone at a median of three years of follow up. Both of these studies will require further follow up for more conclusive interpretations (such that standard therapy might be changed).

In patients with advanced disease a number of trials have been completed in the past year which have investigated high doses of chemotherapy, demonstrating the feasibility of this approach. It is too soon to evaluate the overall role of high-dose therapy to breast cancer.

The risk of recurrent disease for patients with node negative breast cancer is of a magnitude that the need to discriminate those patients with little risk of recurrence from those at significant risk is an important task. This would allow more "tailored" therapy, perhaps sparing a majority of patients the costs and toxicities of therapy and indicating the need for more aggressive or different therapeutic approaches for those with poor prognosis tumors or those with tumors which are clearly resistant to standard treatment approaches. This work has been ongoing in a retrospective fashion based on past clinical trials. Potential markers that have been evaluated in the past year include analyses of DNA content, growth fraction, and cathepsin D. Confirmatory studies are now ongoing prospectively in the current generation of node negative adjuvant trials.

The role of adjuvant therapy and the current status of prognostic factors for patients with node negative breast cancer was discussed at an NIH Consensus Development Conference on Early Stage Breast Cancer on June 18 - 21, 1990. In addition, past clinical trials evaluating approaches to breast conservation were presented. It is likely that the results of this conference will clarify our current understanding of adjuvant therapy and will help establish the research agenda for the next five years for these patients.

Future Plans

<u>High dose chemotherapy:</u>

The role of high-dose chemotherapy with autologous bone marrow rescue given as adjuvant therapy for patients with very high risk of recurrence following local resection (Stage II or IIIa with $\geq$ 19 axillary lymph nodes) will be

evaluated in a randomized Phase III trial. In order to design the best trial(s) possible, a strategy meeting will be held by the Cancer Therapy Evaluation Program in September 1990. This should not only identify the best possible current design but should help identify additional development trials (role of IL-3, role and timing of peripheral blood stem cell harvesting) which need to be done.

The current adjuvant trials will continue to accrue for the next 1-3 years with the exception of CALGB 8541 and NSABP B-19. The CALGB would like to replace their study with a high-dose regimen for those with greater than 10 axillary lymph nodes. They will likely continue to test dose intensification (at lower doses than those proposed in the transplant study) with colony stimulating factors for those with less than 10 axillary lymph nodes. A final design has not been agreed upon. The NSABP will hold a meeting to plan a replacement study for B-19 on June 22 - 23 in Pittsburgh.

Chemoprevention of breast cancer:

The Cooperative Groups will be asked to help plan, coordinate and carry out a trial of tamoxifen as a chemopreventive agent for women over age 50. The primary endpoint of the study will be incidence of breast cancer. Because tamoxifen lowers serum cholesterol and blood lipids, a secondary endpoint will be incidence of cardiovascular morbidity.

Cooperative Human Tissue Network:

The Cooperative Groups will be approached about contributing to this tumor bank so that there is a more available resource for accessing tumor samples in patients with clinical follow up so that new therapeutic and prognostic factors can be evaluated in a time efficient manner.

Drug Development:

Ongoing trials will continue to evaluate new drugs in an optimal setting. Promising drugs CTEP hopes to have tested include navelbine and a new anthrapyrazole. Early results with taxol in patients with metastatic breast cancer are promising.

ADULT LEUKEMIAS

Accomplishments

An intergroup (SWOG, ECOG, CALGB) trial was activated to compare autologous bone marrow transplantation with standard consolidation for adult AML patients in first remission. Those with an HLA-identical sibling will be eligible for allogeneic BMT.

CALGB investigators have recently published (Blood 77:2242, 1991) their data demonstrating that adults with AML whose blasts co-express lymphoid markers have a more favorable prognosis.

Fludarabine has impressive activity in both refractory and previously untreated CLL. A national intergoup study has been activated in previously untreated patients with advanced disease to compare chlorambucil (the standard agent) vs. fludarabine vs. the combination of the two agents. The doses in the combination are based on a disease-specific phase I trial recently completed by SWOG. The results of this randomized study should redefine standard therapy for CLL. Participants include CALGB, SWOG, ECOG and NCI-Canada.

The availability of recombinant human growth factors (G-CSF, GM-CSF, IL-3) has permitted new approaches to the treatment of adult leukemias. These agents are in clinical trials to shorten the duration of neutropenia, thereby allowing a greater number of patients to enter complete remission. Several trials are specifically focusing on elderly patients since they are at the greatest risk for treatment-related deaths from myelosuppression.

Future Plans

SWOG and CALGB investigators have demonstrated the expression of the multi-drug resistance phenotype in relapsed patients with AML and CML in blast crisis. Studies are being planned to attempt to overcome this resistance clinically through the use of such agents as cyclosporine.

The high response rate reported for all trans-retinoic acid (ATRA) in acute promyelocytic leukemia has stimulated plans for two national intergroup clinical trials. Previously untreated patients will be randomized to either conventional induction chemotherapy or ATRA. Patients who achieve a complete remission will receive consolidation chemotherapy followed by a secondary randomization to maintenance with ATRA or observation. Relapsed patients who have not previously received ATRA will be given this agent, followed by a low-dose consolidation for responders, and a secondary randomization to either ATRA or observation. Participating groups include SWOG, ECOG, CALGB, CCSG, POG, NCI-Canada, and the Australian Leukemia Consortium.

ADULT MALIGNANT LYMPHOMA

Accomplishments

SWOG and ECOG recently completed a large 4-arm trial comparing CHOP with m-BACOD, MACOP-B and ProMACE/CytaBOM in adults with previously untreated NHL. THe results of this study are under analysis and will redefine the standard approach to these patients.

Plans

The availability of recombinant human growth factors may permit an increase in delivered dose intensity, which may translate into higher response rates and improved survival. CALGB is completing a phase I trial of a new CHOPE regimen with GM-CSF and will soon compare the intense regimen with a more "standard" dose CHOPE. SWOG and ECOG are conducting similar phase I trials in untreated patients with G-CSF and GM-CSF to support increased doses of ProMACE/CytaBOM.

SWOG investigators are exploring the possibility of pharmacologically reversing multi-drug resistance in NHL and myeloma using agents such as verapamil and quinidine.

MALIGNANT BRAIN TUMORS

Accomplishments

Interstitial versus External Beam Radiation Therapy for Gliomas--
Interstitial irradiation for malignant tumors in the brain has been administered widely throughout the country. From center to center the isotopes, surgical techniques, and dosimetry are highly variable. The BTCG currently has a Phase III comparison of external beam versus external beam plus interstitial irradiation for malignant gliomas which is more than half completed.

Efforts to improve the effectiveness of cranial irradiation through hyperfractionation or halopyrimidine radiosensitization are undergoing study in the RTOG. RTOG has initiated a comparison of twice daily fractionated irradiation with chemotherapy versus standard irradiation plus chemotherapy. Entry of 500 patients is expected over 3 years. Other Cooperative Groups and Cancer Centers continue to explore phase II chemotherapeutic agents in malignant gliomas.

Future Plans

The current standard treatment of primary CNS lymphoma is surgery and/or radiation therapy. Several group studies are testing multiagent chemotherapy with radiation. Discussion with these investigators was held at ASCO in May, 1991 to intergrate their programs into an intergroup study.

GASTROINTESTINAL CANCERS

Accomplishments

<u>Esophageal Cancer</u>

Phase III comparisons in the RTOG and ECOG for localized esophageal cancer tested radiation alone with radiation plus chemotherapy. Both studies show a benefit for the combined modality arm. Two large intergroup trials in local-regional esophageal cancer are underway. One incorporates the observed superiority of combined modality therapy (DDP, FU, RT) over RT alone and compares this combination regimen to one in which of all three components are maximally intensified. The other trial tests surgery with or without neoadjuvant DDP/FU. While these trials run concurrently, they draw on different referral patterns and will not compete with each other for patients.

Colorectal Cancer

NCCTG Rectal Trial (86-47-51) and INT-0114

The NCCTG rectal adjuvant trial employed combined radiation + chemotherapy and used a 2 x 2 factorial design. Preliminary analysis suggests no additional benefit of the MeCCNU to 5-FU compared to 5-FU alone. It also evaluated the benefit of continuous infusion 5-FU during radiation therapy compared to intermittent bolus 5-FU; analysis of this comparison is too early to evaluate. High priority status was assigned to this trial and stimulated accrual, such that the trial completed accrual 06-30-90. The replacement trial (INT-0114) will accrue 1200 patients to a comparison of pelvic irradiation plus one of four chemotherapy regimens, 5-FU, 5-FU/levamisole, 5-FU/leucovorin, or 5-FU/levamisole/leucovorin. This study is accruing rapidly and will complete earlier than initially targeted.

NCCTG (89-46-51) and Intergroup (INT 0089) Colon Adjuvant Trials

Three Cooperative Groups (ECOG, SWOG, CALGB) are comparing 5-FU/leucovorin on two schedules versus now standard 5-FU/levamisole, versus 5-FU/leucovorin/levamisole. Accrual of 2,700 is expected in approximately 3 years. The NCCTG is testing FU/levamisole with or without leucovorin and also whether 6 or 12 months' therapy is sufficient.

Gastric Cancer

Accomplishments

The intergroup gastric adjuvant protocol testing FU/Leucovorin + Radiation Therapy versus surgery alone was activated in June, 1991.

LUNG CANCER

Accomplishments

Several trials in patients with lung cancer were completed in the last year. Studies of new chemotherapeutic agents have been performed, including amonafide, didemnin B, gallium nitrate, 4'-iodo-4'-deoxydoxorubicin, piroxantrone, teniposide, thioguanine, trimetrexate, and others. Interesting phase III trials have examined the possible benefit of gamma-interferon treatment in small cell lung cancer patients who have achieved a complete response to chemotherapy (NCCTG-86-20-51), and whether treatment with an intensive chemotherapy regimen is superior to treatment with a standard chemotherapy regimen (EORTC-07871). The final results of these trials have not yet been published.

Future Plans

Several new studies have started during this time. The chemotherapeutic agents deoxyspergualin, edatrexate, fazarabine, and taxol are being examined. Randomized phase III trials are examining the benefit of postoperative chemotherapy in locally advanced non-small cell lung cancer (INT-0115), the effects of prophylactic cranial irradiation on small cell lung cancer patients

in complete remission (EST-3589), and whether or not oral etoposide and intravenous cisplatin is superior to intravenous etoposide and intravenous cisplatin, in the treatment of extensive stage small cell lung cancer (CALGB-9033).

A large intergroup trial of postoperative retinoid therapy in patients with completely resected, stage I non-small cell lung cancer is being developed. It will determine whether postoperative retinoids decease the incidence of second primary tumors, and will examine some aspects of tumor genetics and histologic features for use as potential intermediate endpoints in chemopreventive trials.

V. CONTRACTS

Extramural Clinical Trials Office--EMMES

EMMES currently serves the operations and data management functions for the extramural IL-2/LAK cell trials, the suramin studies and other Group C protocols. The IL-2/LAK studies were conducted at approximately 17 institutions and accrued over 240 patients. The suramin studies are just being activated, related in part to difficulties in establishing the pharmacologic monitoring at each institution. There are now 2 centers participating, with additional institutions anticipated in the near future. There are currently three Group C protocols which are actively accruing patients. EMMES was involved in developing the case study forms for several of these and serves the operations and data management functions for all of them. These protocols include pentostatin for hairy cell leukemia, VM-26/ara-C for relapsed acute lymphocytic leukemia, and fludarabine for refractory chronic lymphocytic leukemia. Accrual to the fludarabine study has been particularly active with greater than 600 cases to date. Over 5,000 patients have been entered on the levamisole study.

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INVESTIGATIONAL DRUG BRANCH

DESCRIPTION

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and evaluating them for efficacy and toxicity. It does this by: (1) coordinating and monitoring the trials of new agents developed by the DCT; (2) planning with members of the Clinical Investigations Branch overall strategies for new agent studies in specific tumor types; (3) regulating the distribution of investigational new drugs for which DCT is the sponsor; (4) maintaining close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new agent development proceeds in a coordinated way.

The Investigational Drug Branch is divided into three sections: two medical sections, one for cytotoxic agents and one for biologic response modifiers, which are concerned with the clinical aspects of the drug development process, and the Drug Management and Authorization Section, which regulates the distribution of investigational new drugs to all NCI-sponsored investigators. The professional staff of the Branch includes 11 physicians, 5 pharmacists, 1 Ph.D. and 1 registered nurse.

BIOLOGICS EVALUATION SECTION (BES)

IMMUNOREGULATORY CYTOKINES

INTERLEUKIN-2 (IL-2)

An NCI-sponsored extramural Phase II trial of high dose IL-2 alone in patients with renal cell carcinoma confirmed the activity of the drug originally reported from the Surgery Branch, NCI. Complete or partial remissions of advanced tumor were seen in approximately 20% of 71 patients entered on study. Responses obtained in the Surgery Branch studies have been durable, with some patients relapse-free up to 4 years following initiation of treatment. These data form the basis for a licensing application submitted to the FDA. A study comparing a standard high dose IL-2 regimen with a lower dose IL-2 regimen, to determine if similar efficacy can be obtained with less toxicity, was initiated in the Surgery Branch.

The major focus of IL-2 development continued to be combinations with other biologic agents. In general, preliminary results have not suggested marked beneficial effects of adding interferon-alfa, interferon-gamma, interleukin-4 or tumor necrosis factor to IL-2 containing regimens. However, studies of interferon-alfa/IL-2 administered at doses sufficiently low to allow outpatient therapy have demonstrated response rates of 20-25% in renal cell carcinoma, with some patients experiencing durable remissions. Phase III studies comparing this regimen to interferon or interleukin-2 alone are contemplated, as are comparisons to higher dose, more toxic IL-2 alone regimens. Data from combinations of IL-2 and monoclonal antibodies are still

being collected - antibodies in these trials include LYM-1 (lymphoma), R24 and MG22 (melanoma), and OKT3 (a non-specific activator of T cells which enhances the immunopotentiating effects of IL-2). A preliminary assessment of an R24/IL-2 trial in patients with malignant melanoma conducted at the BRMP, NCI suggested response rates may be increased to 40%. Combination trials with other cytokines such as GM-CSF, interleukin-1, and M-CSF were approved and will be initiated in the coming year. Studies combining cancer vaccines and IL-2 are in progress.

Regional therapy trials of IL-2 alone in ovarian, head and neck, and bladder cancer are continuing. Agents with potential to block the toxicity of IL-2, such as amino-arginine derivatives, are under preclinical development. Trials of IL-2 in patients with acute leukemia in remission following chemotherapy only or autologous marrow transplantation are in the planning stages.

A major impediment to the continued development of IL-2 has been the decline in available drug supply as some pharmaceutical sponsors decrease or stop production. Negotiations with alternate suppliers are in progress.

IL-4

IL-4 has been disappointing in clinical trials to date. Phase II studies of high dose IL-4 in metastatic melanoma and renal cell carcinoma have been essentially negative. Preliminary results combining IL-2 with IL-4 at high doses have not indicated superior activity over that seen with IL-2 alone. Recently, IL-4 was shown to have anti-tumor activity in some patients with lymphoma and Hodgkin's disease. Clinical trials have been initiated.

INTERLEUKIN-1-ALPHA AND INTERLEUKIN-1-BETA (IL-1)

The development of these very similar molecules has proceeded rapidly over the past year, principally in efforts directed toward determining if IL-1 can minimize the side effects of existent anticancer treatments. Trials to define the toxicity of both types of IL-1 when administered alone are largely completed. Based on data already gleaned from these efforts, studies are now underway to evaluate the effects of these agents to protect against the toxicity of conventional- and high-dose chemotherapy. Protocols to assess the benefit of IL-1 in enhancing the rapid regrowth of bone marrow cells after bone marrow transplantation are about to begin. Like TNF, IL-1 may protect normal tissues against the damaging effects of radiotherapy while enhancing the toxicity of radiation to malignant cells. As the first step in trying to exploit this differential action on normal and cancerous tissues, study protocols have been written to assess the safety of administering IL-1 in conjunction with anti-tumor, radio-labelled monoclonal antibodies. Other studies, to examine a role for IL-1 when used with more conventional radiotherapy techniques, are in the early planning stages. Because IL-1 may also have direct antitumor activity, it will also be important to evaluate this action. Trials are underway to determine if IL-1, given either by vein or directly into tumor sites, may cause tumor regressions in patients with melanoma. In addition, plans have been made for studies to evaluate possible synergistic anti-tumor effects of IL-1 when used together with a number of chemotherapeutic agents (carboplatin, methotrexate, and etoposide).

ERYTHROPOIETIN (EPO)

Epo is the hormone, produced by the kidneys, that stimulates the maturation of red blood cells from their precursors in the bone marrow. Because cancer patients often experience anemia and many require blood transfusions during treatment of their cancers, considerable interest has developed in the administration of Epo to minimize the consequences of this anemia. CTEP is currently sponsoring a trial of Epo and G-CSF in children with AIDS and has approved a study of Epo and G-CSF in adult patients undergoing therapy for HIV-associated lymphoma. Also planned are studies to determine if Epo may have utility in reducing the blood transfusion requirements of patients undergoing bone marrow transplantation and radiotherapy. A study of the combination of Epo and G-CSF to counter the anemia and low white blood counts suffered by patients with myelodysplasia is about to begin active patient enrollment.

TUMOR NECROSIS FACTOR (TNF)

Phase II trials designed to define the anti-tumor activity of TNF when given as a single agent are generally complete or nearing completion. Though the effectiveness of TNF when given alone has been limited, promising leads for benefit when TNF is given together with topoisomerase II inhibitors (e.g., VP-16 and actinomycin-D) are being pursued. Recent data suggesting synergy between TNF and the topoisomerase I inhibitor, topotecan, have stimulated interest in the clinical evaluation of the concurrent administration of these agents. A trial to examine the combined activity of TNF when administered with other cytokines, such as interleukin-2 and interferon-alpha has also recently been approved. An important role for TNF in cancer care could be supportive; among the CTEP goals for evaluation of TNF are examinations of its actions to protect against the toxicity of radiation therapy or chemotherapy and to alleviate the accumulations of fluid in the linings of the lung and abdomen that are sometimes the cause of considerable morbidity for cancer patients.

COLONY STIMULATING FACTORS

GRANULOCYTE (G-CSF) AND GRANULOCYTE MACROPHAGE (GM-CSF) COLONY STIMULATING FACTORS

A great deal of information regarding the toxicity and effectiveness of these agents when used to reduce the side effects of chemotherapy or to allow higher doses of chemotherapy has now been developed, and both agents have now been approved by the FDA for commercial sale. Both drugs appear to ameliorate the depression in white blood counts seen with chemotherapy regimens, and as a result may have positive effects in countering the problems of infection that can be associated with low numbers of white cells. Little effect to counter the chemotherapy-induced reduction in platelets, cells involved in blood clotting, has been observed with G- or GM-CSF; this information, though not positive, is valuable, because it has spurred the evaluation of newer agents such as IL-1, IL-3, IL-6, and stem cell factor, which, when used alone or in conjunction with the CSF's may provide protection against lower platelet

counts. Preliminary information has also implied that the CSF's might protect against the mouth sores and diarrhea which occur after some forms of chemotherapy. Clinical trials to evaluate the true ability of the growth factors to moderate these toxicities are underway.

Studies evaluating the role of the CSF's in permitting oncologists to administer higher doses of chemotherapy over time in a safer fashion have demonstrated particular utility with certain single agents, such as cyclophosphamide and adriamycin, and with combination chemotherapy regimens for breast cancer, lung cancer, bladder cancer, multiple myeloma, and lymphoma. These studies have suggested that the greatest benefit of these factors may be in allowing chemotherapy treatments to be given in a rapidly repeating manner, ensuring that patients do not suffer delays or reductions in chemotherapy dose due to side effects. Again, valuable negative information has also been obtained which suggests that the use of the CSF's alone to counter the bone marrow suppressive effects of certain chemotherapeutic agents such as thiotepa, melphalan and carboplatin, will not prove particularly advantageous. These data are important because they also point to the need to develop combination protective regimens involving the CSF's and IL-1, IL-3, IL-6, and stem cell factor, as well as TNF and non-biological agents like WR-2721.

In addition to raising white blood counts, G-CSF and GM-CSF appear to stimulate the release into the bloodstream of primitive bone marrow cells which are the forebears of white cells, red blood cells and platelets. Such cells are relatively easily collected from the blood of outpatients and seemingly can be used, after chemotherapy given in doses so high that it destroys the normal bone marrow, to reconstitute bone marrow function when reinfused. Such methods for collecting these cells may have advantages over the current procedures, which require that patients be hospitalized and taken to the operating room for collection of bone marrow for transplantation prior to receiving the high-dose chemotherapy. Efforts are underway to define the role of the growth factors in making the collection of these blood cell progenitors more efficient and potentially more cost-effective, and to delineate the competency of these cells as replacements for bone marrow transplantation. Use of the growth factors may also allow production of these cells in large numbers outside of the patient's body; reinfusion of the greater numbers of cells could then potentially be even more useful in protecting patients against the infectious and bleeding complications which occur with extremely high-dose chemotherapy.

Certain chemotherapeutic agents preferentially destroy tumor cells as they are dividing. Because the growth factors can bring leukemic cells into the cell division cycle, the administration of a growth factor for several days prior to treatment with such chemotherapeutic agents is being explored with the expectation that this sequencing of growth factor and then chemotherapy may enhance the effectiveness of the chemotherapeutic drugs. Trials to define the toxicity of this type of treatment have been successfully performed and larger studies to test the therapeutic benefit are now actively accruing patients. Additional studies to define a similar role for the CSF's and the cytotoxic agent, Ara-C, in the treatment of myelodysplasia (pre-leukemia) are about to begin.

Beyond their use in increasing white blood cell numbers the CSF's may have important utility in enhancing the anti-infectious or tumoricidal activities of inflammatory white blood cells such as macrophages and neutrophils. Studies to examine the ability of the growth factors for the therapy of infection in cancer patients have been proposed and are under consideration. Because new, broadly active oral antibiotics (such as ciprofloxacin) have also become available in recent years, considerable interest has developed in the use of these compounds to prevent the infectious complications of cancer chemotherapy. Critical questions must now be raised regarding the relative roles of the prophylactic use of such antibiotics as compared to the CSF's in preventing infections. In addition, it is possible that the combined use of two of the agents together might have considerable benefit to prevent infections during a course of chemotherapy and allow the delivery of higher amounts of chemotherapeutic agents. Early planning for comparative trials to address these issues is now underway within the cooperative groups.

Though the CSF's are thought to have little intrinsic antitumor activity, the possibility exists that these agents, particularly GM-CSF, may augment the anticancer effects of macrophages and neutrophils. The CTEP has approved three trials to address this possibility; one of these will assess the antitumor activity of GM-CSF when given with monoclonal antibody therapy also directed against the tumors.

INTERLEUKIN-3 (IL-3)

IL-3 has now entered early trials to assess its toxicity. Current indications are that it can be used safely to augment bone marrow precursors to white blood cells, red blood cells, and platelets. Multiple trials to better define the side effects of this important molecule while evaluating its ability to protect against the low blood counts associated with cancer chemotherapy have been received and reviewed by the CTEP and should begin shortly. Particularly important will be a determination of how best to use IL-3 together with G- or GM-CSF for maximal effect in moderating the bone marrow toxicity of high-dose chemotherapy. Also important will be determinations of the role of IL-3 relative to the colony stimulating factors, G- and GM-CSF, in mobilizing stem cells to enhance peripheral blood progenitor cell collection, and in treating myelodysplasia and leukemia. Protocols to evaluate these uses based on pre-clinical information and the experience acquired with the better studied colony stimulating factors are about to begin patient enrollment.

INTERFERONS

INTERFERONS (INTERFERON-ALPHA AND INTERFERON-GAMMA)

Interferon-alpha is licensed in this country as treatment for hairy cell leukemia and Kaposi's sarcoma. Clinical research investigations in a wide variety of other cancers are also being pursued; these include chronic myelogenous leukemia (CML), renal cell cancer and malignant melanoma. Pre-clinical studies have also indicated that interferon-alpha may synergize with certain biologicals, most notably the anti-tumor activity of IL-2. Phase I studies have documented the anti-tumor activity of the combination of IL-2 and interferon-alpha in renal cell carcinoma and malignant melanoma; multiple

trials exploring this combination in other disease states and different administration regimens are ongoing. Interferon also demonstrates some synergistic activity when combined with certain cytotoxic chemotherapeutic agents, most notably 5FU. Recent trials have indicated that 5FU plus interferon-alpha has substantial activity in the advanced colon cancer setting. Additional studies in other malignancies and/or utilizing other administration schemes for this combination are being explored. Interferon-alpha may also be capable of augmenting the tumor differentiating properties of retinoic acid; trials to explore this hypothesis are in the design phase.

Interferon-gamma is being employed in a wide variety of clinical research cancer trials, both alone and in combination with other agents; it has recently been licensed in the U.S. for the treatment of certain immunodeficiency states, most notably the chronic granulocytes disease (CGD) syndrome. The most striking report of single agent activity of interferon-gamma was a recent Austrian trial indicating activity of low doses of interferon-gamma when given on a once a week schedule in the setting of advanced renal cell cancer; confirmatory U.S. trials are underway. Interferon-gamma has also been combined with other biologicals, including IL-2. A large Phase I evaluation of this combination was recently completed and modest activity of this combination was suggested; a Phase II evaluation of this combination in the renal cell cancer setting is currently being implemented. Interferon-gamma has also been coupled with chemotherapeutic agents, most notably as consolidative biological therapy to PACE chemotherapy for small cell lung cancer. There have been patients who were partial responders patients to PACE chemotherapy who converted to CR status following interferon-gamma administration; trials to confirm this observation are in the design phase. Interferon-gamma has also been used in clinical trials to increase tumor-associated antigen expression (both CEA and TAG-72 antigens) on adenocarcinomas; this property may prove useful in association with monoclonal-antibody mediated treatment strategies.

MONOCYTE ACTIVATORS

MTP-PE

The incorporation of a substance first derived from bacterial cell walls, muramyl tripeptide (MTP), into liposomes which serve as a delivery vehicle, results in a drug which is capable of activating monocytes and tissue macrophages. In dogs which are at high risk for recurrence of the bone tumor osteogenic sarcoma, administration of MTP-PE significantly delays tumor recurrence and prolongs survival. Following the completion of initial toxicity trials of this agent in man, CTEP is introducing MTP-PE into two major cooperative group trials. The first trial, in children who are undergoing surgery and chemotherapy for osteogenic sarcoma, represents a clinical setting analogous to that in dogs where the dramatic antitumor effects were noted. The second trial will be a randomized surgical adjuvant trial in adults with soft tissue sarcoma.

CYTOKINES UNDER PRECLINICAL DEVELOPMENT

INTERLEUKIN-3/GM-CSF FUSION MOLECULE (pIXY)

This novel compound is a genetically engineered combination of IL-3 and GM-CSF into a single molecule. Current indications from laboratory and animal work suggest that pIXY may have significantly higher potency than either IL-3 or GM-CSF alone in stimulating production of white blood cells and platelets. The CTEP is actively negotiating with the pIXY manufacturer to acquire sufficient amounts of this agent to begin early clinical development, primarily as an adjunct to high-dose chemotherapy.

STEM CELL FACTOR (SCF -- ALSO KNOWN AS MAST CELL FACTOR, *C-KIT* LIGAND, STEEL FACTOR)

SCF has only recently been discovered and produced by genetic engineering methods. It appears to act to stimulate growth of some of the most immature cells in the bone marrow, cells that give rise, after many divisions, to leukocytes, macrophages, red blood cells, platelets, and lymphocytes. Use of this agent clinically may focus on expansion of populations of all blood cells by sequential or combined use of SCF and existent factors (G-CSF, GM-CSF, IL-6, Epo) to produce very large quantities of blood cells. It is hoped that such treatment methods will ultimately eliminate the bone marrow suppressive side effects of chemotherapy altogether. The manufacturers of SCF will soon apply to the Food and Drug Administration for permission to begin clinical investigations with the drug. The CTEP plans to maintain active discussions with the manufacturers to obtain this agent for clinical testing.

INTERLEUKIN-6 (IL-6)

IL-6 will enter clinical trials in 1991. This new cytokine has potent effects on platelets, thus there is potential to give standard and high (and perhaps more effective) doses of chemotherapy without the risk of bleeding from low platelets. IL-6 in combination with IL-3, another cytokine just entering clinical trials, is capable of enhancing hematopoetic stem cell survival, thus preventing or ameliorating other hematologic toxicity from chemotherapy. This approach may also have utility in gene transfer experiments to correct inborn genetic defects, since IL-3 plus IL-6 enhance survival and engraftment of the gene-transfected stem cells.

IL-6 was also shown to have direct anti-proliferative effects against some tumor cell lines, and in animal models produced tumor regression through induction of a tumor response similar to IL-2, but with less toxicity. There is evidence of synergistic anti-tumor activity when combined with IL-2.

ACTIVATED CELLS

ADOPTIVE IMMUNOTHERAPY

Adoptive immunotherapy focused less on non-specific effector cells (i.e., LAK cells which kill most tumors and spare normal tissue) and more on cytotoxic T cells (CTL or tumor-infiltrating lymphocytes, TIL cells) which kill a single host tumor by recognizing specific antigens present on the tumor cell. A recent analyses of the Surgery Branch randomized trial of IL-2/LAK versus IL-2 alone in patients with metastatic melanoma showed a trend toward higher long-term survival rates in the IL-2/LAK arm. Accrual continues to this trial. IL-2/LAK has also shown promise in extending survival when administered by the intracavitary route to patients with brain tumors, and more data is being gathered in a Phase II study. Another non-specific effector cell derived from T lymphocytes is undergoing clinical investigation at the BRMP, NCI. The cells are prepared by incubating peripheral blood lymphocytes ex vivo with anti-CD3 for 24 hours. Preliminary data suggest that, following adoptive transfer, these cells can be expanded to large numbers in vivo by systemic administration of IL-2.

The Surgery Branch of the NCI initiated trials with tumor-infiltrating lymphocytes (TIL) transfected with the gene for tumor necrosis factor. A previous study with TIL, transfected with a neomycin resistance gene for tracking purposes, showed that adoptively transferred TIL can be found in peripheral blood and tumors several months after their administration. The TNF transfected TIL are expected to deliver large amounts of cytotoxic TNF directly to the tumor. Other methods of improving the generation of TIL in vitro, such as addition of TNF or IL-4, are under investigation. Studies will also examine the role of pre-harvest vaccination with autologous tumor or pre-harvest systemic treatment with IL-2 or other cytokines in producing more specific and potent TIL cells for therapy.

Although preliminary trials of TIL cells were done in patients with RCC and melanoma, new studies are being conducted in patients with ovarian cancer. TIL in combination with IL-2 will be administered directly into the peritoneal cavity to maximize the interaction between effector cells and tumor.

ANTIGEN-DIRECTED THERAPIES

Numerous technical developments have led to new generations of monoclonal antibodies, antibody fragments, and antibody conjugates. As they have become available, these constructs have been entered into active clinical and biological investigation.

UNCONJUGATED MONOCLONAL ANTIBODIES

The binding characteristics, biological activities, and pharmacokinetics of murine antibodies to a wide range of hematopoietic and solid tumor-associated antigens have been studied over the last several years. Some of the most interesting of these antibodies have been chimerized, and these chimeric antibodies, with their promise of longer circulating half-lives, reduced

immunogenicity, and increased immunobiological activity, are now being introduced into NCI-sponsored trials. Phase I trials have begun with one of these chimerics directed against the TAG-72 adenocarcinoma-associated antigen. At the same time, trials investigating the combination of monoclonal antibodies together with immunostimulatory cytokines, including IL-2, interferons, GM-CSF, and M-CSF, have been initiated.

In an alternative strategy, monoclonal antibody against the T-cell associated CD3 antigen, already in clinical use in large doses for the treatment of acute renal allograft rejection, is now being studied in much lower doses for its ability to activate T- lymphocytes. The antibody, administered together with IL-2, may allow enhanced antitumor immune responses, as predicted in preclinical studies. Several clinical trials to investigate this approach are now underway.

RADIOLABELLED MONOCLONAL ANTIBODIES

Information important to the development of the therapeutic use of monoclonal antibodies labelled with radioisotopes has been generated in a series of trials sponsored under NCI contracts. Phase I trials establishing maximally-tolerated doses, toxicity characteristics, and the relationship of drug pharmacokinetics to these parameters have been completed for whole mouse and mouse-human(chimeric) antibodies, and for antibody fragments. Various issues which have affected the use of these antibodies, including antibody half-life in the circulation, the importance of antibody affinity, and the complicating factor of antibody immunogenicity, have all been studied. Phase II trials of the most promising antibody-isotope combinations have been initiated in patients with colon and breast carcinoma, and ovarian carcinoma trials are being developed. In other DCT-sponsored trials, significant responses in patients with advanced, chemotherapy-refractory non-Hodgkin's lymphoma have been achieved, and further clinical trials are being developed using this approach.

Despite the promising results from animal models and the evidence from diagnostic studies suggesting relative tumor localization of these agents, the therapeutic use of radiolabelled antibodies has developed slowly in the clinic, due both to the cost and the organizational complexities of these studies. The results of these initial therapeutic trials, and the data from preclinical studies predicting that it should be possible to significantly increase this activity by the use of better antibodies, the concomitant use of myeloprotective or myelostimulative cytokines, or by the use of radioisotopes other than Iodine, are stimulating the development of other clinical trials in this area against both lymphoid and solid tumors.

IMMUNOTOXINS

Clinical responses have been achieved against lymphoid tumors in recently-completed Phase I clinical trials using conjugated antibodies against several different B- and T-lymphocyte associated antigens. One of these immunotoxins, against the CD-5 antigen, is now being studied in Phase II trials in chronic lymphocytic leukemia, in pediatric T-cell leukemia and lymphoma, and in the prevention of acute GVH disease. Another B4-blocked ricin is being studied in

B cell non-Hodgkin lymphomas, where Phase I trials have demonstrated activity.

NON-SPECIFIC IMMUNOSTIMULANTS

LEVAMISOLE

The results of two large scale Cooperative Group studies demonstrated a marked reduction in the recurrence rate of Dukes' C colon carcinoma following treatment with 5-FU and levamisole, and a New Drug Application for Levamisole was approved in June 1990. Despite these positive results, the mechanism of action of Levamisole is not yet known. Preclinical and clinical studies to evaluate possible mechanisms of action and interaction of Levamisole with 5-FU continue to be pursued; trials to evaluate different Levamisole dosages and schedules are currently underway. In addition, the role of levamisole as adjuvant treatment in gastrointestinal and other types of malignancies continues to be evaluated.

DIFFERENTIATING AGENTS

TRANS-RETINOIC ACID

In last year's narrative attention was drawn to the plans for confirming the remarkable clinical activity of the oral Vitamin A analog all trans Retinoic Acid (TRA) to induce remission in adults with acute promyelocytic leukemia. Since then this activity has been confirmed; over 80% of both adults and children with this malignancy who have failed initial chemotherapy can be successfully treated with minimal side effects. Many of these patients attain complete clinical remissions with this drug. The Cancer Therapy Evaluation Program of the Division of Cancer Treatment over the last year has rapidly moved to bring the drug into clinical study in the United States, filing for an IND, making it available to patients with relapsing APL through a compassionate use mechanism developed with the FDA, and establishing maximally-tolerated doses for the drug through Phase I trials. Drug has been made widely available to basic scientific investigators. Further clinical investigations were solicited and letters of intent for over 50 clinical trials of tRA alone or in combination with other differentiation-inducing agents or cytotoxic chemotherapeutic agents have been approved by CTEP. These trials involve a wide range of malignancies, and for the most part include detailed biological studies to investigate the biology of retinoid action in the malignant cells. National clinical trials involving the major cooperative groups have been developed for both newly-diagnosed and relapsing patients with APL. The NCI has worked actively with the pharmaceutical company involved to gather data supporting licensing approval for tRA for this clinical indication.

This surge in clinical interest in the retinoids as therapeutic agents in malignancy has been paralleled by dramatic breakthroughs in the understanding of retinoid biology. A series of nuclear receptors for the retinoids has been

described, the genes cloned, and the chromosomal location mapped. In APL it has been demonstrated that the characteristic chromosomal translocation associated with this malignancy, the reciprocal transfer of material between chromosomes 15 and 17, directly involves RAR alpha, a major retinoid receptor. In addition to making TRA widely available to laboratory investigators, a meeting of basic investigators interested in retinoid biology was held by CTEP to further facilitate clinical-laboratory cooperative studies in the series of clinical trials recently approved. The recent recognition of differential binding of distinct synthetic retinoids to different retinoid receptors, coupled with the developing information that different retinoid receptors are expressed at varying in different normal and malignant tissues, provide a rationale for the patterns of toxicity seen with this class of agents, and also for the possibility that individual retinoids may be active in different malignancies. The DCT is working actively to introduce a series of new generation retinoids into clinical trial, to investigate this possibility clinically. The parallel developments of recognition of the potential therapeutic applications of the retinoids in established malignancy, and the new progress in understanding of the underlying biology of retinoid effects make possible the future development of more rational, less toxic, differentiation-based therapeutic strategies towards cancer.

COMBINATIONS OF BIOLOGICALS AND CYTOTOXICS

VACCINES

Vaccines are used to elicit a host immune response against tumor, which alone or in combination with other biologic agents may cause regression of advanced disease, or prevent the recurrence of disease in the adjuvant setting. Large randomized studies are in place for patients with surgically resected disease and at high risk for recurrence: in colon cancer an autologous whole cell tumor vaccine is being compared to 5-fluorouracil plus levamisole, and in melanoma a membrane lysate containing vaccinia virus determinants (as an adjuvant) is being compared to observation alone. A second randomized cooperative adjuvant trial in melanoma will examine the efficacy of an allogeneic vaccine derived from two melanoma cell lines.

In order to develop vaccines more effectively there is a need to define and purify the tumor antigens recognized by the host, to determine the best method of administration for development of a potent response, and to define host characteristics that determine the nature of the immune response. Monoclonal antibodies have been developed (called anti-idiotypes) which mimic the structure of tumor antigens and thus can be administered to elicit specific immune responses to tumor. Techniques have also been developed to define the antigens on host tumor cells recognized by serologic responses to an allogeneic vaccine composed of surface proteins from several melanoma cell lines. These results, and identification of tumor antigens recognized by TIL cells, will allow the production of pure tumor antigens by recombinant technology. It should be remembered that adoptive immunotherapy is a form of vaccination where immune sensitization to tumor occurs ex vivo.

Animal models have shown that administration of recombinant cytokines (IL-2, IL-1) markedly increases the potency of the vaccine, resulting in an enhanced in vivo antitumor response. Trials of vaccine in combination with IL-2 are ongoing. Recently, transfection of cytokine genes directly into tumor cells, so that the tumor cell secretes the cytokine, was shown to increase the immunogenicity of the tumor cells used for vaccination. A clinical trial using this approach has been proposed by investigators in the Surgery Branch.

DEVELOPMENTAL CHEMOTHERAPY SECTION (DCS)

In addition to coordinating and monitoring clinical trials for 97 cytotoxic agents with active INDs in the Investigational Drug Branch, the DCS is also exploring several broader drug development initiatives, including: 1) dose intensification; 2) new methodology for Phase I trials; 3) strategies to overcome drug resistance.

Dose Intensification has been facilitated by a number of bone marrow protective strategies such as bone marrow transplant and colony stimulating factors. High dose therapy is being evaluated in a number of randomized trials which should help define the importance of this approach to patient outcome. In addition, the dose-response relationship will be evaluated in early Phase II drug development by comparing high dose versus standard dose-response rates for new cytotoxics being developed. This will ensure that the Phase II evaluation of these compounds is not compromised by the use of a suboptimal dose.

Phase I Methodology is being reexamined with the goals of completing dose-finding trials more efficiently in order to evaluate new agents more rapidly and increasing the likelihood that patients on Phase I trials receive biologically active and potentially beneficial doses. Attention is being focused on pharmacologically guided dose escalations, intra-patient dose escalations and other strategies which will reduce the number of patients and the time required to establish maximum tolerated doses in Phase I trials.

Strategies to Overcome Drug Resistance vary according to the classes of drugs and include the following:

REVERSAL OF MULTIDRUG RESISTANCE

Overexpression of a cell membrane glycoprotein of 170 kilodalton molecular weight, a product of the multidrug resistance gene, mdr-1, has been associated with clinical resistance to therapy in certain tumors. This has limited the utility of a number of the most active anti-cancer therapies available. Agents such as R-verapamil and cyclosporin A, which specifically and competitively bind to p-glycoprotein, have successfully reversed multidrug resistance in vitro. The NCI is currently in negotiation with the Sandoz Corporation to finalize a collaborative agreement to develop cyclosporin analogues which are less immunosuppressive than the parent compound, but effectively reverse

multidrug resistance in vitro.

Trials are underway in Chronic Myelogenous Leukemia in Blast Crisis (CML-BC) and lymphoma with either cyclosporin A or R-verapamil in combination with cytotoxic chemotherapy. Additional disease sites which also have marked overexpression of P-170 will be evaluated using these and other MDR-reversal agents [to be developed] in combination with appropriate cytotoxics.

REVERSAL OF RESISTANCE TO ALKYLATING AGENTS

L-BUTHIONINE SULFOXAMINE (BSO)

Intracellular elevations of glutathione (GSH) have also been shown to be associated with primary and acquired resistance in some experimental models of human cancer. Studies indicate that administration of buthionine sulfoximine (BSO), a potent inhibitor of the first and rate-limiting step of GSH biosynthesis, to animals of cultured cells results in tissue and cellular GSH depletion, suggesting the potential for reversing resistance mechanisms associated with increased levels of GSH> The compound has been shown to reverse the acquired resistance of human ovarian cell lines to either melphalan and/or platinum. Clinical trials are ongoing to evaluate a combination of BSO together with L-PAM for the potential of reversing drug resistance. Preliminary data indicate that BSO induces in vivo depletion of intracellular GSH in peripheral lymphocytes and tumor cells. The dose + schedule of BSO that optimizes GSH depletion are currently being defined, preclinical toxicology studies are now completed for a combination of BSO and carboplatin and Phase I trials will be initiated.

ETHACRYNIC ACID

A number of studies are being sponsored with this agent in an attempt to inhibit the enzyme glutathione - S transferase in an effort to cause glutathione depletion and decrease resistance to alkylating agents and platinum compounds.

MECHANISMS TO OVERCOME ANTIMETABOLITE RESISTANCE

BIOCHEMICAL MODULATION OF 5-FU (Dip, LV, AZT, PALA, IFN, Levamisole, Urd)

Biochemical modulation involves the combination of inactive or minimally active compounds with chemotherapeutic agents that have established antitumor activity in order to enhance the therapeutic effectiveness of the active drug. 5-FU has been at the center of this research. The modulating compound may alter the drug's extracellular or intracellular metabolism, acting as a cofactor in enzymatic reactions (e.g. Leucovorin), inhibiting critical enzymes (e.g. PALA or IFNa), blocking transport systems (e.g. Dipyridamole), replacing nucleotides in DNA (e.g. AZT) or RNA, selectively rescuing or protecting normal tissues (e.g. Uridine) or other mechanisms not yet determined (e.g. Levamisole).

The combination of Leucovorin and 5-FU has now become the standard regimen for patients with advanced colorectal adenocarcinoma and clinically significant activity has also been documented with 5-FU in combinations with alpha Interferon and PALA. Building on these results, studies with combinations 5-FU of two or more modulators against several histologies (particularly gastrointestinal) are ongoing. In the adjuvant setting, 5-FU in combination with the anthelminthic drug, Levamisole, has become standard therapy for patients with resected stage B2 and C colon cancer. Although several attempts have been made to document a biochemical or immunomodulatory interaction, the mechanism of this synergy remains an enigma. The addition of Leucovorin to 5-FU/LV and Levamisole is being studied in current adjuvant trials.

Specific cytotoxic agents of particular interest which are being developed in DCS are described below:

TOPOISOMERASE I INHIBITORS

TOPOTECAN

Camptothecin and its analogues are a group of novel compounds; they are the only known inhibitors of topoisomerase I, an enzyme necessary to mammalian cells for DNA replication. Topotecan is an analogue which was selected for development because it has several advantages over the parent compound: 1) it is water soluble, 2) it has a broad spectrum of preclinical activity and 3) it has significant cytotoxicity regardless of the route of administration. Preclinical data suggest that efficacy is improved when tumor cells are exposed to this agent for prolonged periods. Based on these data, intermittent and continuous infusion schedules are being explored in the Phase I evaluation of this agent.

Several responses have been observed on one schedule recently completed which was administered as a daily 30 minute infusion for 5 days (dx5). Although other Phase I schedules have not yet been completed, the dx5 schedule will be used to launch the Phase II development program scheduled to begin this summer. As additional schedules come to completion in Phase I, selected regimens will be evaluated in randomized trials in targeted disease sites (ovary, breast, NSCL) to define the optimal schedule. The schedule identified in this way will then be used to explore efficacy in previously untested malignancies, in tested malignancies for which response was found to be unimpressive on the schedule used and in combination with other antineoplastic agents.

TOPOISOMERASE II INHIBITORS

AMONAFIDE

Amonafide induces topoisomerase II mediated DNA cleavage and also inhibits macromolecular synthesis. A broad Phase II evaluation of this agent is underway. Toxicity data suggest that this drug is well tolerated with reversible myelosuppression as its dose limiting toxicity. Objective responses

have been observed in a Phase II trial in patients with breast cancer, a second Phase II trial is ongoing to define the level of activity of this compound in this disease site more precisely. Amonafide is also being evaluated in high doses as a preparative regimen for bone marrow transplantation.

Amonafide is eliminated primarily via metabolism to an active N-acetyl metabolite. Preliminary data suggest that the severity of toxicity and potential for activity may be predicted based on the patient's acetylator phenotype; further research is ongoing to correlate acetylator phenotype, pharmacokinetics and pharmacodynamics in the context of clinical trials. Utilization of this approach will hopefully assist in individual patient dosing and optimize amonafide administration.

FOSTRIECIN

This novel compound, produced by Streptomyces pulvaraecus, inhibits macromolecular synthesis and is thought to inhibit DNA topoisomerase II. It shares the same method of entry into cells as methotrexate, the reduced folate carrier transport system. Because of its unique structure, novel proposed mechanism of action, and need for the reduced folate carrier system to gain cell entry, fostriecin was chosen for further evaluation. Phase I clinical trials are scheduled to begin in the Fall '91 when formulated drug will be available.

TENIPOSIDE

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol was recently activated for VM-26 in combination with Ara-C for the treatment of patients with refractory or first relapse acute lymphoblastic leukemia. Forty-three patients have been accrued to date. Thirteen of 29 evaluable patients have had a complete response to treatment. Several patients have experienced durable complete remissions some of which have been observed up to 18 months.

Confirmatory trials in small cell lung cancer have been initiated based on data from the Finsen Institute which demonstrated extraordinary single agent activity of VM-26 in small cell lung cancer (J Clin Oncol 4:524, 1986). These trials are ongoing and are too early for meaningful data interpretation. Although one trial reported inactivity in this disease site, two trials reported a response rate of 24% and 40% respectively.

MERBARONE

The precise mechanism of merbarone cytotoxicity is unknown, although some preclinical data suggest that it functions as a novel topoisomerase II inhibitor. Preclinical data indicated that efficacy was best using an intermittent infusion schedule. Two Phase I studies were conducted to evaluated a 5-day continuous infusion schedule. Because of severe phlebitis, the drug could not be administered via peripheral vein and administration via a central venous catheter is required. To further assess the problem, both

trials also evaluated the feasibility of a daily 2 hour infusion for 5 days. Although this was also a tolerable regimen the maximum dose delivered was less than half the dose which could be delivered as a continuous infusion. A broad Phase II screening program is currently underway utilizing the 5-day continuous intravenous regimen.

MITOTIC SPINDLE TOXINS

TAXOL

This unique natural product derived from the bark of Taxus brevifolia has shown promising antitumor activity. Results of 3 Phase II studies indicate that 30% of women with relapsed ovarian cancer who had been treated with multiple regimens (radiation, chemotherapy) responded to taxol. Responses, including complete responses, were seen in women who were refractory to conventional therapy. To pursue this activity, a Phase III comparison of standard therapy (cytoxan + cisplatin) against the combination of taxol + cisplatin is underway in newly diagnosed patients, and trials of taxol in combination with other agents for ovarian cancer are planned

One recently reported Phase II trial in advanced breast cancer demonstrated a 56% response rate to taxol, including responses in some patients previously failing adriamycin. A second Phase II trial is underway to determine if these data can be confirmed. In addition, we are sponsoring two Phase II trials combining taxol with adriamycin in different sequences to build upon the early promise of these results.

A broad Phase II evaluation of taxol in most diseases is underway, this being implemented in phases as the drug, which is available in extremely limited quantities becomes available. Current disease sites include colon, lung, prostate and head and neck. Bladder, sarcoma, pancreas and lymphoma trials will start shortly. Phase I studies of pediatric solid tumor and leukemias, intraperitoneal delivery for ovarian patients, and dose-escalation of taxol with growth factors are ongoing.

INTERCALATORS

PIROXANTRONE (OXANTRAZOLE)

Piroxantrone is one of a new class of intercalating agents, the anthrapyrazoles. Of the three most active agents in this class developed by Warner-Lambert, piroxantrone is the one currently in development through the NCI. Piroxantrone was the first agent to undergo development utilizing pharmacologically guided dose escalations according the Blood Level Working Group method. It was estimated that 9-12 fewer patients were required for the Phase I evaluation. The regimen currently being evaluated in broad Phase II testing is 150mg/m2 IV bolus every 3 weeks with potential for dose escalation. One of the other analogues has demonstrated substantial activity in breast cancer. It is expected that piroxantrone will also be active in this disease as there was no difference between these agents preclinically.

LIPOSOMAL DOXORUBICIN

Preclinical studies with liposome encapsulated doxorubicin have shown that the maximally tolerated dose of doxorubicin can be increased by approximately 2.5 fold. This was accompanied by an alteration in the tissue distribution of doxorubicin, with less accumulation in cardiac tissue. Superior antitumor activity was noted in some, but not all, preclinical models. Several Phase I trials with liposomal doxorubicin are exploring different schedules to define the best use of this agent.

One schedule, single bolus every 3 weeks, has been completed recently. The maximum tolerated dose was found to be the same as the free form of adriamycin. Trials are planned in a limited number of disease sites to test the advantage of this agent, identified preclinically: 1) preferential uptake by the liver and 2) increase uptake tumor cells compared to normal tissue.

PYRAZOLOACRIDINE

Pyrazoloacridines are a class of agents which were specifically synthesized with the intent of designing agents which had selective superior efficacy in solid tumors. Pyrazoloacridine, named after the class of agents, was one of the most active agents in this regard. This agent recently passed the Decision Network to enter clinical evaluation. The IND for this agent has been reviewed and accepted by the FDA. The first Phase I trial is underway at Wayne State University using a single bolus every 3 weeks regimen. A second trial will be conducted at Johns Hopkins using the same schedule.

ANTIMETABOLITES

AZIDOTHYMIDINE(AZT)

Thymidine salvage plays a role in protecting tumor cells from the cytotoxic effects of 5-FU (see paragraph on biochemical modulation of 5-FU). An IND for AZT in cancer patients was filed in March 1990. Studies are ongoing to evaluate this modulation of 5-FU cytoxicity.

2-CHLORODEOXYADENOSINE (CDA)

CdA is a purine analogue that was synthesized by investigators at the Scripps Clinic in La Jolla, CA. Although its exact mechanism of action has not yet been elucidated, a marked dNTP imbalance in affected cells and consequent DNA double strand breaks appears implicated. The toxic effect of CdA appears to require phosphorylation by deoxycytidine kinase(dCk) to the triphosphate level(CdATP). CdATP accumulates in cells such as lymphocytes that have high levels of dCk. Incorporation of CdATP into DNA and modulation of the immune system may also play a role in this drugs antitumor effects.

Preliminary clinical studies, performed under the Scripps IND, have identified dramatic activity against hairy cell leukemia (over 100 patients treated).

Less dramatic activity has been seen against cutaneous T-cell lymphoma and low grade non-hodgkin's Lymphoma. CTEP has submitted an IND application to the FDA for CdA. A Phase I study for patients with solid tumors, using a 5-day continuous infusion schedule will be started once the IND is approved. CTEP also plans to make CdA available to patients with hairy cell leukemia through the Group C or "Special Exception" mechanism until the drug becomes commercially available.

CARBOXYPEPTIDASE ($CPDG_2$)

$CPDG_2$ is a zinc-dependent enzyme that was isolated from a pseudomonas strain in the 1970s. It hydrolyzes the C-terminal glutamate residue from MTX and other folate analogues (but not nonclassical antifols). A recombinant form of this enzyme, $CPDG_2$, which is a dimer of 84,000 daltons, has recently been cloned from pseudomonas sp strain RS16. It does not cross the blood brain barrier or cellular membranes. Although an IND has yet to be filed for this agent, potential uses for CPDG2 in patients include intrathecal rescue from an intrathecal methotrexate overdose, systemic rescue from high-dose methotrexate therapies and potentiation of non-classical antifols, such as Trimetrexate by depletion of the endogenous folate pool. Toxicology studies have been completed and a decision on proceeding to an IND application will shortly be made.

CYCLOPENTENYL CYTOSINE (CPE-C)

CPE-C is an inhibitor of CTP synthetase that has demonstrated antitumor activity in Ara-C resistant leukemias and B16 melanoma. AN IND application is being prepared and a Phase I protocol for the NCI Clinical Center has been approved by CTEP.

DEOXYCOFORMYCIN (PENTOSTATIN; dCF)

dCF is the first adenosine deaminase inhibitor investigated therapeutically in man. Although early clinical trials demonstrated significant toxicity, well-tolerated and effective regimens have been developed for hairy cell leukemia (HCL) and the drug is available from CTEP for patients with HCL who have failed alpha interferon. A New Drug Application has been submitted to the FDA for this indication.

D1694

This folate analogue directly inhibits thymidylate synthetase, in contrast to classical antifols such as methotrexate that inhibit dihydrofolate reductase. It was highly cytotoxic to all wild type tumor cell lines (L1210, WIL2, HeLa & MCF7) following continuous exposure to the compound in thymidine free culture media and produced growth delays in human xenograft ovarian, colon and lung tumors. Hematologic and gastrointestinal abnormalities were the most frequently encountered toxicities in rodents and dogs. The first Phase I study was opened in Great Britain in April 1991. The first U.S. trial is scheduled for August 91 and will be performed at the National Cancer Institute.

EDATREXATE (ETX: formerly referred to as 10-EdAM)

Edatrexate is an analogue of methotrexate (MTX) that has more efficient intracellular transport, greater selectively for tumor cells over normal tissues and that undergoes more extensive polyglutamation once inside cells than MTX. It is more active than MTX against a number of murine tumors and human tumor xenografts and has shown impressive in vivo synergy with alkylators.

The Phase II evaluation of ETX is proceeding rapidly. To date twenty-two studies in seventeen histologies have been approved and three others are in review. In addition, 9 Phase I studies of combinations involving ETX have been approved or are under review, including two studies of leucovorin rescue of this agent. Finally, the Ciba-Geigy Company is sponsoring two Phase III studies in NSCLC of a regimen that produced a high response rate in Phase II evaluation. Patients are randomized to receive Vinblastine and Mitomycin C, with or without ETX. Accrual to these studies is near completion.

FAZARABINE (Ara-AC)

This analogue of Ara-C demonstrated interesting antitumor activity in a number of preclinical solid tumor models. The Phase II evaluation in solid tumors is ongoing, with studies approved or in review for eight histologies. The Phase I studies in leukemia have not yet resulted in reproducible dose-limiting toxicity in spite of multiple escalations beyond the dose used in the solid tumor studies. As the preclinical activity of Ara-AC was very dependent on the schedule used, the duration of the infusion (currently 72 hours) may be inadequate and 4 and 5-day infusions will be evaluated shortly.

FLUDARABINE PHOSPHATE (FAMP)

This agent is the halogenated phosphate derivative of vidarabine, which has the advantage of resistance to deamination by deaminase and improved solubility. The compound has undergone extensive clinical evaluation as an anticancer agent since its introduction into the clinic in 1983. While early trials demonstrated significant myelosuppression and episodes of severe neurotoxicity (including cortical blindness and coma), recent clinical investigations revealed significant activity against lymphoproliferative malignancies, particularly chronic lymphocytic leukemia (CLL) and low-grade non-Hodgkin's lymphoma (NHL). The single agent response rate in 149 evaluable previously treated CLL patients was 56%. CTEP has provided this agent for over 800 previously treated CLL patients through the Group C mechanism. Although the drug is generally well tolerated, myelosuppression, pulmonary and neurotoxicity has been seen. Earlier this year, the FDA approved this agent and commercial supplies should be available shortly. Phase III randomized trials comparing fludarabine with other standard agents (e.g. chlorambucil, cyclophosphamide(in CLL and NHL are ongoing.

TIAZOFURIN (TCAR)

Investigators at Indiana University have obtained impressive responses with TCAR using a 15-day schedule in patients in the myeloid blast crisis phase of chronic myelogenous leukemia. Significant antitumor activity was noted in 9/10 cases, although the duration of response to each course of therapy was only a few weeks, necessitating repeated treatments to maintain the responses. There was a direct correlation between attaining 90% IMPDH inhibition (and/or 80% depletion of intracellular GTP) and inducing a hematologic response. There were no responses in patients whose intracellular GTP levels could not be decreased or maintained below 20% control. The addition of allopurinol was necessary both to inhibit GTP salvage pathways and to prevent uricemia. The observation of down-regulation of c-Ki-ras and c-myc oncogenes in one particularly sensitive patient and the response pattern in most patients points towards a differentiating rather than a cytotoxic effect for tiazofurin, consistent with a return to the chronic phase. As reported by Tricot et al (Can Res 49:3693-3701,1989), toxicity has been substantial and severe with this regimen. Of the first 16 patients treated (including patients with ANLL), sudden coma was seen in two patients, pleuropericarditis in two, cardiac arrest in two others and seizures (possibly related to hypertension) occurred in another two patients. The advanced state of the patients' disease on this study undoubtedly contributed to the toxicity profile. The investigators have informed us that subsequent patients on this regimen have fared much better and that life-threatening toxicity related to the tiazofurin can be avoided or ameliorated in most if not all patients, primarily by early treatment of hypertension and by discontinuing the infusions at the first sign of trouble. However, given the small number of patients treated so far, only preliminary conclusions can be made regarding the incidence of toxicity and a better picture of the actual risks associated with this regimen should become available as the number of treated patients increases.

URIDINE(Urd)

Uridine may improve the therapeutic index of 5-Fluorouracil by preferentially rescuing normal tissues over tumor cells or by modulating the 5-FU metabolic pathways, selectively rescuing RNA but not DNA. Considerable work has already been completed in preclinical models and an intravenous formulation of Uridine is currently being evaluated in conjunction with 5-FU-based chemotherapeutic regimens. However, the mode of administration, which requires a central line to prevent phlebitis, has proved very cumbersome and has hindered patient accrual. Several investigators have expressed interest in evaluating the impact of this nucleotide on 5-Fluorouracil metabolism when administered orally. For these reasons, an IND for an oral formulation of Uridine was filed and studies were opened to determine to tolerability of the oral uridine when administered with 5-FU and other cytotoxics. These trials are ongoing.

ALKYLATING AGENTS

HEPSULFAM

Hepsulfam is a bis-sulfamic acid ester with structural similarity to busulfan. The compound appears to act as a bifunctional alkylator and has demonstrated good activity in in vivo murine tumor models. Phase I trials of hepsulfam are currently ongoing and its activity will be screened in a spectrum of various tumors.

TETRAPLATIN (Tp)

Tetraplatin is a new platinum IV compound which was selected for clinical development based on evidence of non-cross resistance in tumor models which were resistant to other platinum compounds. Its preclinical toxicity profile appears similar to carboplatin. Phase I trials are underway to define the appropriate Phase II dose for additional clinical trials.

NOVEL COMPOUNDS

IPOMEANOL

Three Phase I trials are currently active: single bolus q 21 days at Johns Hopkins and daily x 5 bolus q 21 days at NCI-Navy and Ohio State. The latter trials have only recently begun accrual.

Early pharmacokinetic data indicated that, while the dose in mg/m^2 represented approximately 70% of the LD_{10} in female mice, the AUCs at that dose represented only 4% of the AUC at the LD_{10} in female mice and 10% of the AUC at the lowest non-toxic dose in dogs which suggests that human handling of the compound may be somewhat different. The daily x 5 trials have been amended to allow a higher starting dose based on the daily x 1 experience and will use more aggressive dose escalations and intrapatient dose escalations until a dose which produces biologic activity is identified. After some initial experience is gained with the daily x 5 schedule, trials designed to induce tolerance will be initiated.

SURAMIN

Suramin is a polysulfonated napthylurea which has been in clinical practice since 1920 for the treatment of trypanosomiasis. Interest in its antitumor effects were stimulated by the finding that suramin caused Addison's Disease in AIDS patients and by subsequent work which demonstrated that it was a growth factor antagonist. The activity of a number of growth factors appears to be blocked by suramin, including basic-FGF, PDGF, TGF-beta and EGF.

Toxicity includes thrombocytopenia (in previously treated patients), coagulopathy, mild alterations in renal function and neurotoxicity (polyradiculopathy progressing to flaccid paralysis requiring intensive care unit support at high serum levels). The prevention and management of these toxicities was improved when dose adjustments were made on the basis of pharmacologic monitoring to maintain serum suramin levels <300 mcg/ml and careful monitoring of the prothrombin time to maintain it at < 17.5 seconds. Other toxicities have included proteinuria, vortex keratopathy, rash, anorexia/malaise, hepatitis and adrenal insufficiency.

Responses to suramin have been seen in adrenal, renal, and prostate cancers and lymphoma. A number of Phase II studies have begun in disease sites where growth factors are felt to play an important role or where there are in vitro data suggesting suramin activity. Tumor biology or other correlative basic science endpoints have been incorporated into all of these clinical trials.

Blood level monitoring will be done weekly on all patients on Phase II trials so that infusion doses can be individualized to maintain levels >200 and <300 mcg/ml and, therefore, ensure that therapeutic levels are achieved as rapidly as possible while reducing the risk of neurotoxicity which correlated with peak levels >300mcg/ml.

Additional Phase I trials are underway in an attempt to define a schedule which produces the desired blood levels with reduced toxicity and greater patient convenience. Several innovative trial designs are using population based pharmacokinetics to model dosing regimens which will then be adapted to individual patients using adaptive control feedback algorithms. These trials are exploring the maximum achievable and tolerable serum concentration and the maximum duration of maintenance therapy at the optimal concentration. Pharmacodynamics will be correlated with pharmacokinetics in all studies.

PYRAZINE DIAZOHYDROXIDE (PZDH)

PZDH is a more hydrolyically stable analog of pyridine-2-diazohydroxide and has a broad spectrum of curative antitumor activity in animal tumors. While it is known to produce single-strand DNA breaks, the actual mechanism of cytotoxicity is unknown. Initial Phase I trials of PZDH are currently ongoing to determine the maximum tolerable dose and proper dose for Phase II trials. Upon completion of these Phase I trials, the drugs activity will be evaluated in a spectrum of various tumors.

TEREPHTHALAMIDINE

The agent is one of a class of phthalanilide derivatives shown to have preclinical antileukemic activity. It underwent Phase I testing in the early 1960's and responses were seen in patients with lymphomas and a patient with a germ cell tumor. The drug was abandoned because of the unusual toxicity of eye muscle paralysis but further anima testing demonstrated that the risk of this could be reduced by slowly infusing the drug rather than giving it as an I. V. bolus. With the current wide availability of continuous infusion pumps, it was felt that this agent should be re-examined. There are also preclinical data to suggest that combining terephthalamidine with inhibitors of polyamine

biosynthesis such as MGBG and DFMO may potentiate its antitumor action, whereas combining it with Suramin may selectively block in toxicity. The drug has begun Phase I testing on a five day continuous infusion every three week schedule.

CHLOROQUINOXALINE SULFONAMIDE

This is the second compound with outstanding activity in the human tumor cloning assay to be selected for clinical development. Its mechanism of action remains totally unknown. the compound is especially active preclinically against melanoma, ovary, breast and lung tumors. Two Phase I trials using a 1-hour infusion every four week schedule are nearing completion with several minimal responses in lung and colon cancers being reported. As the does limiting toxicity of CQS is not myelosuppression, it may be possible to give the drug weekly. A revised Phase I trial on this schedule is underway. Once the MTD and best schedule is defined, the drug will undergo broad Phase II testing against solid tumor. The IDB is initiating preclinical synergy studies in collaboration with the DTP to explore possible combination of CQS with other cytotoxic agents active against solid tumors.

RADIATION AND CHEMOTHERAPY SENSITIZERS

PORFIROMYCIN

This is an N-methyl derivative of mitomycin-C. Both porfiromycin and mitomycin underwent clinical evaluation in the 1960's. Since both compounds demonstrated a similar spectrum of clinical antitumor activity and mitomycin C is more potent than porfiromycin, the clinical development of porfiromycin was not pursued. Preclinical data by Sartorelli, et al. suggested that porfiromycin was preferentially toxic to hypoxic cells compared to well oxygenated cells. Based on these data, investigators at Yale University have initiated a Phase I trial of porfiromycin as a radiosensitizer in head and neck patients. This study is nearing definition of the MTD with the dose-limiting toxicity being myelosuppression.

SR 2508

A randomized trial in patients with head and neck cancer is nearing completion which will establish the efficacy of SR 2508 and radiotherapy versus radiotherapy alone. Several pilot studies are ongoing. Phase I studies have defined the maximally tolerated dose of DR 2508 given with brachytherapy based on preclinical data which suggest that DR 2508 may be more effective when given with low dose rate radiotherapy. This approach is particularly promising in prostate cancer and brain tumors and Phase II trials are being conducted in these disease sites. Phase I trials have been completed with the combination of SR 2508 and cyclophosphamide and one study is ongoing to better define the optimum delivery schedule and mechanism of chemosensitization by this agent. The ECOG is planning Phase II trials in both small cell lung cancer and breast cancer. Several innovative pilots are underway which add SR

2508 to platinum based regimens based on preclinical data that the drug chemosensitizes both cisplatin and carboplatin. Trials are now underway designed test the application of these combinations to ovarian cancer and autologous bone marrow transplants.

PHOTODYNAMIC THERAPY

Photodynamic therapy is a new modality for the treatment of cancer in which a light-activated drug is administered to a patient and becomes cytotoxic only upon exposure to light, which in most clinical applications is supplied by a red laser. Photofrin II is the most clinically advanced of these compounds and has demonstrated activity against a wide range of surface and intraluminal malignancies. A Phase I study at the NIH Clinical Center in intra-abdominal malignancies is leading towards a GOG trial of photodynamic therapy for post-operative residual disease in ovarian cancer and the SWOG is planning a bladder cancer trial. A major limitation of this approach is the necessity to deliver light to the tumor. The IDB is working with the RRP and DTP to help develop newer phototherapeutic drugs with physicochemical properties that offer the possibility of broader applications of this technique to cancer treatment.

BUDR AND IUDR

Bromodeoxyuridine and iododeoxyuridine are members of the class of halogenated pyrimidines being developed as radiosensitizers which enhance DNA strand breakage after radiation exposure. Recently reported results have shown that adding BUDR to radiation therapy combined with chemotherapy prolonged the median survival of patients with anaplastic astrocytomas by 21%. A phase III trial is being conducted collaboratively by three groups to verify these results and compare IUDR and BUDR in this setting.

Both these agents also have utility in studying tumor cell kinetics in vivo, which may prove to be an important technique for improving diagnostic and prognostic accuracy in a wide range of tumor types. A considerable amount of data exist modeling the behavior of brain tumors which may lead to better radiation and chemotherapy treatment planning. In order to move this field forward, IDB and CTEP have established a collaborative development plan with the Diagnosis Branch in DCBD to organize and oversee cytokinetic trials with these agents.

HMBA

Hexamethylene bisacetamide is the first of a group of polar-planar differentiating agents to enter clinical trials. Some efficacy for this agent has been demonstrated via an intravenous route in patients with myelodysplastic syndromes. With the recent development of an oral formulation, it may be possible to give doses of this drug continously for extended periods of time, the preferred schedule for differentiating agent. Diseases to be studies with this approach include myelodysplasia, bladder, head and neck, and lung cancer. Additional studies will be done to better understand the action of metabolites of this drug, attempt modulation of its

effect on protein kinases, and determine if its efficacy can be enhanced by combining it with the differentiating agent trans-retinoic acid

DRUG MANAGEMENT AND AUTHORIZATION SECTION (DMAS)

GROUP C DRUGS

Over the past year Group C drug authorization and distribution for individual patients has continued at a high level. Group C drugs are those that have reproducible efficacy in a tumor type, that alter the pattern of care of the disease, and those that can be safely administered by physicians without specialized supportive care facilities. Group C protocols are standardized treatment designs which use Group C drugs to treat a specific tumor or stage of disease. DMAS participates in the protocol writing and establishes management procedures unique for each protocol that are used by the section in screening and registering individual patients. This program is both national and international in scope. Qualified physicians register with DMAS, agree to obtain patient informed consent, and, as necessary, Institutional Review Board approval. Physicians are required to report Adverse Drug Reactions to NCI but in most cases there is no additional patient reporting.

The current drugs in Group C status are Amsacrine in refractory AML, Azacytidine in refractory AML, Pentostatin in Hairy Cell Leukemia, Erwinia Asparaginase in ALL for patients sensitive to E. Coli Asparaginase, Teniposide in refractory ALL and Fludarabine in refractory CLL. Hexamethylmelamine for use in refractory Ovarian Carcinoma became commercially available in the past year.

This year extensive section resources continued to be directed toward registering physicians and patients for the use of Fludarabine. Since the inception of the Fludarabine Group C protocol, 1 1/2 years ago, more than 2,800 patients have been registered and approximately 8,000 vials of drug have been shipped each month. Numerous additional patients were entered on Fludarabine Special Exception protocols for such indications as refractory indolent lymphomas, prolymphocytic leukemia, and CLL patients that do not meet the eligibility criteria for the Group C protocol.

It is worthy to note some of the unique features of each of these drugs that impact on the effective management procedures. For example, with fludarabine the entry criteria are very specific, often requiring further laboratory tests and a subsequent return phone call by the registering physician. With physician return calls, a patient's fludarabine registration can take several days to complete.

A typical Group C registration involves the investigator first calling DMAS to discuss a patient's case with a pharmacist. Attempts are made to refer the patient to an existing clinical trial, however if this is not possible and the patient qualifies for the Group C protocol, the entry request is approved. If not already registered, the physician is requested to complete an FDA 1572 form (Statement of Investigator) and must be a board eligible/certified oncologist or hematologist or a specialist such as a urologist or gynecologist

who routinely treat cancer patients. A patient registration letter and adverse drug reaction reporting form is sent and the quantity of the initial drug shipment is calculated and drug is shipped. Shipment records are maintained and reorder requests are considered as long as the investigator remains in active status and in good compliance.

Over the past year, for all Group C drugs, more than 3,000 new patients were approved to receive treatment and 7,400 drug reorders were honored by the DMAS.

SPECIAL EXCEPTION PROTOCOLS

Special Exception Protocols are also sometimes referred to as "Compassionate protocols". They are used for patients in instances when a patient has failed all conventional treatments for a specific form of cancer and when the patient is not eligible for, or for various reasons, refuses entry onto a clinical trial. In order for DMAS to consider a Special Exception request the drug must have demonstrated activity in the specific disease. The Special Exception mechanism is also used in instances when a patient fails to meet the entry criteria for a Group C protocol or when combination or multimodality treatment is being considered.

The procedure for obtaining Special Exception approval is somewhat analogous to that of Group C, although the patient entry criteria are not as well defined. The requesting physician calls DMAS and discusses the case with a pharmacist. The prior treatment, performance status, blood chemistries and organ functions are reviewed and the proposed treatment is presented and a rationale for the proposed treatment is given. Unlike Group C where the patient is joining an established treatment protocol, Special Exceptions require that the physician register with DMAS, complete and return a single patient protocol, obtain Institutional Board Approval and patient informed consent. Special Exception protocols are filed with FDA and physicians must report any Adverse Drug Reactions and report to DMAS on the outcome at the end of treatment.

In the past year DMAS registered numerous Special Exception patients for Fludarabine in cases when patients were diagnosed with diseases other than CLL for which there is data to support treatment, most commonly refractory lymphomas and prolymphocytic leukemia. Also in the past year, significant numbers of patients were registered for the use of Trans-retinoic acid in Acute Promyelocytic Leukemia.

About 1,800 Special Exception Protocols were approved and an additional 1,400 requests for Special Exception reorders were honored by DMAS in the past twelve months.

TREATMENT REFERRAL CENTER (TRC)

The Treatment Referral Center (TRC) was established in the Drug Management and Authorization Section of the Investigational Drug Branch and became operational the end of January 1991. The TRC was organized to: 1) manage

inquiries about specific agents in short supply (e.g., taxol for breast and ovary cancer), 2) help to ensure that appropriate treatment options are considered and 3) optimize patient referral to centers of clinical and research excellence.

For certain diseases, initially breast and ovarian cancer, the TRC will develop algorithms consisting of major cooperative group trials, standard therapies and "Treatment Referral Center Protocols. To date, for ovarian cancer, this algorithm has been developed and "Treatment Referral Center Protocol" are being developed.

NEW COMPUTER SYSTEM DEVELOPMENT

The DMAS has largely completed the first major redesign of their computer system in 15 years. The DMAS directed its computer support contractor in the system development, equipment was purchased, installed, and the Oracle data base management system was programmed to meet the section's computer needs. The DMAS offices in Executive Plaza were wired for a Local Area Network (LAN). The LAN was connected to the contractor facility via modems. Final implementation of the new system began in October 1990 but was delayed for six months due to a transition to a new computer support contractor. The LAN is scheduled to be in place and operational by early July 1991.

THE ELECTRONIC CLINICAL DRUG REQUEST ORDERING SYSTEM (ECDR)

The electronic drug ordering and verification system for the transmission of drug requests from investigators to NCI was greatly expanded in the past year. There are currently more than 120 institutions authorized to order drugs using the ECDR and more are being added. The system has been extended to include international locations. The system has simplified the drug ordering procedure and has reduced overall drug distribution time from weeks to days. It has been well received and has helped to minimize the need for investigators to maintain large drug inventories and it has thus helped reduce drug costs to NCI.

ENHANCED PROTOCOL REVIEW PARTICIPATION

The DMAS continues to expand it involvement with the CTEP Protocol Review Committee. For several years it has been providing weekly drug cost estimates for all protocols being considered by the committee to assist in determining the drug cost consequences of proposed treatment regimens. The section has taken an increasingly important role in reviewing protocols for their the drug information content, treatment plan, dose modifications and drug supply sections. The Protocol Review Committee and subsequently the study chairmen, are advised of the findings and recommendations. This has helped to maintain consistency and accuracy within each protocol and between protocols. This effort has taken considerable amounts of the section's professional time and has significantly contributed to protocol review process.

INVESTIGATOR REGISTRATION

The administration of the primary investigator registrations and annual reregistrations continues to be an important function of DMAS. There are currently 5,606 clinical investigators registered to receive investigational drugs and compliance with registration requirements is 100 %.

DRUG COST EXPENDITURES

The total drug costs have increased from $ 4.0 million in FY 88 to $ 4.4 million in FY 89 as a result of increased drug distribution to Cooperative Groups, CCOP's, the NCI intramural program and NIAID for AIDS (AIDS distribution was transferred to NIAID in July, 1989). FY-90 costs are running at an projected annual rate of $ 4.8 million. The increase can partly be attributed to the distribution administration charges associated with Levamisole.

Drug Distribution Data for the Past Year

Number of Drug Orders (Line Items)	New Group C Orders (Reorders)	New Special Exception Protocols (Reorders)	Total Containers (Vials, Ampules, Btls) Distributed
23,700 (34,398)	2,976 (3,592)	1,764 (7,404)	932,165

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REGULATORY AFFAIRS BRANCH

The Regulatory Affairs Branch is responsible for: (1) preparing and submitting Investigational New Drug Applications (INDs) to the Food and Drug Administration (FDA) for assisting in the initiation of clinical trials with anticancer and antiAIDS agents and complying with all FDA regulatory requirements pertaining to these agents; (2) implementing, coordinating and administering the monitoring of clinical trials with anticancer agents sponsored by the Division of Cancer Treatment, NCI. The Branch assures that clinical trials are conducted according to NIH and NCI policies and procedures and Federal regulations.

The Regulatory Affairs Branch is composed of the Drug Regulatory Affairs Section and the Quality Assurance and Compliance Section. The Drug Regulatory Affairs Section is responsible for:

1. Liaison between the Division of Cancer Treatment, NCI, and both the Center for Drug Evaluation and Research and Center for the Biologics Evaluation and Research of the FDA;

2. Submission of INDs to FDA after analyzing the adequacy of the data for cytotoxic and biologic anticancer agents developed by the Division of Cancer Treatment, NCI, and other NCI divisions, particularly the Division of Cancer Biology, Diagnosis and Centers;

3. Submission of INDs to FDA after analyzing the adequacy of the data for antiAIDS agents;

4. Coordination of responses to correspondence from FDA regarding IND applications and amendments;

5. Compliance with adverse drug reaction regulations;

6. Liaison with the preclinical sections of the Division of Cancer Treatment, particularly the Developmental Therapeutics Program and the Biological Response Modifiers Program;

7. Liaison with pharmaceutical companies to provide preclinical and clinical data and any other information required to complete approval for New Drug Applications;

8. Liaison with intramural clinical groups in NCI and NIH on regulatory issues concerning agents of particular interest; and

9. Liaison with extramural investigators on regulatory issues concerning agents of particular interest.

The Quality Assurance and Compliance Section is responsible for:

1. Planning, organization and administration of a program for monitoring the quality of clinical data for all clinical trials with anticancer agents sponsored by the Division of Cancer Treatment;

2. Attendance at 10-20% of on-site audits performed by the Cooperative Groups;

3. Carrying out the on-site audits of Cancer Centers and other single institutions conducting clinical research utilizing DCT-sponsored investigational agents;

4. Carrying out special mail and on-site audits of Group C Protocols;

5. Carrying out special on-site audits of promising Phase II clinical studies to confirm response rates before decisions are made about future Phase III studies;

6. Serving as the Project Officer for a contract with the Clinical Trials Monitoring Service;

7. Liaison with the Office for Protection from Research Risks (OPRR) and the Cooperative Groups to help new physicians/institutions complete assurances to become able to enter patients on study as rapidly as possible;

8. Setting guidelines for the conduct of DCT-sponsored clinical research and serving as an educational resource to the cancer community for site visit monitoring and regulatory requirements for clinical trials;

9. Review of each protocol submitted to CTEP to assure the informed consent form meets federal guidelines and that other regulatory and policy issues are addressed;

10. Liaison with the Scientific Investigations Branch, FDA; and

11. Performing for-cause audits in response to legitimate patients concerns and complaints or information from outside sources.

The professional staff of the Regulatory Affairs Branch includes the following individuals:

Dale Shoemaker, Ph.D., Chief

Drug Regulatory Affairs Section -

Jay Greenblatt, Ph.D., Head

Maryellen Franko, Ph.D.

Jan Casadei, Ph.D.

Elizabeth Moore, R.Ph., M.S.

Quality Assurance and Compliance Section -

Dorothy Macfarlane, M.D., Head

Joan Mauer, B.S., M.T.

Gary Smith, M.G.A.

A summary of the activities for FY '91 includes:

1. Forty-one INDs for cytotoxic and biologic anticancer and antiAIDS agents were prepared and submitted to the Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research of the FDA.

2. The INDs for five agents were inactivated.

3. During CY '90 250 adverse drug reactions were reported to FDA. An additional 1217 adverse drug reactions were received, reviewed and held for reporting to FDA through the Annual Reports.

4. Seven special audits were carried out to confirm the data and response determinations in promising Phase II trials.

5. On-site audits were made to 15 Cancer Centers or other single institutions which are conducting trials with DCT-sponsored investigational agents.

6. Reviewed the reports from Cooperative Group on-site audits at 167 member institutions, 130 affiliates and 32 CCOPs (or CCOP components).

7. Meetings were held with the Division of Anti-Viral Drug Products of FDA to determine the preclinical data and the IND format required for the agents used to treat patients with AIDS and the preclinical data required to support proposed amendments to active clinical studies.

8. Reviewed approximately 500 protocols and informed consent forms for regulatory and NCI policy issues.

9. Meetings were held with the Center for Biologics Evaluation and Research to determine the preclinical safety testing of monoclonal antibodies prior to Phase I trials. As a result of these discussions a formal national meeting to discuss preclinical and clinical issues concerning monoclonal antibodies will be held this fall. The meeting will be co-sponsored by the FDA, NCI and NIAID.

DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY '91, an Investigational New Drug Application (IND) was submitted to the Center for Drug Evaluation and Research, Food and Drug Administration (FDA), for each of the following compounds:

Agent	NSC Number
Anthrapyrazole DuP 941	NSC 357885
Bryostatin I	NSC 339555
Chlorodeoxyadenosine	NSC 105104
Compound CAI	NSC 609974D
Cyclopentamyl Cytosine (CPE-C)	NSC 375575
DDI/AZT	NSC 612049/NSC 602670
Fostriecin	NSC 339638
4-HPR	Not Assigned
Hydrea IV	NSC 32065
ICI D1694	NSC 639186
Pyrazoloacridine	NSC 366140
Sulofenur	Not Assigned
Taxotere	NSC 628503D
Terephthalamidine	NSC 57155

INDs were submitted to the Center for Biologics Evaluation and Research, FDA, for the following agents:

Agent	NSC Number
Erythropoietin	NSC 628281
GM-CSF	Not Assigned
IFN-Gamma	NSC 635256
IL-1 Alpha	NSC 640032
IL-2	Not Assigned
IL-2/IL-4	NSC 600664/620201
IL-3	NSC 641115
IL-4	Not Assigned
IL-6	Not Assigned
M-CSF	NSC 635258
MoAb Anti-B4 Blocked Ricin	NSC 639185
MoAb CC83	Not Assigned
MoAb HD37-SMPT-dgA	NSC 639181
MoAb COL-1	NSC 624343
MoAb D612	NSC 64116
MoAb R24/GM-CSF	NSC 608918
MoAb 14.18 Chimeric	NSC 623408
MoAb 250	NSC 624786
MTP-PE	NSC 628280
PEG-L-Asparaginase (M)	NSC 624239
PEG-L-Asparaginase (K-H)	NSC 625239
TIL Cells Transduced with Neomycin Resistance Gene in Combination with IL-2 and IL-4	Not Assigned
TIL Cells Transduced with TNF Gene	Not Assigned
TNF	NSC 635257
Tumor Cells Transduced with IL-2 Gene	Not Assigned
Tumor Cells Transduced with TNF Gene	Not Assigned
Tumor Primed Anti-CD3 Activated Lymphocytes	NSC 618843

INDs Inactivated.

The INDs for the following agents were inactivated:

Agent	NSC Number
Heroin	IND 14,463
Interferon Beta	IND 2533
Monoclonal Antibody OVB-3 PE	IND 2688
Monoclonal Antibody 171A	IND 2176
Monoclonal Antibodies LiCo 16.88 and 28A32	IND 2387

The Regulatory Affairs Branch currently maintains 180 active INDs for both cytotoxic and biologic anticancer and antiAIDS agents.

Adverse Drug Reaction Reporting.

The Section is responsible for reporting adverse drug reactions to FDA. During CY '90 250 adverse drug reactions were reported to FDA. An additional 1217 ADRs were received and processed and held for the Annual Reports to FDA. A package outlining the reporting of adverse drug reactions was prepared and mailed to over 5,000 DCT investigators. The data from these reports are being entered into a data base on a personal computer.

Additional Activities.

Revisions were made to the internal procedures for adverse drug reactions (ADRs). Letters continue to be submitted to FDA with whatever summary information we have for ADRs reported by telephone or as a brief communication. A followup submission is made which contains detailed information on the event. This allows the CTEP to better meet the FDA's required reporting timeframes. All ADRs are prepared for review weekly by the Head of the Biologics Evaluation Section and the Head of the Developmental Chemotherapy Section, Investigational Drug Branch. Their review along with that of the Section is essential for determining trends, frequency, etc. Continuing discussions were held with CTEP staff to review suggestions on ways to streamline the ADR process.

Procedures are in place to systematically update Clinical Brochures, particularly for those agents just entering Phase II trial and for agents of particular interest. The revised Clinical Brochures are provided to all investigators currently using the particular agent.

Guidelines have been developed and implemented for the procedures to follow to provide investigational agents to foreign investigators. In addition, development continued on specific guidelines to be used by the CRC/EORTC which will be implemented through Dr. Yoder at the NCI Liaison Office in Brussels, Belgium.

Discussions were held with the CTEP staff, particularly with the Investigational Drug Branch, to determine the tasks to be carried out on the contract for the pharmacokinetic study of anticancer agents.

The Sections's staff continues to disseminate information and guidelines for the process validation and monitoring of TIL cell generation to all NCI investigators performing human studies with IL-2/LAK, IL-2/TIL and modifications of LAK and TIL cells, i.e., educated LAK and expanded lymph node cells.

The guidelines for the manufacture and testing of monoclonal antibodies were revised to reflect recent changes in FDA policy regarding the preclinical testing and validation of monoclonal antibodies. A monoclonal antibody

guideline was also established for monoclonal antibody contractors, companies and grant applicants. The guidelines provides recommendations on the information required to submit an IND for these agents.

The Section's staff reviews all new Biologic Response Modifiers Program monoclonal antibody contracts for compliance with FDA requirements. The staff also reviews contracts between investigators from the Division of Cancer Biology, Diagnosis and Centers and monoclonal antibody manufacturers.

A major activity of the Section has been to work with Dr. Anderson, Dr. Blaese and Dr. Rosenberg in obtaining FDA approval for clinical therapies involving retroviral gene insertion. To date the following INDs have been approved for treatments involving retroviral gene insertion:

1. "TIL Cells Transduced with the Gene Coding for Neomycin Resistance" for following the trafficking of TIL cells;

2. "T-Cells Transduced with the Gene Coding for Adenosine Deaminase" for the treatment of children with Severe Combined Immunodeficiency Disease; and

3. "TIL Cells Transduced with the Gene Coding for Tumor Necrosis Factor".

In addition, three INDs for intramural and extramural clinical studies are currently being prepared for other gene therapy protocols. These include:

1. "Tumor Cells Transduced with the Gene Coding for Interleukin-2";

2. "Tumor Cells Transduced with the Gene Coding for Tumor Necrosis Factor"; and

3. "TIL Cells Transduced with the Gene Coding for Neomycin Resistance in Combination with Interleukin-2 and Interleukin-4".

Section staff have worked closely with intramural and extramural investigators, pharmaceutical companies, contractors and the FDA to coordinate all facets of gene therapy submissions and to obtain FDA approval.

Procedures for providing preclinical and clinical data to pharmaceutical companies in the most timely manner continue to be implemented. ADRs are sent to the companies at the same time as they are submitted to FDA. Similar procedures are now in place for all protocols and protocol amendments approved by CTEP.

The Section's professional staff serves on the Developmental Therapeutics Program Quality Control Committee which reviews and approves certificates of analysis for all biologic and cytotoxic anticancer and antiAIDS agents sponsored by the Division of Cancer Treatment, NCI.

The Section's professional staff participated in numerous meetings with pharmaceutical companies to outline the Branch's operating procedures and explain its role in CTEP's drug development process. In addition, a document which outlines the roles to be carried out by CTEP and by the pharmaceutical company for co-development of an agent was developed and implemented.

Procedures for implementing Codevelopment Agreements with pharmaceutical companies have been developed and utilized.

QUALITY ASSURANCE AND COMPLIANCE SECTION

The Quality Assurance and Compliance Section is responsible for on-site monitoring of all clinical trials sponsored by the Division of Cancer Treatment. This includes all trials conducted by the Cooperative Groups, and studies conducted at Cancer Centers or other individual institutions which utilize DCT/NCI-sponsored investigational agents.

The Section is also responsible for setting guidelines and standards for the conduct of clinical trials in order to assure data quality and compliance with regulatory requirements for clinical research. The Protocol and Information Office (PIO) part of the Section is responsible for the administrative support of the protocol review process. It also maintains a record of each protocol sponsored by the DCT from the time it is submitted for review through publication of trial results.

Cooperative Group On-Site Monitoring.

In the case of the Cooperative Groups, DCT has delegated the responsibility for organizing and conducting the monitoring program to each group. Each institution is to be monitored at least once every three years. During the past year, the Cooperative Groups site visited 167 member institutions, 130 affiliates and 32 CCOPs (or CCOP components).

The Quality Assurance and Compliance Section continues to co-site visit with the Cooperative Groups in 10-20% of the scheduled visits to assure the adequacy of the audit procedures. In addition, the Cooperative Groups submit a report on each on-site audit to the Section for review and comment if deemed appropriate.

An audit results database for Cooperative Groups is maintained and includes results of all audits conducted since January 1985.

Phase I and Single Institution Study Monitoring.

The Quality Assurance and Compliance Section directly oversees the monitoring of Phase I and Cancer Center studies. Phase I studies are monitored three times per year. During the past year, 15 visits to Cancer Centers or other single institutions conducting trials with DCT/NCI-sponsored investigational agents were accomplished.

Additional Monitoring Activities.

Seven special audits were carried out to examine the data and verify response determinations in promising Phase II trials. These included: Lym-1 monoclonal antibody in lymphoma, Suramin in prostate cancer, Taxol in breast cancer, Tiazofurin in CML, DFMO in brain cancer, Taxol and Cisplatin in lung cancer and combination chemotherapy in melanoma.

Protocol and Information Office.

All protocols submitted to CTEP were reviewed by Section staff. Protocols are thoroughly reviewed for regulatory issues, some of which include: a standard ADR reporting section, referral to Common Toxicity Criteria, age restrictions, supplier of drugs, inclusion of multicenter guidelines, etc. Section staff are responsible for the review of the informed consent documents to ascertain that the document's contents are in compliance with federal regulations and accurately reflect the research protocol.

All amendments to DCT/NCI-sponsored clinical protocols are reviewed in-depth by Section staff. Informed consent documents are reviewed for Division of Cancer Prevention and Control-sponsored trials.

The New Drug Study Group application is included with the LOI approval letter for any institution wishing to do independent studies which is not an NCI-supported Cancer Center. Applications are reviewed and approved by Section staff in cooperation with Investigational Drug Branch staff.

Additional Activities.

Section staff are considered prime resources for dealing with and handling inquiries and problems by both intramural and extramural people in regards to FDA regulations and HHS Office of Protection from Research Risks (OPRR) regulations. Many inquiries deal with Institutional Review Board procedures, informed consent, and broad regulatory compliance issues.

Adverse drug reaction (ADR) reporting from Cooperative Groups and other investigators using DCT-sponsored investigational agents is monitored closely.

The Section staff completed quality assurance review of 77 patient cases entered on two Group C protocols for Fludarabine Phosphate in CLL and Pentostatin in HCL.

An electronic data collection mechanism was developed for obtaining quarterly accrual and demographic data from each DCT-sponsored Clinical Trials Cooperative Group.

Two for-cause audits were conducted.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1990 - September 30, 1991

The Clinical Oncology Program (COP) is the intramural treatment research arm of the National Cancer Institute. The Program, which is comprised of six Branches, conducts basic and clinical research in medicine, pediatrics, surgery, pharmacology, radiobiology, endocrinology, immunology, genetics and molecular biology in the context of developing curative therapies for cancer. A laboratory under the supervision of Dr. Samuel Broder operates under the auspices of the Office of the Associate Director (OAD). This Office also supports a Biostatistics Data Management Section, supervised by Dr. Seth Steinberg.

PROGRAM ACCOMPLISHMENTS

OFFICE OF THE ASSOCIATE DIRECTOR

Laboratory Investigator-Initiated Research Activities

The OAD virtually reprogrammed all its scholarly activities in response to the AIDS epidemic and to the Department's focus on AIDS as a number one priority. A portion of the investigator-initiated research activities in the laboratory of the OAD is summarized under the following heading:

Development of Anti-retroviral Agents for the Therapy of AIDS and Its Related Diseases:

AIDS and AIDS-related diseases are caused by the third known pathogenic human retrovirus, now called human immunodeficiency virus (HIV). During the past year, Dr. Broder's laboratory has continued to develop technology for the rapid detection of drugs or biologics which can suppress the replication of HIV in vitro. In addition, his group has continued its efforts to bring promising drugs to clinical trials as quickly as possible. At this time, it is possible to say that the general scientific perspective on the development of anti-retroviral drugs has changed: the question no longer is whether clinically active drugs can be developed for the treatment of AIDs, but how many agents will be found and how best to prioritize development of these agents and combination of agents.

A more complete discussion of various agents and therapeutic strategies will be taken up in the laboratory project report

section. In summary, Dr. Broder's laboratory has focused on the development of certain anti-retroviral nucleoside analogues, their biochemical pharmacology, and their application to the therapy of patients with AIDS and related disorders. In addition, certain targeted therapies designed to inhibit HIV binding to cells or to the suppression at the genomic level have been explored. Finally, a Retroviral Disease Fellowship has been initiated within the COP for individuals interested in pursuing laboratory and clinical research on HIV and other human retroviruses. This program will be integrated with that of the Medicine Branch. During the past year, the clinical activities of the laboratory have focused on several areas: (1) the continued development of antiviral therapies for HIV infection, (2) the study of and development of novel therapies for AIDS-related tumors including Kaposi's sarcoma and (3) the assessment of potential surrogate markers for clinical trials in AIDS.

One family of anti-retroviral nucleosides is referred to as dideoxynucleosides. The first in vitro assessment of these drugs against HIV was undertaken in this laboratory about seven years ago and has been discussed in previous annual reports. About six years ago, one member of this family, AZT, was used by COP for the first time to treat patients with chemotherapeutic agent approved for prescription status. Two other dideoxynucleosides developed in this laboratory, ddC and ddI, are now undergoing large-scale Phase II/III studies devised to assess their efficiency formally.

We are continuing to evaluate dideoxynucleosides as single agents, and in addition, are studying them as building blocks in combination regimens. Starting in February of 1988, we initiated a small clinical study of the purine analog 2',3'-dideoxyadenoxine (ddA), and later that year, we initiated a Phase I study of the related compound 2',3'-dideoxyinosine (ddI). Both ddA and ddI had been shown in our laboratory to have potent clinical activity against HIV infection in vitro but to have little toxicity against T cells. Both undergo anabolic phosphorylation in cells to ddA-5'-triphosphate, which is believed to be the active moiety at the level of reverse transcriptase. In addition, both drugs can be cleaved into dideoxyribose and the free base under acid conditions (such as are found in the stomach). However, while adenine, the free base of ddA, can cause renal damage, hypoxanthine, the free base of ddI, is a physiologic metabolite and is reasonably well handled by the body. For this reason, ddI seemed the preferred form for oral administration, and we have concentrated our clinical efforts on this compound.

The Phase I study of ddI conducted by our group demonstrated that at doses which were well tolerated, patients had increases in T4 cells and total lymphocytes, decreases in HIV p24 antigen (a measure of the viral load), and other evidence of immunologic, virologic, and clinical improvement. In the majority of patients, the increases in CD4 cells were sustained for at least

9-12 months. In addition, some patients had a reversal of HIV-dementia. At very high doses, the limiting toxicities of ddI were found to be painful peripheral neuropathy, occasional pancreatitis, and occasional hepatitis. Doses of 200 to 750 mg/day of ddI, however, are associated with activity but rare toxicity, and these are the doses being used in the Phase II/III trials.

As a result of this Phase I study (with supporting data from two other studies), three Phase II/III studies of ddI were initiated in October of 1990. In addition, ddI is being made available to patients who cannot tolerate AZT or who have deteriorating disease while on AZT under the regulatory mechanisms of a "treatment IND" and "Expanded Access Program." At the same time, we are continuing to investigate the clinical use of this drug, both alone and with other agents. We have found, for example, that the T4 rises on ddI are most consistent in patients who have previously received AZT for less than one year. Even patients with long-term prior AZT therapy, however, generally have virologic responses to ddI as measured by HIV p24 antigen. We have also observed that the survival of patients receiving ddI is quite good: overall the median survival of AIDS patients given ddI was approximately 26 months. This is quite striking considering that the median T4 count of the patients at entry was $35/mm^3$. It is substantially better than the 6 to 12 month median survival in untreated AIDs, and it suggests that the drug will be found to be efficacious in the controlled Phase II/III trials.

It is likely that the optimal therapy against HIV will involve combinations of drugs and agents. Last year, we initiated a study of a regimen of AZT with acyclovir, ddI and ddC in patients with AIDS or severe AIDS-related complex. These drugs have different toxicities, and this is one rationale for their combined use. In addition, there is evidence that HIV from patients on long-term AZT therapy which has become resistant to AZT preserves its sensitivity to ddI and ddC. Preliminary results from this study suggest that patients feel better, have increases in their T4 cells, and have decreases in HIV p24 antigen on the regimen. There is a suggestion that the CD4 increases on this regimen persist for approximately one year.

With the development of a variety of agents with anti-HIV activity, one of the principal tasks has been on how to prioritize the vast number of combination regimens. However, even the most basic questions in this area remain unanswered. For example, it is unclear whether it is best to employ simultaneous therapy with two dideoxynucleosides or to use them in an alternating fashion. To address this question, we have recently started a protocol to explore two pilot regimens: AZT alternating with ddI, and AZT given simultaneously with ddI. Patients are being randomized between the two regimens, and in addition to gaining experience with both approaches, it is possible that we may gain a sense as to which should be studied

further. Preliminary data from the trial, which started in the beginning of 1991, have revealed that patients have initial clinical and laboratory improvement on both regimens. Neither of the regimens is at this point clearly superior to the other.

We have recently been investigating the development of tumors in patients with AIDS or AIDS-related complex on long-term HIV therapy. We have recently observed that 8 of 55 patients on long-term AZT containing regimens developed non-Hodgkin's lymphomas. The most recent data from this group suggests that when assessed by the method of Kaplan and Meier, the chance of developing a non-Hodgkin's lymphoma is approximately 30% in patients with AIDS or severe ARC who are maintained on AZT-based therapy for 3 years. We believe that these most likely represent "opportunistic" lymphomas which have arisen as profoundly immunosuppressed patients remain alive longer. This situation is analogous to certain childhood immunodeficiency diseases such as Wiskott-Aldrich syndrome in which the cumulative incidence of lymphomas has increased as patients have remained alive longer. AIDS-related lymphomas are typically high grade, occur in extranodal sites, and are difficult to treat. In collaboration with Dr. Dwight Kaufman and members of the Medicine Branch, we are exploring a regimen of combination chemotherapy, AZT and GM-CSF for the treatment of AIDS-related non-Hodgkin's lymphomas.

Another area of interest has been the therapy of Kaposi's sarcoma (KS). This continues to be a major source of morbidity and mortality in patients with HIV infection. There is recent evidence that KS cells respond to a variety of growth factors, including fibroblast growth factor, interleukin-6, and an as yet undefined factor produced by HTLV-infected cells. In addition, KS cells produce a variety of cytokines which may lead to the typical KS lesions. There is evidence that the activity of fibroblast growth factor can be blocked by pentosan polysulfate, a polyanionic compound. In addition, pentosan polysulfate has been reported to have anti-HIV activity <u>in vitro</u>. Finally, we and others have found that this compound can inhibit the growth of a KS cell line <u>in vitro</u>. Based on those reports, we initiated a trial of pentosan polysulfate, administered by continuous infusion and then by subcutaneous injection, in patients with HIV-associated KS. We found no activity against HIV, as assessed by CD4 counts or p24 antigen. Also, we did not observe any complete or partial responses. However, some patients did appear to have minimal responses or stabilization of their disease, which may be the sort of tumor response one may see if one acts by blocking tumor growth factors. We plan to continue to study related agents which may block growth factors and/or may inhibit tumor angiogenesis. In particular, we have developed a Kaposi's sarcoma cell line, and are exploring this as a means of assessing anti-KS therapies.

One of the difficulties in assessing anti-retroviral therapies is that Phase II/III trials now rely on clinical endpoints such as mortality or the development of opportunistic infections. As a

result, such trials must involve large numbers of patients and continue for a long period of evaluation. In addition, patients must be followed until adverse events occur on what may be less optimal therapies. An alternative approach is to use surrogate endpoints. However, there is little information available on the relationship between potential surrogate endpoints (such as the CD4 count) and mortality in AIDS. We have examined the records from a cohort of 55 patients enrolled on our first 3 long-term studies of AZT-based therapies. So far, 44 of those patients are known to have died, and the approximate CD4 count was known proximal to the time of death in 41 of these patients. We found that all but one of these 41 patients had less than 50 CD4 cells/mm^3 at the time of their death. It was extremely unlikely that this would have happened by chance alone. Thus, a CD4 count of 50 or below may be a mortality risk indictator for patients on AZT-based therapy. In addition, these results suggested that maintaining the CD4 count above 50 cells/mm^3 might enable a substantial prolongation of life for HIV-infected patients.

Since our initial observation that AZT could at least partially reverse HIV-dementia in certain patients and the subsequent finding that monocyte-derived cells were important target cells for HIV infection, particularly in the brain, our laboratory has had an interest in studying the infection of monocytes by HIV. We have observed that dideoxynucleosides can effectively inhibit HIV replication in monocytes in spite of their poor phosphorylation in these cells; this apparently occurs because monocytes have very low levels of competing deoxynucleoside-5'-triphosphates. We had previously found that granulocyte-macrophage colony-stimulating factor (GM-CSF) can enhance HIV replication in monocytes but at the same time can potentiate the activity of AZT. This past year, we have extended this work to show that other cytokines may have different effects on either HIV replication or the activity of dideoxynucleosides. For example, macrophage colony-stimulating factor also enhances HIV replication, but does not enhance the activity of AZT. Thus, each potential cytokine must be studied separately. We are now conducting a clinical trial to explore the combination of AZT and GM-CSF in patients with HIV infection and neutropenia. In addition, we are now exploring the effects of cytokines, HIV, and anti-HIV drugs on the production of cytokines. Cytokines such as interleukin-6 may be one factor leading to the development of NHL in HIV-infected patients, and these studies may lead to a therapeutic strategy for reducing the incidence of NHL.

Logical extension of current approaches for therapy of HIV infection would be the use of combinations of multiple antiviral agents which have different antiretroviral mechanism(s). In the past 5 years, we and many other scientists focused on reverse transcriptase of HIV as a principal target; however, the HIV protease also represents a crucial virus-specific target for new therapies of AIDS. Such an approach is one way of inhibiting the production of mature, infectious virions in chronically HIV-

infected cells. Recently, antiretroviral peptide analogs have been synthesized based on the knowledge of physiology and structure of HIV-1 protease. Two-fold (C_2) symmetric protease inhibitors belong to one class of such protease inhibitors. Several (C_2) symmetric compounds have been shown to exert a potent activity against several strains of HIV-1 in vitro in the literature. We have now found that some C_2 symmetric compounds have been shown to exert a potent activity against various HIV-1 strains including monocytotropic HIV strains and AZT-resistant HIV variants in a variety of culture systems in collaboration with scientists in Abbott Laboratories. We have also found that such symmetric HIV protease inhibitors could synergize with other drugs in vitro. When several symmetric protease inhibitors were tested in combination with AZT or ddI against primary HIV isolates, their antiviral activities were synergistic in some cases and additive in others.

In other studies, a major effort is underway to develop lipophilic nucleoside analogues which may have improved central nervous system penetration as compared to the prototype drugs - as such, these compounds may be particularly useful in patients with HIV-related encephalopathy.

Emergence of drug-resistant variants must always be considered possible especially in the case of HIV, the highly mutable pathogenic retrovirus. Indeed, it was reported in 1989 that AZT-resistant variants of HIV-1 were isolated from patients with AIDS or ARC who had been under AZT treatment for more than 6 months. Several dideoxynucleosides including dideoxycytidine (ddC) and dideoxyinosine (ddI) have now been administered to HIV-infected individuals. It is possible that HIV can also develop resistance against these dideoxynucleosides. Attentions have now been focused on whether combination antiretroviral therapies can retard or inhibit development of drug-resistant HIV variants and whether resistance against a particular drug wanes if the drug is discontinued. We addressed these issues by using specimens from patients who received long-term therapy with dideoxynucleosides including AZT in NCI. We found that in 4 out of 5 patients receiving AZT/ddC for 15 - 41 months, viruses were highly resistant to AZT; the other patients isolated showed possible low level AZT resistance.

However, all these strains appeared to be sensitive to ddC and ddI. All HIV strains isolated from 6 patients who received ddI alone for 12 - 16 months appeared to be sensitive to ddI. Two of the 6 patients had received AZT for 2 - 3 months before receiving ddI therapy. These two patients had developed resistance to AZT (although they remained sensitive to ddI), and the AZT resistant HIV strains appeared to resume the sensitivity to AZT off AZT therapy. Our data suggest that HIV develops resistance to AZT more readily than to ddC or ddI, while these data do not provide basis for concluding that AZT-ddC or ddI are inferior, equivalent, or superior to AZT as therapy of AIDS.

It has been shown that quantitative analysis of HIV proviral DNA in cultured cells or clinical specimens is feasible by using polymerase chain reaction (PCR). We have recently established a rapid and simple method for determining the proviral DNA content in peripheral blood mononuclear cells (PBM) from patients with HIV-1 infection using PCR technique. Using this technology, we demonstrated that the amount of HIV proviral DNA in PBM from patients with AIDS or ARC significantly decreased after initiation of antiviral therapy with dideoxyinosine (ddI or didanosine). However, the amounts of proviral DNA in PBM do not necessarily reflect the viral load in patients and it is possible that HIV is expressed in PBM only to a limited extent. Therefore, we asked where HIV most infected its target cells and HIV mRNA is mostly expressed by employing various tissues at biopsy or autopsy of HIV-infected individuals. We then found that the lymph node was at least one of the main sites for active replication of HIV-1 in patients with AIDS or ARC. We also found that HIV-1 replicates at a greater level in lymphoid organs, in particular in lymph nodes, as compared to peripheral blood mononuclear cells. This suggests that PBM may not be a good source for viral load quantitation.

We then turned our attention to quantifying virus particles in circulation using RNA-PCR, and established a methodology to quantitate HIV-1 virion particles (VP) in plasma of HIV-1 infected individuals and assessed its potential to monitor the changes of viral load in patients with AIDS or ARC during antiretroviral therapy against HIV infection. Our plasma RNA PCR technique could detect HIV RNA in plasma in all HIV infected individuals at varying clinical stages (59/59), while ELISA failed to detect p24 Ag in 29 and 19 of 46 plasma samples with and without acid-pretreatment, respectively ($p<0.0002$). We also found that the number of HIV-1, VP in plasma was markedly higher in patients with AIDS or ARC than in asymptomatic patients ($p<0.01$). When we monitored the changes in the viral particle numbers in plasma in patients with AIDS or ARC who received ddI therapy, there was a significant decrease in patients up to 70 weeks ($p=0.02$). The plasma HIV VP numbers determined by our plasma RNA PCR technique may provide information on the plasma viremia status of patients with HIV-1 infection. However, more research is required to evaluate the utility of this technique in monitoring the changes of viral load in patients receiving antiretroviral treatment.

Infection with HIV is characterized by a period of clinical latency associated with low to undetectable expression of the virus. There is an early acute phase of infection with viremia, however, after the first month of infection, a stage of a low level viremia with few infected cells detected in peripheral blood is followed. The mechanisms responsible for maintenance of this apparent viral latency have been as yet poorly understood. If specific events in the infected cells that lead to activation of the latent state into a productive one are identified, it might be possible to intervene the progression into the state of

immune deficiency in patients with HIV infection. Several factors have been implicated in the activation of HIV expression. These include immune activation, cytokine-mediated viral induction, transactivation by certain DNA viruses, heat shock, and irradiation of infected cells with ultraviolet or x-ray. Another possible mechanism of activation of HIV expression could be demethylation of proviral DNA sequence in a cell. In order to demethylate DNA of HIV-infected cells, 5-azacytidine (AZC) and its analog were employed. A persistently HIV-infected T cell line, ACH2, produced a low level of HIV in vitro. However, following exposure to AZC or its analog, a profound potentiation of viral production occurred as assessed by production of envelope glycoprotein, reverse transcriptase, p24 Gag protein and reverse transcriptase. Alteration in the DNA methylation pattern in ACH2 was confirmed by Southern blot hybridization using the isoschizomer restriction enzymes Msp I and Hpa II and an HIV-specific probe. AZC-induced potentiation of viral expression did not occur in any of the other chronically infected cells that produce high levels of HIV-1. AZC is, by its own right, a toxic agent. We further found that the toxicity of AZC per se was, at least in part, associated with the observed enhancement of HIV expression in ACH2 cells. Indeed, it was subsequently found that certain other toxic substances including methotrexate, 5-fluorouracil, vinca alkaloids can also potentiate the expression of HIV-1 in ACH2 cells. These data suggest that DNA methylation/demethylation may play a role in regulating HIV expression at least under certain circumstances, although its relevance to in vivo events remains to be further investigated.

In other studies, we have found that certain dideoxynucleosides can inhibit the replication of hepatitis B virus (HBV) in a human hepatocytic cell line that chronically produces infectious HBV virions in vitro. HBV causes acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The latter complication causes over one million deaths worldwide every year. Among dideoxynucleoside analogues we tested, dideoxyguanosine (ddG) was the most potent agent diminishing viral replication by as much as 95% as assessed by the amount of episomal HBV DNA without impairing cellular growth. AZT was least effective against HBV; Northern blot analysis revealed no apparent difference in the pregenomic viral RNA profile, suggesting that these dideoxynucleosides suppress reverse transcription in the replicative cycle of HBV. These data suggest that some 2',3'-dideoxynucleosides can exert a potent antiviral activity against HBV in vitro at least under certain circumstances. Because HBV infection has become an increasingly serious problem among gay men and intravenous drug abusers, many of whom are co-infected with HIV-1, these data may have direct clinical implication in treating such superinfected individuals with dideoxynucleoside analogues.

CLINICAL PHARMACOLOGY BRANCH

This year represents the first full year for the reformulated Clinical Pharmacology Branch.

1. Major Personnel Changes:

Recruitments were made of Ingrid Jordan as Branch Secretary, Janet Amber as Editorial Assistant, and Holly Hartz as Clerk-Typist.

In addition to the founding members of CPB, we have been joined by Oliver Sartor, M.D., and Dvorit Samid, Ph.D.

2. Major Clinical Advances:

--Previous studies with suramin had maintained blood levels of 275-300 μg/ml for only a few days. We have now demonstrated that this blood level is tolerated for four weeks. We have also developed a three compartment Bayesian model which predicts blood levels with considerable accuracy (Cooper).

--Preclinical studies have indicated considerable synergy between suramin and alkylating agents or doxorubicin (Sinha). A randomized clinical trial comparing suramin plus doxorubicin with suramin plus thiotepa has been initiated (Sausville).

--Preclinical studies have shown synergy between suramin and somatostatin analogs. A clinical trial testing this concept has been approved and is awaiting final negotiations with the drug company (Sartor).

--Patients with untreated metastatic prostatic cancer are in either hormone-responsive or hormone-unresponsive populations. The tumor-responsive population may be treated with androgen withdrawal, which is currently standard treatment for this disease. Suramin has demonstrated activity against hormone-unresponsive tumor populations and could potentially improve response rates and response duration in these patients. A clinical trial testing this idea by combining suramin with leuprolide and flutamide is in progress (Myers).

3. Major Laboratory Advances of Clinical Import:

--Carcinoma of the Prostate:

Antitumor activity of potential clinical value has been found for receptor agonists (Trepel), nonhydrolyzable ATP analogs (Trepel), cAMP analogs (Trepel), the quinoid ansamycin antibiotics (Trepel), and phenylacetate (Samid). Preclinical evaluation of these agents and mechanism of action studies are proceeding. Naturally occurring heparin sulfates have been isolated which also suppress the growth of prostate carcinoma (Cooper and Ranson). Phenylacetate was found to have considerable activity

against prostate carcinoma in vitro at levels already achieved in infants with urea cycle abnormalities

--Carcinoma of the Lung:

Behring Werke sent us a semisynthetic isoflavone that inhibited tyrosine kinases in cell-free systems. In lung cancer, this agent appears to have another mechanism of action. Nevertheless, it was a potent agent against a wide range of lung carcinoma cell lines in vitro. It also showed activity against human lung carcinoma lines in nude mice when administered orally or IV. We are rushing preclinical development of this compound as fast as possible (Sausville).

--Carcinoma of the Stomach:

As part of a broad search for other tumors responsive to suramin, we have found that carcinoma of the stomach is uniformly sensitive to suramin with IC_{50}'s below 200 μg/ml. In collaboration with Dr. Allegra of the Medicine Branch, we are now actively seeking patients with this tumor for suramin treatment.

4. Basic Science:

The basic science which led to these advances are outlined below:

Prostate Carcinoma:

--cAMP causes terminal differentiation in prostate carcinoma cell lines. This process is associated with release of active TGF-beta 2.

--Purinergic type 2 agonists trigger terminal differentiation in prostate carcinoma cell lines.

--Prostate carcinoma cells express many markers of neuroendocrine differentiation, including dense core granules, neuron-specific enolase, S100 and neurofilament proteins.

--Prostate carcinoma cells express high levels of $pp60^{c\text{-}src}$ and have high levels of *src* kinase expression.

Small Cell Carcinoma of the Lung:

--Nine of 12 SCLC lines, but only four of 14 non-SCLC lines, are sensitive to growth inhibition by cholera toxin. Expression of ganglioside G_{M1} predicts for cholera toxin sensitivity in SCLC, but not in non-SCLC.

HTLV-1-associated ATLL:

--Conversion of IL-2-dependent ATLL to IL-2-independent ATLL is associated with a switch from $p56^{lck}$ to $p56^{lyn}$.

Miscellaneous:

--The benzoquinoid ansamycin antibiotics, geldanamycin and herbimycin A, are very cytotoxic against neuroectoderm-derived tumors and tumors with neuroendocrine properties, including medulloblastomas, neuroepitheliomas, colon carcinoma, melanoma, and prostatic carcinoma.

MEDICINE BRANCH

During the past year clinical activities in the Medicine Branch remained largely targeted on four general disease areas: breast cancer, colorectal cancer, lymphoma, and ovarian cancer. Approaches were focused on the following themes: (1) modulation of the activity of 5FU in gastrointestinal cancer; (2) the effect of high-dose or dose-intense therapy in a variety of tumor types, including breast cancer, ovarian cancer, and lymphoma; (3) modulation of multidrug resistance; (4) early clinical trials of new agents. Selected findings and activities are described briefly below. In collaboration with investigators in the BRMP and the ROB we began planning a new generation of lymphoma studies that are expected to see activation in early FY 92. In addition to these ongoing activities, several new areas will receive active attention in the coming months. In collaboration with the Surgery Branch and the ROB pilot multimodality studies in esophageal, gastric, and pancreatic cancers have been designed and are about to be implemented. In late FY 91 the laboratories of H. Mitsuya and the laboratory and clinical activities of R. Yarchoan were incorporated into the Medicine Branch; these activities in the HIV area will see significant expansion as a result of the availability of additional space in the newly constructed A-wing of Bldg 10.

NOTEWORTHY CLINICAL RESULTS

1. **Interferon can be combined with 5FU and leucovorin** in the treatment of patients with large bowel cancer with acceptable toxicity. The response rate in previously untreated patients is about 45%. Pharmacokinetic studies show that the use of interferon in this manner increases total exposure to 5FU by about 1.3-fold. It is not yet clear whether this response rate is higher than one might expect from FU-leucovorin or FU-interferon. A separate trial is examining the possibility that administration of hematopoietic colony-stimulating factors may permit a further increase in the administered dose intensity of 5FU.

2. Clinical trials in the use of **modulating agents to reverse drug resistance** are an area of significant activity. In lymphoma, a regimen (EPOCH) based on continuous infusion of three natural products (doxorubicin, vincristine, etoposide) along with prednisone and cyclophosphamide has produced high response rates in a group of heavily pretreated patients with drug-resistant disease. Addition of R-verapamil to EPOCH at the time of

cessation of further response on EPOCH alone has resulted in further significant tumor shrinkage in approximately 4 of 11 patients. In a number of patients in whom serial tumor biopsies were available in the course of therapy, levels of tumor-associated P-glycoprotein appeared to rise dramatically with progression of disease. Additional studies in resistance reversal are being considered for several other tumor types, including breast cancer, renal cell carcinoma, adrenocortical carcinoma, and acute myelocytic leukemia.

3. Studies of **dose-intense chemotherapy regimens** are proceeding in several tumor types; the aim in all is to deliver maximum tolerated doses of drug, with or without the aid of myeloid growth factors (G-CSF or GM-CSF) to minimize the duration and depth of neutropenia. Examples of ongoing studies of this approach include: (a) FLAC + GM-CSF in advanced breast cancer. This regimen has produced high response rates in a large cohort of women and, in the subset of patients with locally advanced disease only, a pathologic CR rate of about 30%. This regimen is now being studied in patients with Stage II disease. (b) Doxorubicin + taxol + G-CSF in advanced breast cancer (Phase I). (c) Piroxantrone + G-CSF in breast cancer (Phase I). (d) Carboplatin + GM-CSF in refractory ovarian cancer. This study, now completed, established an MTD of 800 mg/m^2 in association with GM-CSF and yielded a higher response rate than would be expected for conventional doses in this population. (e) Taxol + G-CSF in refractory ovarian cancer. Data thus far has established that substantially higher doses of taxol (250 mg/m^2 every 3 weeks) can be given on time with G-CSF than without the growth factor; the study is now trying to determine whether the therapeutic effect is a function of dose. (f) ProMACE-CytaBOM (short-course) in previously untreated patients with intermediate and aggressive non-Hodgkin's lymphoma. Results thus far have clearly established that the drugs in this regimen can be given in a more dose-intense fashion than with previous scheduling of this regimen. (g) 5FU + leucovorin + GM-CSF in GI malignancies. (h) ICE (ifosfamide, carboplatin, etoposide) with autologous bone marrow reinfusion in patients with lymphoma and selected carcinomas. A phase I trial of this combination has established a safe level for further study. Future efforts will focus on further modulation of toxicity with the addition of interleukin-1 to chemotherapy.

4. **Early clinical trials of new agents** during the past year have included (a) Tetraplatin, an analog of cisplatin that demonstrates the absence of cross resistance in in vitro models; phase I study with this agent is currently in progress. (b) Pentosan for epidemic Kaposi's sarcoma; treatment was well tolerated but yielded no evidence of significant antitumor effect. (c) Fazarabine in breast cancer (phase II). Preliminary results show some antitumor activity and very good patient tolerance.

Plans for the coming year include studies with a diversity of novel structures, including (a) the incorporation of interleukin-3 and interleukin-1 into combination chemotherapy for breast cancer and the ICE autologous bone-marrow transplant regimen for lymphoma, respectively. (b) a phase I study of ^{177}Lu (a beta emitter) conjugated to the murine antibody CC49 in breast cancer. (c) a phase I study of ^{131}I conjugated to murine COL1 in colorectal cancer (both radiolabeled antibody studies will be collaborations between Medicine Branch staff and Drs. Jeffrey Schlom, Tom Goffman, and Jorge Carasquillo. (d) Cyclopentenyl cytosine (CPEC), an inhibitor of cytidylate synthase (phase I). (e) D1694, a folate-analog inhibitor of thymidylate synthase. (f) CAI, a signal transduction inhibitor that inhibits the growth of in vivo tumor models.

LABORATORY STUDIES

1. **Thymidylate synthase (TS)** as a chemotherapeutic target. TS, the major target enzyme of the antimetabolite 5FU, is induced in cells exposed to 5FU; control of this induction is exerted at the level of translation and is modulated by direct interaction of TS with its mRNA. Exposure to interferon blocks the induction of TS by 5FU and results in increased sensitivity to the fluoropyrimidine; this interaction may be exploitable clinically (see below).

Techniques for quantitation of TS in cells and tissue sections have been developed using monoclonal antibodies against TS epitopes. Studies in collaboration with the National Surgical Adjuvant Breast and Bowel Project are attempting to evaluate tissue expression of TS in clinical specimens as a potential prognostic factor and predictor of response to therapy.

2. **Leucovorin enhancement of 5FU cytotoxicity.** Studies examining the determinants of leucovorin efficacy in enhancing the cytotoxicity of 5FU suggest that the duration of exposure of cell lines to leucovorin may be a major factor. Conversion of leucovorin to 5,10 methylene THFA is both time and dose-dependent, but polyglutamylation is principally dependent on time rather than dose.

3. **Dihydropteroate synthase (DHPS)** of Toxoplasma gondii and Pneumocystis carinii is a potentially important target for drug development aimed at better drugs for opportunistic infections in immunosuppressed hosts. Tests using purified T. gondii enzyme have revealed that many sulfone derivatives are potent (IC_{50}<1mM) inhibitors. Efforts are now concentrating on cloning and expressing the P. carinii DHPS with a view toward screening potential inhibitors.

4. **Folate Binding Proteins.** Studies focusing on the biochemistry and molecular biology of human folate binding proteins have resulted in the cloning and characterization of a second member

of what now appears to be a gene family; the coding region of this new, "placental" form of the FBP is 70% homologous to a previously characterized FBP from human KB cells. The exon-intron structure of the coding regions and the sequences of the putative regulatory regions of these genes have been characterized from genomic clones; the overall organization appears very close to the placental gene.

In human there exist both membrane-bound (M-FBP) and soluble extracellular (S-FBP) forms of the folate binding proteins; these are related to each other in a precursor-product fashion. Hydrolysis of the M-FBP to the S-FBP is accomplished by a membrane-bound metalloprotease. Recent work suggests that this conversion involves cleavage of a hydrophobic region at the carboxyl end of the protein, which may serve to anchor the protein in the cell membrane.

5. **Methotrexate Resistance.** In nine methotrexate-resistant KB cell clones grown in the presence of physiological concentrations of folate, analysis of possible resistance mechanisms shows that 9/9 have transport defects; in association with this, these cells exhibited a significant decrease in expression of the FBP (5-70% of wild-type KB). Four of the nine had increases in DHFR activity (1.5-9 fold higher than WT). Sequence analysis of the FBP from each of these lines is in progress. Studies in these mutant lines have also identified a 50 kD membrane-associated protein that is overexpressed in several of these lines; in wt cells folate depletion increases expression and repletion decreases expression. Efforts are underway to clone and characterize this protein, which may have a role in normal folate homeostasis.

6. **Multidrug resistance.** An exhaustive analysis of the primary sequences of p-glycoprotein (the product of the *mdr1* gene) from over 100 sources has shown that the appearance of mutant sequences is a very rare event in the course of selection. In addition, work in colon cancer cell lines selected for resistance has revealed genetic polymorphism at this locus. In the course of selection, overexpression of one or both alleles may occur in association with amplification of an individual allele as resistance evolves.

Differentiating agents such as butyrate can affect the multidrug-resistance phenotype of cells; curiously this effect involves a loss of p-glycoprotein function while levels of the protein are increasing. This effect correlates with progressive declines in the level of Pgp phosphorylation. In related studies, 8-Cl-cAMP downregulates expression of mdr1 mRNA and PgP expression and increases drug accumulation.

The glutathione S-transferase-p enzyme appears to be a marker of drug resistance in breast cancer cells; its content varies inversely with cellular content of estrogen receptor, and preliminary analysis suggests that in patients with node-negative breast cancer, expression of GST-p is associated with a poorer

prognosis. However, increased expression of GST-a, m, or p, at least at the levels of expression achieved in transfection studies, is insufficient by itself to confer the multidrug resistance phenotype on cells.

Co-transfection studies suggest that elevated levels of protein kinase C may increase drug resistance in cells expressing *mdr1*; this increase is associated with increased phosphorylation of P-glycoprotein.

A breast cancer cell line that exhibits >3000 fold resistance to mitoxantrone and enhanced efflux and decreased accumulation of drug shows only low-level resistance to doxorubicin and etoposide and does not express mdr1 but does show membrane material that cross reacts with polyclonal antibody against conserved regions of p-glycoprotein.

7. **Platinum Resistance.** Work here and elsewhere has suggested that cellular resistance to platinum compounds may be mediated by a variety of mechanisms. Recent studies here have focused largely on the importance of repair of platinum-DNA adducts as a clinically relevant determinant of resistance. Data suggest a correlation between expression of members of the ERCC gene family and response to platinum-based therapy. Of great interest are data suggesting that the extent of platinum-DNA damage in circulating leukocytes may correlate with the drug's antitumor effect; this suggests that major determinants of platinum resistance are not confined to tumor tissue.

In separate studies, the selection of a number of platinum-resistant cell lines and sensitive revertants has revealed a 55 kD protein as a correlate of the resistant phenotype; this protein is currently being purified and sequenced.

8. **Transcriptional Regulation of the *c-myc* Oncogene.** Productive efforts to identify and characterize downstream recognition sequences within Intron I and corresponding trans-acting factors have now identified a cluster of four cis elements that bind nuclear proteins (MIF 1-4). MIF-3 is a strong negative regulator of the *c-myc* promotor. Cells from patients with Burkitt's lymphoma frequently have mutations in the recognition sequences for MIF1-4. Homology exists between the MIF-1,2, and 3 sites and also between the MIF-3 and 4 sites. Phosphorylation is apparently important for at least some of these interactions, since phosphorylation of a serine residue on MIF-1 is required for binding with its recognition sequence. A more detailed understanding of these interactions may enable the construction of synthetic peptides or other reagents to interfere with *c-myc* expression.

It also appears that exposure of cells to certain differentiating agents, such as retinoic acid, TPA, and DMSO, results in dramatic alteration of complex formation between nuclear proteins and c-myc DNA. It is not yet clear whether this effect represents a

change in activity of binding proteins already present or the induction of new proteins. It has been shown, however, that undifferentiated HL60 cells contain a nuclear protein distinct from MIF-1 that binds to the MIF-1 recognition sequence, whereas MIF-1 appears upon induction of differentiation. Since it is known that c-myc expression varies with the state of differentiation (induction of differentiation is typically accompanied by downregulation of c-myc expression), understanding the mechanism of c-myc regulation under these circumstances is potentially of therapeutic significance.

9. **Cytogenetics.** Chromosomal abnormalities in cell lines and fresh tissue from patients with non-small cell lung cancer. A wide variety of structural abnormalities were found; these were non-randomly distributed among a number of chromosomes and, within chromosomes, favored certain sites (3p14.2, 3q21, 19q13, and 11p15, among others). Similar studies in esophageal cancer have documented the existence of chromosomal abnormalities in virtually every chromosome examined. These studies provide important information to guide the search for genes responsible for the malignant state.

NCI-NAVY MEDICAL ONCOLOGY BRANCH 1991

Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

Chromosomal aberrations are more likely to occur in chromatin regions that are "open," active, and accessible. This concept has been the foundation of a successful program of gene identification and characterization within our laboratory. This strategy has led us to the discovery of four interesting and important human genes. Three of these genes are members of the basic domain-helix-loop-helix (bHLH) family of transcription factors, a family known to act in nodal points of tissue specific developmental processes. One of these genes, SCL, appears to play a role in early hematopoietic development, the other two are more likely to be active in early human nervous system development. We have also identified a gene, SIL, which may be the first known tissue specific topoisomerase, and which forms a fusion message with SCL subsequent to an interstitial deletion of chromosome 1 in approximately 20% of children with T-cell acute lymphoblastic leukemia. (Kirsch, Bertness, Nakahara, Aplan, Lipkowitz, Bier, Moghadam, Tchorz).

Gene Rearrangements as Tumor Specific Markers

Structural alterations and expression of immunoglobulin (Ig), T-cell receptor (TCR) and various growth affecting genes are studies in normal, "premalignant," and malignant tumors and cell lines.

A. We have shown that hybrid genes are formed by site specific recombination between variable segments from one immune receptor locus and joining segments from another. We have demonstrated that such events occur in the peripheral T-cells of all normal individuals but are 100 times more frequent in the peripheral T-cells of patients with ataxia-telangiectasia (AT). These hybrid genes 1) affect and alter the repertoire of immune receptor diversity, 2) suggest that an underlying defect in AT may be chromatin "hyperaccessibility," and 3) provide a possible screening test for people at an increased risk for the development of lymphoid specific chromosomal translocations, and therefore lymphoid malignancy. We have recently completed a pilot study of individuals involved in the agriculture industry in which we have demonstrated an acquired transient "AT-like" picture in individuals exposed to a variety of pesticides and herbicides. These individuals are the same population for which epidemiological studies have suggested an increased risk of leukemia and lymphoma.

B. We have identified a gene, SCL, involved in a nodal point in hematopoietic development. In collaboration with the Children's Cancer Study Group (CCSG) and the Southwest Oncology Group (SWOG) we have used the SCL probe in tumor genotyping studies on patients with lymphoid disorders and found SCL disruption to occur in 20-30% of childhood T-cell ALL pronounced SCL expression in M7 AML and CD34+ CML blast crisis.

Gene and transcript mapping. We have localized numerous genes of interest to specific regions of human chromosomes. Most recently using biotinylated probes we have mapped a putative neurogenic gene to human chromosomes 1q21. Furthermore, we are using RNA tissue in situ hybridization as a means of detecting transcripts of interest in individual cells. We are also engaged in a protocol to assess the utility of an SCL based PCR assay to determine and follow minimal residual disease in a subset of CCSG patients. (Kirsch, Aplan, Lipkowitz, Lombardi, Bier, Seibel and Nakahara).

Clinically Relevant Immunohistochemical Markers in Lung Cancer

Our goal is to define immunohistochemical markers that will best type lung cancer for diagnosis, prognosis, and selection of therapy. Small cell lung cancer (SCLC), characterized by neuroendocrine (NE) features, is responsive to chemo- and radiotherapy. Some non-SCLC also express NE features. The hypothesis is that these tumors might be more responsive to cytotoxic treatment than other non-SCLC.

A. Characterization of markers. In a retrospective study a comprehensive group of 113 lung cancers were tested for the immunohistochemical expression of 17 antigens using a sensitive avidin-biotin-peroxidase technique. Logistic regression analysis was used to separate tumors into the proper categories (SCLC and carcinoid tumors versus NSCLC) based on the

immunohistochemical markers. As a result 95% of the tumors were correctly predicted using the cell counts and staining intensities of only six markers. The results suggested that 1) individual marker counts are not useful in tumor classification, 2) "specific" NE markers such as serotonin and neuropeptides bombesin, calcitonin, ACTH, vasopression, neurotensin are not useful, 3) the best NE markers are a panel of "general" NE markers (Chromogranin A, Leu 7, NSE) which are present in NE cells throughout the body.

B. Clinicopathologic correlation. This panel of "general" NE markers was applied to the non-SCLC cases on protocol 83-15 in our branch. Although the numbers were small, the response rate to chemotherapy was 50% (4/8) in the patients whose tumors were positive for NE markers versus 16% (6/38) in those with negative NE markers. Moreover, patients with NE positive tumors developed metastases significantly earlier ($p2 < 0.027$). The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. Immunohistochemistry provides a highly effective and specific technique to achieve this goal. (Linnoila, Mulshine and Gazdar).

Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation.

Cellular differentiation is a complex process for which the molecular mechanisms are poorly understood. How changes in growth potential are related to expression of the differentiated phenotype is at present unknown. We have focused our attention on questions such as the role of oncogenes in the differentiation process of murine erythroleukemia (MEL) and F9 teratocarcinoma cell lines. We were able to demonstrate that in both cell lines, high levels of expression of a transfected c-myc gene blocks HMBA, DMSO or Retinoic Acid (RA) induced differentiation.

Based on these findings and the published reports on the homology between C, N, and L-myc protooncogenes, we investigated the ability of the related L- and N-myc genes to substitute for c-myc in blocking MEL differentiation. Our results clearly indicate that constitutive high levels of transfected L- and N-myc mRNAs block inducer-mediated differentiation. These studies strongly suggest that down regulation of c-myc expression in MEL cells is a necessary event for terminal differentiation. We used a number of deletion mutants of the human c-myc gene for mapping the regions responsible for its apparent critical role in MEL and F-9 teratocarcinoma cell differentiation. In MEL cells, our results suggest that the first 40 amino acids of the c-myc protein are dispensable for blocking differentiation, the other domains of the protein are necessary for this function. In addition, we are developing a new approach for identifying proteins that interact with the c-myc protein during differentiation. (Segal, Bar-Ner, Cultraro and Dunn in collaboration with D. Segal, NCI).

Treatment of Extensive Stage Small Cell Lung Cancer

Although a dose-response curve clearly exists for alkylating agents in the initial chemotherapy of small cell lung cancer, the therapeutic benefit of higher than standard doses of the more recently introduced regimen of etoposide/cisplatin (VP16/PLAT) is uncertain. We randomized at least partially ambulatory patients with extensive stage SCLC and without major organ dysfunction to receive either VP16 80 mg/m squared + PLAT 27 mg/m squared Days 1-5 q 3wks or VP16 80 mg/m squared Days 1-3 + PLAT 80 mg/m squared Day 1 q 3 wks for the first 6 wks of therapy. Nonambulatory patients and those with organ dysfunction were assigned standard dose treatment. All patients received the standard-dose regimen during wks 7-12. From wks 13-24, patients in complete response (CR) continued standard-dose VP16/PLAT, while all other patients received a new 3-drug regimen that led to further improvement in response in only 5 cases. CR's were given prophylactic cranial irradiation. One hundred and eight patients have been entered (88 of whom were randomized). With a median follow-up of 55 mos, preliminary results are:

	N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt
High	40	25%	85%	12 mos	1,600	53,000
Standard	43	21%	81%	11 mos	2,500	161,000
Nonrand	25	4%	72%	6 mos	1,800	89,000

CR rates (p=0.86) and survival (p=0.93) were similar in patients randomized to high- and standard-dose therapy. There were 2 treatment-related deaths in the high and one in the standard dose arm. We conclude 1) standard-dose VP16/PLAT is at least as active as any regimen we have ever utilized for extensive stage SCLC and produces only modest myelotoxicity, and 2) there is no evidence of superior efficacy when planned drug doses are increased by 67% during the first 6 wks. (Ihde, Gazdar, Linnoila, Phares, Minna, Oie and Russell).

Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects.

We have chosen oncogenes to explore the biologic and biochemical functions of 2 dominant (L-myc and c-jun). Transcriptional and translational products of L-myc have been characterized and are now being correlated with biologic functions. Ultimately, truncated fragments of this gene will be tested for potential transformation suppression function.

Likewise, we have recently described the transforming function of c-jun in mammalian cells and are now mapping this function by deletion mutation. Correlation of this function with other known activities of c-jun, such as transactivation will be done. Mutants of c-jun capable of inhibiting AP-1 transactivation and cellular transformation will be characterized. (Birrer, Brown, Szabo, Sanders and Preis).

Etiology of Cutaneous T-cell Lymphomas

The cutaneous T-cell lymphomas (Mycosis Fungoides and the Sezary Syndrome) with early stage skin lesions may comprise a polyclonal rather than a monoclonal population. It is currently unclear whether the disease arises from an event in a T-cell precursor, or whether it arises out of a T-cell response to an event, or possibly a viral infection, in an accessory cell. These early stage skin infiltrations are currently studied with PCR amplification and sequencing of T-cell receptor rearrangements in the skin. p53 mutations have been detected in several patients with advanced stage disease suggesting a role for this tumor suppressor gene. We are exploring the possibility that a retrovirus may be implicated in the pathogenesis of this disease by studying patient materials for retroviral-like sequences and by culturing cells from patients and attempting to isolate retroviral activity. In addition, we have studied response to growth factors and cytotoxic activities of a number of pharmacologic agents in MF cells and in Hut 78, an MF cell line, in an attempt to derive new therapies for patients. (Foss, Kuehl, in collaboration with Dr. Gallo and Dr. Sausville, NCI).

Molecular Genetic Events in Lung Cancer

A. We have recently reported atrial natriuretic factor mRNA expression and immunoreactivity in tumor and tumor cell lines from small cell lung cancer patients with hyponatremia who did not produce arginine vasopressin. High pressure liquid chromoatography (HPLC) analyses of the tumor cell lines and tumors from patients with hyponatremia and mRNA expression of atrial natriuretic factor have revealed that intracellular and extracellular peptide appears to be the 28 amino acid form of atrial natriuretic peptide, the form that normally circulates in human plasma. These studies are the first to characterize the ectopic production of atrial natriuretic peptide in small cell lung cancer patients and may have identified the third factor (natriuretic factor) that has been hypothesized in the syndrome of inappropriate antidiuretic hormone (SIADH). The receptor for atrial natriuretic factor is also present on small cell lung cancer cells and the cells respond to exogenously added atrial natriuretic factor with an increase in intracellular cGMP, similar to the normal receptors on vascular smooth muscle cells. Therefore, there appear to be functional ANF receptors on the surface of small cell lung cancer cells.

B. We reviewed the clinical course of 234 lung cancer patients. In contrast to none of the 123 non-small cell lung cancer (NSCLC) patients, 18 of 111 (16%) small cell lung cancer patients had hyponatremia. Ten of these 18 had tumor cell lines available and 8 expressed ANF mRNA, 8 expressed AVP mRNA, and 6 of 10 cell lines produced both ANF and AVP mR NA. All of the 10 cell lines produced ANF mRNA, AVP mRNA, or both. Studies of 10 tumor cell lines from the 93 SCLC patients without hyponatremia showed 9 produced ANF mRNA and one produced AVP mRNA. From these studies

we have observed that all tumor cell lines studied from SCLC patients with hyponatremia produce ANF mRNA or AVP mRNA, or both. Atrial natriuretic peptide may be the previously hypothesized third factor and play an important role in the pathogenesis of hyponatremia in some patients with SIADH. (Johnson, Gazdar, Ihde, Mulshine, Ohsaki and Richardson).

In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

A protocol combining twice-a-day radiotherapy plus VP 16 and cisplatin for limited-stage small cell lung cancer continues. Thirty-eight patients have been entered onto study and 28 of 36 (78%) patients who have completed therapy have achieved a complete remission. The projected median survival is 30 months with a median potential follow-up of 27 months. One patient has died from combined modality pneumonitis.

A phase I trial using dihydrolenperone, an agent identified as being active against human lung cancer by the human tumor colony-forming assay (HTCFA) has been completed. Thirty-two patients have been studied at 6 dosage levels. The principal side effects have been somnolence and hypotension in all patients. Six patients have had to stop therapy because of somnolence and none because of hypotension. There have been no objective responses to date.

In vitro testing with dihydrolenperone showed 50% inhibition of growth of non-small cell and small cell lung cancer lines at 25-165 ug/ml. Pharmacokinetic determinations show peak absorption at 3-5 hours and plasma levels were more than 100-fold less than the levels where in vitro activity against lung cancer cell lines was observed.

From these studies we conclude that the HTCFA has identified a compound with novel side effects, the maximum-tolerated dose is 50 mg per square meter, and achievable plasma levels are much less than that required for in vitro activity. (Johnson, Ihde, Gazdar, Minna, in collaboration with Drs. Glatstein and Salem, NCI and Drs. Strong and Parker, FDA).

Mechanisms of Oncogene Action in Tumorigenesis

We have undertaken a study to identify critical genetic events in the pathogenesis of human cancer. We have currently focused our research efforts on studying the mechanism and implication of inactivation of the retinoblastoma (Rb) gene in human cancer. Our recent findings are as follows: 1) essentially, all small cell lung cancer tumors have absent or aberrant Rb protein products; 2) we have identified and characterized a series Rb mutants (defective in phosphorylation and oncoprotein binding); 3) we are investigating the possibility of these Rb mutants to function as transforming genes (dominant negative effect); 4) we have successfully transfected a wild-type or mutant Rb gene in a

SCLC cell line to study its biological effect; 5) we are using our Rb open reading frame reagents to identify putative cellular proteins that normally interact with the Rb protein and presumably modulate its growth inhibitory effect; and 6) we have generated a large number of in vitro Rb mutant proteins to characterize different functional domains of this protein. In addition, we continue to maintain a research effort studying mechanisms of L-myc gene activation. We have identified a 200 Kd cellular protein that binds to a specific region of the L-myc protein and we are currently attempting to clone this protein. Further we have preliminary evidence that the Rb tumor suppressor gene may also bind to the L-myc protein as well. (Kaye, Lin, Otterson, Shimizu, Kratzke)

New Drug Discovery Project

The primary goal of this group is to identify new agents of potential clinical use in treating solid tumors. A major effort over the past 5 years has been the use of an in vitro assay which may be helpful as a preclinical screening model for antitumor agents. The model has been used to predict the clinical activity of 7 chemotherapeutic agents against 11 human colorectal carcinoma cell lines which have been developed in this branch. Using the model, we have shown that leucovorin enhances the in vitro cytotoxicity of the fluoropyridines versus our panel of colorectal cell lines. A study was also performed to detect possible synergy between etoposide and cisplatin in a panel of 8 human bronchogenic carcinoma cell lines. Extensive analysis revealed no in vitro synergy, a finding at variance with standard feeling. Schedule dependent drug interaction has been documented between methotrexate and 5-fluorouracil. Persantin has been shown to enhance the cytotoxicity of 10-EDAM in human lung cancer cell lines. Clinical trials are planned to explore this.

At present, we are involved in several trials of new experimental therapeutic agents: a radiolabeled monoclonal antibody ^{90}yttrium-T101) in mycosis fungoides and chronic lymphocytic leukemia; 4-ipomeanol in lung cancer. A phase I trial of hepsulfam has recently been completed, and maximally tolerated schedule identified as 360 mg/m^2 i.v. every 5 weeks. Dose limiting toxicity was leukopenia. (Kramer, Gazdar, Johnson, Ihde, Mulshine, Sladek).

Biology, Growth and Chemosensitivity.

A. There appears to be an increase in the incidence of adenocarcinoma subtype of NSCLC in the USA. In particular, tumors with features characteristic of bronchiolo-alveolar carcinomas appear to be increasing. The relatively large number of cell lines that we have established that have ultrastructural and biochemical evidence of origin from peripheral airway cells (Clara or Type II pneumocyte) parallels the epidemiology of the disease. Markers for differentiation in lung cancers include expression of Clara and surfactant genes (in adenocarcinomas) and N-cam in neuroendocrine tumors. In addition, expression of CEA is much higher in neuroendocrine lung cancers than in others.

B. SCLC cell lines retain their chemosensitivity patterns for many years, and in vitro testing is predictive of patient response and survival. Thus, panels of cell lines (SCLC, NSCLC, colorectal and gastric carcinomas) are useful reagents to screen putative new phase I and II drugs using the MTT tetrazolium dye assay.

C. Mutations of ras genes are an important negative prognostic factor in NSCLC, and occur independently of p53 gene mutations. (Gazdar, Linnoila, Ihde, Mulshine, Johnson and Kramer).

Molecular Pathology of Pre-malignant Lung

Our goal is to define the molecular events that occur in the bronchopulmonary epithelium in the premalignant state. This involves mapping the expression of growth factors and their receptors, oncogenes and tumor suppressor genes at the cellular level in the progenitor cells of lung cancer in the non-neoplastic lung. This helps to understand the order of events leading to malignant transformation, and provides tools for early detection of cancer and cancer-susceptible individuals as well as basis for the early intervention.

Characterization of the system. Surgically resected pairs of malignant and corresponding non-neoplastic lung from the same patient composed of all NSCLC types were studied by RNA-RNA in situ hybridization for the expression of myc-family oncogenes and the peripheral airway cell (PAC) cell(progenitor cells) differentiation. C-myc oncogene was overexpressed in 8 out of 17 tumors, 2 of which also expressed L-myc, while 15 out of 17 lungs showed low levels of c-myc both in airway epithelium and alveoli. N-myc levels both in tumors and lung tissue remained undetectable. The expression of PAC differentiation genes SP-A (the major surfactant associated protein) and Clara cell protein was focal in 4 tumors and restricted to type II cells in alveoli and bronchiolar cells of the lung, respectively. By immunohistochemistry, 5 tumors were positive for p53 staining signifying the possible presence of a mutated form of this suppressor gene. These results suggest that 1) expression of myc in NSCLC is a common event, 2) low levels are present in most cells in the lung. and 3) PAC differentiation is restricted to subpopulations of bronchopulmonary cells. (Linnoila, Mulshine, Gazdar, Minna and Jensen).

Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies

The efforts of this Branch have been central to the recognition of gastrin-releasing peptide as an autocrine growth factor for small cell lung cancer. Dr. Cuttitta developed a monoclonal antibody (2A11) to the active portion of that peptide and

demonstrated that the immunoglobulin could block the mitogenic effect of GRP in vitro and in vivo. In collaboration with Hybritech, Inc. (San Diego, CA), we have initiated a clinical trial to test whether one can control autocrine-mediated malignant proliferation of small cell lung cancer using a monoclonal antibody. Our Branch has a long-standing interest in the role of growth factors in cancer, so that information from 2A11 antibody clinical trial could be a foundation from subsequent anti-growth factor trials.

The phase I portion of the 2A11 antibody trial identified 250mg/m_2 of the monoclonal as the optimal dose. The Phase II portion of the 2A11 evaluation has recently started. We have previously reported the diagnostic application of lung-associated monoclonal antibodies derived at this Branch for use in the early detection of lung cancer. We have patented the method for this approach with collaboration from Johns Hopkins and in conjunction with the Lung Cancer Study group will proceed to rapidly follow up on this critical area. We have followed up on this work with several publications including a report characterizing the fine binding affinity of one of the antibodies used for early lung cancer detection. (Mulshine, Gazdar, Linnoila, Cuttitta, Ihde, Kramer, Avis, Treston, Scott with collaboration with Dr. Glatstein, NCI).

Non Small Cell Lung Cancer Therapy Project

A primary objective of this Branch is to improve the state-of-the-art in the therapy of lung cancer. In the past, this Branch had focused this effort in the study of small cell lung cancer. With the advances both in the therapy of the small cell patients and in the study of small cell lung cancer biology, we decided to generalize the Branch effort to include the systemic evaluation of non-small cell lung cancer. This pilot studies the feasibility and value of using in vitro criteria to select therapy for patients with metastatic non-small cell lung cancer. (Mulshine, Linnoila, Oie, Russell, Englee, Jensen).

Identification of genes expressed selectively in plasmacytomas.

Using subtractive cDNA cloning, we have identified and partially characterized two genes that are expressed in mouse plasmacytomas but not pre-B or B lymphomas:

EGP314 - This gene is the murine equivalent of an 314 amino acid pan-epithelial membrane glycoprotein which had been identified in all human epithelial cells, but was not thought to be expressed in human hematopoietic cells. Although we do not detect expression in normal spleen cells, lipopolysaccharide stimulated cultures of murine spleen cells express significant levels of the

2 kb mRNA, consistent with its expression in normal plasma cells. This gene is expressed in one of two human myeloma cell lines, but not in Burkitt's lymphoma or lymphoblastoid cell lines. Since the structure of the glycoprotein shows homology to nidogen, an extracellular adhesion factor, we speculate that it may be involved in the communication between epithelial cells and plasma cells that is necessary for transport of secretory immunoglobulin into lumenal spaces, e.g. gut, lung, etc.

Gene 326 - This gene encodes an approximately 83,000 Dalton protein with a stiking homology to known proteins, i.e. a sequence of 40 amino acids which is homologous to a sequence repeated several times within a number of other proteins, many of which have been shown to function as beta subunits of a trimeric G protein complex. Surprisingly, we cannot detect expression of this gene in normal tissues, with the possible exception of mouse testes. Although mouse cell lines (with the exception of mouse plasmacytomas) do not express this gene, it is expressed in at least 6 of 8 Burkitt's lymphomas and a human myeloma cell line which has a c-myc translocation. Since this gene does not seem to be expressed in normal mouse plasma cells, it appears that its expression may be linked to the tumorigenic process which generates murine plasmacytomas and human Burkitt's lymphomas (Kuehl, Bergsagel, Kobrin, Brents).

Major Staff and Administrative Changes in FY 1991

In April 1991, after a twenty-two year association with the National Institutes of Health, Dr. John D. Minna accepted the position as Director of the Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center at Dallas, Texas.

Dr. Bruce Johnson has been appointed Acting Chief of the NCI-Navy Medical Oncology Branch.

Dr. Adi Gazdar retired from NIH and has also joined the Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center at Dallas, Texas, as Professor of Pathology.

Dr. Barry Kramer accepted the postiion as Associate Director for Early Detection and Community Oncology Program, Division of Cancer Prevention and Control at the National Cancer Institute.

Dr. Daniel C. Ihde is now the Deputy Director of the National Cancer Institute.

Dr. Stanley Lipkowitz became a senior staff member in the Branch.

Dr. David Curiel accepted a position as Assistant Professor at the University of North Carolina at Chapel Hill.

Dr. Jay Lynch completed his medical staff fellowship training and joined the faculty of the University of Florida.

Ms. Duiona Baker assumed the position of Administrative Officer for the Branch.

Postdoctoral fellows who completed their research training include: Dr. Marc-Henri Stern (Paris, France); Dr. Tetsuya Mitsudomi (Japan); Dr. Itsuo Chiba (Japan).

Dr. Michael Boger, LCRR, USN, of the Oncology Division for the Naval Hospital, retired after 20 years of service in the United States NAvy to relocate to Spartansburg, South Carolina.

PEDIATRIC BRANCH

Clinical Studies

1. NCI 83P-CCG 134P: Treatment of newly diagnosed acute lymphoblastic leukemia in high-risk patients. The major aim of this study is to demonstrate that high-risk patients can be effectively treated on a regimen which uses CNS preventive therapy devoid of cranial radiation. An additional objective is to determine whether there is a difference in the outcome of patients at high risk for early treatment failure according to whether they do or do not have features consistent with "lymphoma leukemia syndrome." The protocol involves the use of an aggressive, early intensification phase of therapy and intensive systemic maintenance therapy, together with CNS specific treatment. The latter consists of periodic administration of systemic high-dose methotrexate, systemic high-dose cytosine arabinoside and intrathecal cytosine arabinoside and methotrexate. With a median potential duration of study of 4.3 years, the event-free survival is projected at between 55 and 60 percent at three years. The occurrence of isolated CNS relapse in only three of the 107 patients enrolled in this study to date, indicates that this study has been successful in demonstrating effective central nervous system preventive therapy can be achieved in high-risk patients without the use of cranial radiation.

2. NCI 84A-CCG 144: This protocol treats newly diagnosed patients in the "average-risk" category, randomizing them to one of two forms of CNS preventive therapy, either high-dose systemic methotrexate infusion or intrathecal methotrexate alone. The median potential duration on study is 30 months. A total of 176 patients have been randomized. There is no significant difference in either the CNS or bone marrow relapse rate in either treatment arm. These data have demonstrated that average-risk patients can receive effective CNS preventive therapy with intrathecal methotrexate alone and do not appear to

require high-dose methotrexate. Further follow up is necessary to answer this definitively.

3. Intrathecal Diaziquone (AZQ): AZQ is a lipid soluble aziridinyl benzoquinone designed for enhanced CNS penetration of the CNS to treat CNS neoplasms. We are evaluating the feasibility of intrathecal AZQ in a Phase I-II trial in patients with refractory meningeal malignancy. Two schedules of administration are being examined: twice a week for four weeks and "CxT", every 6 hours for three doses, weekly x 4. A total of 39 patients have been treated, 28 of whom had acute lymphoblastic leukemia. Demonstrable antineoplastic activity has been observed on both schedules of administration. Seven of the 21 courses delivered on the twice weekly schedule have resulted in complete responses. On the "CxT" schedule, 7 of 24 courses have resulted in complete responses. A maximally tolerated dose has been defined for both schedules. The results of this study indicate that intrathecal AZQ has definite clinical activity in refractory meningeal malignancy, at a dose which is not associated with clinical toxicity.

4. Intrathecal 6-Mercaptopurine (6-MP): Preclinical studies of intrathecal 6-MP, performed in a subhuman primate model indicated that 6-MP could be safely administered by the intrathecal route. Based on these studies we have initiated a Phase I study of intrathecal 6-MP in children with refractory meningeal malignancy. Both a twice weekly and a concentration x time (CxT) schedule (q12h x 6 doses) are been evaluated. To date, 12 patients with CNS ALL treated on the twice weekly schedule have achieved complete responses. No significant toxicity has been observed. These results indicate that intrathecal 6-MP is safe and active against meningeal leukemia.

5. Intrathecal Mafosfamide: 4-hydroperoxycyclophosphamide and mafosfamide, preactivated derivatives of cyclophosphamide, exhibit activity *in vitro* equal to that of 4-hydroxycyclophosphamide. We are currently investigating the feasibility of administering mafosfamide intrathecally. In our nonhuman primate model intrathecal injection of this compound was not associated with either acute or chronic neurotoxicity or with systemic toxicity. The demonstration that cytocidal levels of these agents can be achieved in CSF following intraventricular administration of a non-toxic dose suggests that further study in the clinical setting is warranted. A clinical phase I trial of mafosfamide in patients with refractory meningeal malignancy has recently been initiated, and has rapidly accrued patients.

6. Continuous Intrathecal Infusion: The ultimate extension of the CxT approach is to administer the drug by continuous infusion, an approach studied in a Rhesus monkey model. A new technique was developed in which a cannula is inserted into the

lateral ventricle and then attached to a subcutaneously implanted catheter with a reservoir that is attached to a portable infusion pump containing the drug to be studied. In preliminary studies we have found that with continuous infusion of MTX, ventricular CSF MTX concentrations are maintained at 1 mmol/L for two- to three-fold longer than with the bolus dose, despite the fact that only one tenth of the total bolus dose was administered by infusion. Thus, these studies directly demonstrate the clear pharmacokinetic advantage for continuous intrathecal infusion. A clinical protocol evaluating this approach has recently been initiated, and pharmacokinetic studies in the first patient have confirmed the results of the animal studies.

7. Studies with Thiotepa: We embarked on a multi-institutional Phase II study designed to assess the therapeutic efficacy of Thiotepa against brain tumors. Fifty-six patients have been entered (46 are evaluable for response, 4 are awaiting evaluation). Three of 13 PNET tumors have had a partial response. Ten of the patients in the remaining tumor categories have had stable disease; no other responses have been noted.

8. Phase I Studies: A variety of Phase I trials are being pursued in an effort to develop active, new compounds for the treatment of pediatric malignancies.

Phase I Trial of Piritrexim

Piritrexim, an orally administered, lipid soluble antifolate, was evaluated in a multi-institutional phase I trial in children. Eighteen patients with malignancy refractory to therapy were entered onto the study. The dose-limiting toxicities (DLTs), were myelosuppression and mucositis. The maximum-tolerated dose was 25 mg/m^2/dose, and the recommended dose for phase II trials is 20 mg/m^2/dose. Pharmacokinetic monitoring was performed in 15 of the 18 children entered on study. The results of analysis of pharmacokinetic and pharmacodynamic correlations and of a limited sampling strategy indicated that therapeutic drug monitoring may play an important role in adjusting the dose and schedule of piritrexim in future trials.

Phase I Trial of All-*trans* Retinoic Acid (*t*-RA)

t-RA is an agent that has demonstrated activity *in vitro* as a tumor differentiating agent. We recently initiated and subsequently completed a Phase I trial of *t*-RA in pediatric patients with refractory malignancies. Twenty-one patients were entered at the maximum-tolerated dose (MTD) of 60 mg/m^2. Increased intracranial pressure was the dose-limiting toxicity. Complete responses were observed in two patients with multiple relapsed acute promyelocytic leukemia. Preliminary analysis of

t-RA pharmacokinetics revealed that peak plasma concentrations are relatively low (1 μM) and that the drug is rapidly cleared from plasma. This information will be of value in the planning of Phase II trials.

Phase I Trial of Amifostine/Melphalan

Amifostine has been shown in preclinical trials to protect the bone marrow from the myelotoxicity of melphalan, and in clinical trials to protect from the myelotoxicity of other alkylating agents. Treatment of patients with amifostine prior to melphalan administration may allow for the escalation of melphalan doses beyond those currently tolerable and possibly take therapeutic advantage of the steep dose response curve of melphalan. A phase I pediatric trial is currently being performed to test this hypothesis.

Phase I Study of Topotecan

Topotecan is a new antineoplastic agent with a novel mechanism of action (inhibition of topoisomerase I), which also has been shown to have significant activity against multiple multidrug-resistant leukemia cell lines. Thus, this compound is of significant interest for the potential treatment of solid tumors and refractory hematologic malignancies in the pediatric population.

A Phase I trial and pharmacokinetic study of Topotecan administered as a 24-hour continuous i.v. infusion in pediatric patients with advanced neoplastic disease is in progress.

9. We have analyzed our data on patients with lymphoblastic lymphoma treated on protocol 77-04. The results, although based on a small number of patients (26), are gratifying. Overall survival of patients without bone marrow involvement is 80% at 10 years, and 70% at 10 years for patients with mediastinal masses.

10. We have analyzed all of our previous data relating to patients with small, non-cleaved cell lymphomas to determine the importance of CNS involvement of outcome, and the role of radiation in treatment of CNS disease. Our data strongly support the notion that CNS disease is an accompaniment to extensive systemic disease and does not *per se* represent an obstacle to cure. In addition, patients in whom overt disease in the CNS was irradiated have fared no better than patients who never received radiation, supporting the view that for the small, non-cleaved cell lymphomas, radiation adds toxicity, but no therapeutic benefit.

11. A new protocol, 89-C-41, for patients with non-lymphoblastic lymphomas has been opened to patient accrual for approximately 1 year. This protocol is based on observations made in previous

Pediatric Branch protocols for the non-Hodgkins's lymphomas, including the demonstration of the importance of dose intensity. Treatment consists of alternating cycles of regimens referred to as CODOX-M, a modification of the protocol piloted as protocol 85-C-67, and IVAC, a new regimen based on a pilot protocol, 85-C-62, which was used for the treatment of patients with recurrent non-lymphoblastic lymphomas.

The major goal of the new protocol is to determine whether GM-CSF administration will result in increased dose intensity (i.e., dose rate) in high risk patients, particularly those with bulky disease and/or bone marrow involvement, while at the same time decreasing toxicity. Patients will be randomized to receive either four cycles of CODOX-M/IVAC, or the same treatment accompanied by subcutaneously administered GM-CSF.

12. We continue to monitor results of the completed protocol which studied the intensive program for patients with high-risk pediatric sarcomas. This protocol combined high-dose chemotherapy during induction with total body irradiation (800 rads) and autologous bone marrow reconstitution. Ninety-two percent of the patients enrolled on the protocol were successfully induced. The actual disease-free survival is 50% for those patients free of metastatic disease at diagnosis versus 20% for those with metastatic disease. These results are not significantly different from historical experience and therefore do not stimulate enthusiasm for further investigating a total body irradiation, autologous bone marrow transplant approach to the treatment of these diseases.

13. Protocol 87-C-10, a study of the treatment of moderate risk sarcomas with continuous infusion adriamycin as well as vincristine, cyclophosphamide, ifosfamide, and etoposide has been closed. The primary intent of the protocol was to determine whether continuous infusion of adriamycin would reduce cardiac toxicity. Of the seven patients treated on the protocol, there have been two cases of overt cardiomyopathy with one death. Two other patients have had a significant decrease in the MUGA scan ejection fraction. These results demonstrate that continuous infusion adriamycin is not likely to significantly reduce the cardiac toxicity associated with this agent. To further address this clinical problem, protocol 89-C-07 has been initiated in tandem with the high-risk sarcoma protocol to determine whether the iron chelating agent ICRF-187 will inhibit adriamycin cardiotoxicity. Preliminary results from a study with adults with breast cancer suggests that this is an active cardioprotective agent. Patients entered on protocol 86-C-169, the high-risk sarcoma protocol, will be randomized to receive ICRF-187 or not.

The pilot protocol for the treatment of high-risk sarcomas, 86-C-169, continues to monitor patients. There have been 65 patients entered. It is too early to judge the efficacy of the vincristine, cyclophosphamide, adriamycin, ifosfamide, and etoposide regimen. The major toxicity of the protocol, myelosuppression, is being addressed by a companion study, protocol 88-C-165, which is designed to determine whether the addition of the colony-stimulating factor GM-CSF will reduce the extent of myelosuppression in patients on the sarcoma protocol. Patients are being randomized to either receive or not receive the GM-CSF in conjunction with VAC and IE regimens. To date, 23 patients have been enrolled in this study.

14. Patients on the sarcoma protocol are randomized to receive ICRF-187 with adriamycin or adriamycin alone to learn whether this iron chelating agent will decrease the significant incidence of clinical and subclinical adriamycin associated cardiomyopathy. The patient's cardiac function is monitored closely with radionuclide angiography which is the endpoint for the study. Twenty-eight patients have been entered on this study.

15. The Pediatric and Surgery Branches of the NCI have a long history of studying osteosarcoma. The current study is testing the relative merits of immediate surgery versus neo-adjuvant chemotherapy. As the majority of osteosarcoma patients have resectable tumor at diagnosis, important questions are adjuvant in nature and must be addressed with phase III studies. The numbers of patients required for such studies necessitate multi-institution collaborations. Investigators from the NCI have been intimately involved with the design, conduct and analysis of the MIOS studies.

16. The Pediatric and Surgery Branches of the NCI are collaborating to test the efficacy of IFN-g, IL-2, and TIL in children with recurrent or progressive neuroblastoma. Eligible patients are treated with IFN-g prior to surgery for TIL harvest. In the interim postoperatively and prior to the time that sufficient TIL are grown, approximately 6 weeks, patients receive a single dose of carboplatin in order to prevent rapid, progressive disease. Once sufficient TIL are grown, patients are treated with IFN-g followed by TIL and IL-2 administered in the intensive care unit. Two patients have been treated. The first patient progressed whereas the second patient has responded.

17. There is increasing evidence to support a role for chemotherapy in the treatment of brain tumors. The thrust of this study is to develop a regimen of high-dose cyclophosphamide and GM-CSF that will be used in front-line studies for the treatment of children with high-risk brain tumors. Patients with recurrent malignant brain tumors after radiation therapy for at most one prior chemotherapy regimen or newly diagnosed patients

with high-risk brain tumors such as brain stem glioma or ependymoma are treated with cyclophosphamide at 4.5 g/m^2 administered every 2 to 3 weeks. In addition, patients receive GM-CSF at 5 mg/kg daily from day 3 until the absolute granulocyte count is greater than 1500. To date, 15 patients have been treated. Responses have been seen in medulloblastoma (PNET) and ependymoma. Over 70% of the chemotherapy courses have been complicated with infection. The duration that the absolute granulocyte count is less than 500 is 8 ± 2 days. This study is ongoing to estimate the response rate in the major categories of pediatric brain tumors.

18. To determine the role of new beta-lactam antibiotics in providing simpler, safer and effective therapy for neutropenic cancer patients who become febrile, we have conducted a randomized trial comparing a third-generation cephalosporin (ceftazidime) to a carbapenem (imipenem/ cilastatin) for initial empirical therapy. The goal of this study is to both evaluate the role of these agents in providing safe initial therapy as well as determining whether the numbers of modifications of the primary antibiotic varies in patients with defined infection or prolonged granulocytopenia. From March, 1986 - January, 1990, we enrolled over 500 evaluable episodes of fever and neutropenia, randomizing these to initial ceftazidime (251 episodes) or imipenem (249 episodes). Both regimens provided comparable primary therapy. More modifications of the initial regimen were necessary for patients with documented infection who were randomized to ceftazidime and there were more second infections in this group. These were primarily with gram-positive bacteria. However, there were no differences in infection related morbidity or mortality. On the other hand, there were more complications with imipenem, including a higher incidence of *C. difficile* diarrhea and a higher degree of intolerance due to nausea and vomiting. Overall, both antibiotics appear useful, have different strengths and weaknesses and confirm that various alternatives can be employed to provide safe monotherapy for the majority of febrile neutropenic cancer patients.

19. We have completed the first phase I-II pharmacokinetic study of fluconazole in children with cancer. This study found that fluconazole was safe and well-tolerated. Moreover, fluconazole had a shorter mean plasma half-life than that of adults.

20. Following extensive pre-clinical investigation, we completed a multi-center trial demonstrating the expression of antigenemia due to *Candida* cytoplasmic enolase (a 48 kD Ag) as a new marker of invasive candidiasis in cancer patients.

21. We demonstrated that anti-*Candida* enolase antibody (Ab) [titer>1:100] but not enolase antigen (Ag) was present in serum

of non-neutropenic surgical patients with invasive candidiasis. Patients with invasive candidiasis who were recovering from neutropenia also had rise of anti-enolase Ab and decline of Ag. Anti-*Candida* enolase Ab also was associated with negative serum antigen detection tests and was indicative of favorable outcome in invasive candidiasis. These data indicate that both serum Ag and Ab should be measured in order to optimally utilize *Candida* enolase as an immunodominant marker of invasive candidiasis.

22. We have continued our Phase I-II studies of children with symptomatic HIV infection. Since beginning this project in December, 1986, we have evaluated over 200 children, enrolling the majority into clinical trials.

Our initial study of AZT, administered either by continuous intravenous infusion or on an intermittent schedule, are completed. Both routes of therapy appeared to offer benefit, particularly for children with neurodevelopmental deficits. However, the extent of this benefit, appear to be greater for children treated by the continuous intravenous schedule. To validate this, we are performing a randomized study comparing AZT administered on a schedule that maintains steady-state kinetics in the plasma and CSF to one in which the drug is delivered on an intermittent schedule to children with evidence of encephalopathy or to children who have developed dementia while receiving antiretroviral therapy. To date, 18 patients have been enrolled.

We initiated a Phase I/II protocol to assess the efficacy of subcutaneously administered G-CSF in increasing and maintaining the neutrophil count in HIV infected children who have developed neutropenia as a consequence of AZT. We are also studying the effect of human erythropoietin on overcoming AZT-induced anemia. To date 12 patients have been enrolled with promising early results.

In a search for effective, less toxic regimen, we initiated a Phase I-II trial of dideoxyinosine (ddI) in children in January, 1989. To date, 95 children have been enrolled at several dosage levels (20, 40, 60, 90, 120 mg/m^2/every 8 hours. This protocol enrolled both children who have received no prior anti-retroviral therapy as well as children who have become refractory or intolerant to AZT. Dideoxyinosine was well tolerated and shows promising antiretroviral activity in HIV-infected children. The correlation between response and plasma ddI concentration indicates that bioavailability is an essential consideration for optimizing ddI activity in the treatment of HIV infection. We have also completed the long team follow-up of the entire group, the data which was used to support the NDA for ddI.

We completed a phase I study of recombinant soluble CD4 (rCD4) administered by continuous infusion to children with HIV infection. The initial treatment period of rCD4 alone was followed by the addition of oral ddI at a dose of 270 mg/m^2/day. rCD4 at doses as high as 1000 μg/kg/day was well-tolerated alone and in combination with ddI, however no marked changes in p24 antigen or CD4 counts were observed in patients receiving CD4. The CD4 infusion part of this protocol was ended in May, 1991 and the patients remaining on this protocol continue to receive ddI.

We initiated a Phase I-II dose escalation trial of combination antiretroviral therapy with AZT and ddI in September, 1990. This study is being conducted in collaboration with the Children's Hospital of Los Angeles and Los Angeles County/USC Medical Center. To date 29 children have been enrolled at doses ranging from 60 to 180 mg/m^2 every 6 hours of AZT, and 60 to 135 mg/m2 every 12 hours of ddI. This protocol enrolls children who have not received prior antiretroviral therapy (Arm A), or those who have experienced hematologic intolerance on AZT (Arm B). An interim analysis of this study indicates that this combination is well-tolerated over a wide range of doses, without evidence of new short-term toxicities or of enhancement of known toxicities. Significant increases in CD4 counts, decreases in serum p24 antigen, decreases in viral load in plasma and PBMC's and increases in cognitive function have been observed in 14 patients who reached the initial 12 week major evaluation point. Particularly striking improvements in CD4 cell count were observed in patients at the dose level incorporating the highest dose of ddI. These data suggest that this combination is active *in vivo*, however the longer-term tolerance and the optimal doses remain to be determined in this study.

We initiated a Phase I-II dose escalation trial of oral clarithromycin for pediatric patients with disseminated *Mycobacterium avium* complex infection. This study is being conducted in collaboration with the Children's Hospital of Los Angeles. To date 11 patients have been enrolled at doses of 7.5 and 15 mg/kg/day. A borderline decrease in hearing has been the only significant possible toxicity observed to date.

Improvements in energy levels, appetite and decreased fever have been observed, however recurrence of symptoms has occurred after several weeks in most patients at the first dose level. Tolerance and toxicities of this agent, as well as the durability of clinical response, remain to be determined at the higher doses incorporated into this protocol.

23. NCI 91-C-98, a Phase II study of standard-dose Ara-C in rhabdomyosarcoma was approved in April, 1991. This protocol is based upon laboratory observations demonstrating that exposure of

rhabdomyosarcoma cell lines to 0.5 μM Ara-C induces growth arrest and biochemical and morphological evidence of differentiation, and reverses the transformed phenotype as assayed by tumorigenicity in nude mice. The aim of the protocol is to determine whether Ara-C 100 mg/m^2 SQ daily x 7 days x 2 will be active in recurrent rhabdomyosarcoma.

24. A Phase II study of suramin in relapsed rhabdomyosarcoma patients has been approved by the Clinical Research Subpanel, NCI. This protocol is based upon laboratory observations demonstrating suramin's ability to block the IGF-II autocrine growth loop in rhabdomyosarcoma cell lines. The study is pharmacokinetically guided and aimed at maintaining suramin levels between 300 μg/ml and 200 μg/ml. It is being run in collaboration with Dr. Charles Myers and Dr. Michael Cooper of the Clinical Pharmacology Branch, DCT, NCI. It will be the first study in which suramin is given to a pediatric population.

Pre-Clinical Studies

1. In previous studies, we demonstrated heterogeneous, though generally distinctive, molecular genotypes for each of three leukemias, B-cell precursor ALL of childhood, T-cell ALL of childhood, and ALL of infancy. Within each group, a spectrum of developmentally pre-committed lymphoid precursors from all Ig and T-cell receptor (TcR) genes germline to more mature cells with multiple rearrangements have been identified. A recent study we performed suggested that genotypically less mature leukemias may manifest a more difficult course, and that genotypic heterogeneity may be of clinical relevance. This will be the topic of a prospective investigation in a new NCI protocol for the treatment of high-risk patients with ALL. The utility of immune receptor gene rearrangements as markers for preclinical disease detection and the sensitivity and specificity of the PCR reaction using primers that amplify the hypervariable region of Ig heavy chain also are being evaluated.

2. The p53 gene is a candidate tumor suppressor gene located on chromosome 17 at band p13. Based upon experiments in transgenic mice where a mutated p53 gene under its own promoter resulted in lymphoid tumors, as well as anticipated tumors of lung and bone, the potential role of alterations in this gene in the pathogenesis of childhood acute lymphoblastic leukemia (ALL) is currently being explored. Bone marrow peripheral blood lymphoblasts of 12 children and 2 infants with B-cell precursor ALL, and 11 children with T-cell ALL, have been examined for point mutations by the method of RNase protection using 3 probes spanning the entire p53 coding region, and abnormalities were identified in 2 cases. The nature of these abnormalities was fully characterized by both cDNA synthesis, PCR amplification,

and sequencing of subclones, as well as by direct sequencing of genomic PCR products. These studies have revealed that p53 mutations, expression of these mutations at the RNA level, and loss of heterozygosity may occur in childhood ALL, but at a low frequency. Moreover, a single allele may be susceptible to multiple mutations, as was the case in one child diagnosed with B-cell precursor ALL. Family studies using the same methodology are now in progress in order to determine whether the observed mutations in this gene in childhood ALL are constitutional or acquired. Analyses of polymorphisms located within and in close proximity to this gene are also being developed as a method of screening for loss of heterozygosity and identification of patients warranting more detailed study.

3. Methotrexate (MTX) is the most widely used intrathecal (IT) antineoplastic agent. Accidental IT overdose can produce severe and frequently lethal toxicity. Despite currently recommended interventions, the outcome is often fatal. The carboxypeptidase G class of enzymes rapidly hydrolyze MTX into the inactive metabolite 4-deoxy-4-amino-N^{10}-methylpteroic acid and glutamate. The gene for one member of this class of enzymes, carboxypeptidase-G_2 ($CPDG_2$), has been cloned, and the enzyme purified on a large scale. We evaluated $CPDG_2$ as a potential IT rescue agent for IT MTX overdose in our primate model and studied the CSF pharmacokinetics of MTX with and without $CPDG_2$ rescue. The CSF MTX half-life of 2.3+0.2 hours was decreased to 33.2+6.2 seconds by $CPDG_2$ resulting in a greater than 400-fold decrease in CSF MTX concentration 5 minutes after enzyme administration. Subsequently, groups of three monkeys received either 25 mg IT MTX (equivalent to 250 mg in humans) followed by 150 U IT $CPDG_2$ or 50 mg IT MTX (equivalent to 500 mg in humans) followed by 300 U IT $CPDG_2$. All animals survived without neurotoxicity. Our studies suggest that $CPDG_2$ may prove to be an important addition to the currently recommended strategy for the management of IT MTX overdose.

4. We have also performed additional studies with Carboxypeptidase-G_2. High dose methotrexate (HDMTX) can be safely administered when followed by leucovorin (LV) rescue. As noted above, $CPDG_2$ may act as an alternative form of rescue for HDMTX. $CPDG_2$ has potential advantages over LV rescue: $CPDG_2$ doses not cross the blood brain barrier, raising the possibility that patients could be rescued systemically from HDMTX while selectively excluding CNS tumors from rescue. In contrast to LV, $CPDG_2$ could be used to rescue patients with renal dysfunction and delayed MTX excretion, as it can effectively rescue from systemic plasma MTX concentrations >10μM. The plasma pharmacokinetics of MTX with and without this new purified preparation of $CPDG_2$ have been studied in adult rhesus monkeys. Animals have received up to 8 biweekly doses of enzyme without manifesting allergic

symptoms. The recombinant $CPDG_2$ product may be less immunogenic than its non-recombinant predecessor. Administration of CPDG2 appears safe and well tolerated, and may be useful as an alternative to LV rescue of systemic MTX in patients with renal dysfunction or in the treatment of CNS tumors. These studies of $CPDG_2$ extend previous Pediatric Branch work which demonstrated the ability of intrethecal $CPDG_2$ to rescue monkeys from an intrathecal methotrexate overdose.

5. Pediatric Branch investigators have demonstrated that all trans retinoid acid inhibits the growth of RMS cells without any evidence of differentiation activity, and this activity appears to be stereo-specific in that 13-cis retinoic acid has no effect on these same cells. A Phase I study of all trans retinoic acid was carried out in pediatric patients with refractory malignancies. Detailed pharmacokinetics provided information which will be helpful in the design of Phase I trials.

6. Preclinical studies of several new antineoplastic agents are under way preparatory to the development of Phase I pediatric trials. Agents under study include compounds such as cyclopentenyl cytosine (CPE-C) a cytosine analogue active against cytarabine resistant cell lines, pyrazoloacridine, an anthracycline active against tumor cells resistant on the basis of the multidrug resistant phenotype and Peg-asparaginase, a new asparaginase which provides prolonged asparagine depletion. Pediatric Branch studies have been instrumental in providing pharmacologic information helpful in the design of Phase I and Phase II clinical trials with these compounds.

7. We have demonstrated that EBNA-1 can exert a positive influence on c-myc transcription, its influence being mediated by immunoglobulin enhancer sequences. This provides a potential mechanistic explanation for the association of EBV with the SNCL, since the chromosomal translocations result in juxtaposition of c-myc and immunoglobulin enhancers.

8. We have shown that antisense transcripts are readily detectable from myc gene fragments in transient and stable transfection assays, and that the ratio of sense to antisense transcription is increased in fragments which are structurally similar to a subset of rearranged c-myc genes in Burkitt's lymphoma -- namely those with breakpoints in the first intron. This observation may be important to an understanding of the functional consequences of different types of chromosomal translocation and suggests that antisense transcripts may normally have a role in the regulation of the expression of c-myc.

9. We have demonstrated, by transfection assays, that the intronic enhancer from the immunoglobulin region significantly increases transcription from c-myc fragments that lack the normal c-myc promoters (P1 and P2) but contain the intronic promoter region, P3. Moreover, this enhancer effect is cell line specific, suggesting that some cell lines may lack some or all of the protein factors necessary for enhancer function. These findings are highly relevant to the development of an understanding of the functional consequences of the chromosomal translocations that occur in the SNCL.

10. We have completed a detailed analysis of breakpoints immediately 5' of the c-myc gene, and find them to cluster in a very small (<130bp) region, strongly suggesting the deletion of an upstream regulatory element and retention of a downstream regulatory element necessary to drive transcription from P1 and P2.

11. We have shown that in a high proportion (approximately 45% of IgH genes of SNCL, one IgH allele is unrearranged. This strongly suggests that the translocation occurs in very early B cells (i.e., around the time of D-J joining) such that, in such cases, the translocation prevents on allele from undergoing rearrangement of the IgH locus.

12. We have cloned the mouse bcl-3 gene, the human equivalent of which is involved in a translocation observed in small cell lymphoma/leukemias, and demonstrated that its pattern of expression is remarkably similar to the bcl-2 gene. This raises the possibility that bcl-3, like bcl-2, is involved in apoptotic pathways. The prevention of apoptosis appears to be a potentially important step in the pathogenesis of many neoplasms, perhaps particularly the hemopoietic neoplasms, and further exploration of genes involved in apoptosis is therefore, clearly warranted.

13. We have demonstrated that mutations of the p53 gene are present in some 70% of our cell lines derived from small non-cleaved cell lymphomas (SNCL). since the mutation prevalence appears to be higher in cell lines, derived predominantly from relapse tumors than in primary tumors (30%), these findings are consistent with the possibility that p53 mutations are important to tumor progression in SNCL in the USA.

We have shown that in Argentinean SNCL, some 50% have p53 mutations, and that the pattern of mutations in SNCL appears to differ from that seen in other tumors (e.g., colon cancer). These findings are consistent with the notion that p53 mutations differ with respect to the functional end-result, that there may be differences in the pattern, not only in different tumors but in different geographical regions, and that in some cases, p53

mutations may be necessary to the pathogenesis of the SNCL. The implications for response to treatment clearly need to be examined.

We have further demonstrated that only one of the mutations we have observed in SNCLs -- a mutation at codon 248 -- is not associated with stabilization of the p53 protein in the heterozygous state. This mutation, however, results in high levels of p53 when homozygous. This observation suggests that heterozygous mutations at 248 are innocent, providing an explanation for the clustering of cancer predisposing, inherited p53 mutations (e.g., in families with the Li-Fraumeni syndrome), around this region of p53. Mutations in the Li-Fraumeni "cluster region" also represent a potential predisposing genetic factor to SNCL -- a future research project.

14. We have shown that type 2 EBV is more often associated with HIV positive EBV associated lymphomas (40%) than with non-HIV associated lymphomas in the USA (10%) and Argentina (10%). The prevalence of type 2 EBV in HIV associated lymphomas is similar to that in African Burkitt's lymphoma (40%). Type 2 EBV is known to be quite frequently present in epithelial cells, and we surmise that it is less likely to be established in lymphoid cells (perhaps because of increased immunogenicity) in patients with intermittent (African) or continuous (HIV infected) immunosuppression.

15. Preclinical studies of AZT have demonstrated the effect of probenicid on prolonging the elimination of AZT. However, small animal models have also suggested that probenicid may enhance the CNS penetration of AZT also. We are currently investigating this interesting interaction in our Rhesus model. If the interaction proves to be true, these studies could result in a new approach to the treatment of dementia in AZT sensitive patients.

Another approach to the treatment of HIV dementia in patients who are intolerant of AZT may be the intrathecal administration of the drug. This approach is being evaluated in the monkey model as a continuous intrathecal infusion, and members of the section are collaborating with investigators in the Neurology Institute to develop a protocol to study this approach clinically.

16. Studies of absorption of anti-retroviral compounds in an animal model are being performed. A sustained release oral preparation of the currently available dideoxynucleosides would have the advantage of providing more prolonged exposure to drug over the dosing interval than current oral formulations. However, sustained-release formulations are only of use with drugs that can be absorbed along the entire length of small intestine. Drugs absorbed at a specific site via a carrier are absorbed poorly from a sustained-release preparation. Therefore,

prior to developing a sustained-release formulation, we evaluated the mechanism of absorption of AZT and ddI in an *ex vivo* rat intestinal loop model. These studies reveal that both drugs are passively absorbed, equally in all segments of the small intestine and would, therefore, be amenable to formulation in a sustained-release form.

17. CSF neurochemistry studies in children with HIV encephalopathy are designed to explore the possible biochemical basis for the mental impairment observed in pediatric AIDS patients with HIV encephalopathy, and to identify objective markers of CNS involvement that could be used to monitor the response to therapy. Since the systems potentially involved in this impairment are unknown, the concentrations of three categories of neurotransmitters and their metabolites and related enzymes will be studied. These assays, which have been used to study adult dementia, include "classical" neurotransmitters (acetylcholine, dopamine, noradrenaline, serotonin), neuropeptides (somatostatin, neuropeptide Y, CRF), and amino acids (GABA, aspartate, glutamate, glycine). Levels will be measured in the CSF of children with AIDS before and after treatment with AZT or ddI in order to determine if there is a neurochemical marker of dementia and a correlate for the previously described improvement in mental performance observed in AZT-treated children. Additionally, these values will be compared to those measured in the CSF of children participating

in ongoing clinical trials for the treatment of acute lymphoblastic leukemia.

18. The central nervous system pharmacology of antiretroviral agents is being studied. We have also evaluated the CSF penetration of a series of dideoxypurines, ddA, ddI and ddG. The penetration of ddG, a less water-soluble drug penetrated somewhat better than ddI. We are currently studying a series of halogenated dideoxynucleosides to determine the degree to which these analogs, which were developed to optimize CNS penetration, enter the cerebrospinal fluid. Newer agents such as protease inhibitors and" which were highly active in the NCI drug screen will also be studied.

19. We are in the preliminary phase of developing a cell- and virus-free assay of the crucial viral enzyme integrase, which is responsible for inserting the proviral DNA into the host genome. This step is crucial because it establishes the latent infection which makes HIV disease a life-long infection. Once an acceptable assay of integrase is developed it could be used to specifically screen compounds for their ability to block this important step in the viral life cycle.

20. We have recently demonstrated that the polymerase chain reaction (PCR) can be used to identify chromosomal breakpoint locations in the small non-cleaved lymphomas. Making use of the repeat sequences in the u switch region, we have developed three different sets of oligonucleotide amplimers which are capable of amplifying fragments containing portions of both chromosomes 8 and 14 (i.e., contain the breakpoint itself) and are also able to distinguish between three separate breakpoints in the regimen of the *c-myc* gene- breaks within the first *c-myc* intron, the first *c-myc* exon, or in the immediate *c-myc* 5' flanking sequences.

The clinical importance of these findings stems from the extremely high degree of sensitivity inheritant in PCR. We have been able to detect, in artificially created cell mixtures, the presence of one cell in a million containing the breakpoint location in one of the regions that we are able to detect by PCR. Thus, this technique is not only capable of providing definitive diagnosis, but should enable us to identify very small numbers of cells in tissues not recognized microscopically as being involved. In addition, this technique can be used to follow the presence of minimal residual disease in the bone marrow after therapy, and could be of value in predicting which patients will relapse.

21. We have been able specifically to inhibit the expression of the *c-myc* gene in a subset of Burkitt's lymphomas. This has been accomplished by using an antisense oligonucleotide directed against intron sequences which are present in the mature messenger RNA species in Burkitt's lymphomas with *c-myc* first intron breakpoints on chromosome 8. Both cellular proliferation and *c-myc* protein expression were inhibited in the experiments. These findings demonstrate that the molecular abnormalities in tumors may also provide a target for specific therapeutic endeavors. Because only Burkitt's lymphoma cells, and not normal cells, contain the genetic abnormalities, such therapeutic approaches may be highly selective. We are pursuing pre-clinical studies with anti-sense oligonucleotides using Burkitt's lymphoma xenografts in a nude mouse model.

22. Pediatric Branch investigators have identified that the cell cycle gene $p34^{cdc2}$ is constitutively expressed in neuroblastoma tumor cell lines.

Constitutive expression of $p34^{cdc}$ results in phosphorylation and inactivation of the tumor suppressor gene Rb. Retinoic acid treatment of sensitive NB cell lines decreases expression of $p34^{cdc2}$ which causes Rb to be found in an underphosphorylated and putative active state capable of suppressing tumor cell growth.

23. Continued studies on retinoic acid induced differentiation of NB cells has identified an increase in $TGFb_1$ coinciding with decreased levels of NMYC. These studies indicate $TGFb_1$ may directly mediate growth inhibiting effects of retinoic acid.

24. Amplification of NMYC in NB tumors has been used to identify patients with a poor prognosis. We have identified a correlation between expression of NMYC and the 68kd receptor for laminin by transfecting NMYC expression vectors into cells with few laminin receptors. NMYC transfected cells increase expression of laminin receptor. The correlation of high NMYC levels with high expression of laminin receptor provides a biological framework in which to consider the relationship between NMYC amplification and poor prognosis.

25. We have identified that some NB cells have high affinity receptors for somatostatin. Somatostatin inhibits the growth of these cells by causing a cell cycle delay in exit from G_2.

26. Continued studies on IGF-II expression in neuroblastoma indicates this growth factor can alter the response of cells to growth inhibiting effects of retinoic acid.

27. Studies of insulin-like growth factor II in neuroblastoma have continued and we determined that tumors in which this gene is not expressed seem to have high levels of expression in a variety of cell types making up the stromal tumor compartment. The malignant cells of these same tumors also express high levels of the type I IGF receptor. We believe these results suggest that a paracrine growth mechanism may be of importance in mediating the growth of some neuroblastoma tumors. We are currently investigating the effect of RA induced differentiation on the levels and function of IGF-II in these cells.

28. We have identified IGF-II as an autocrine growth and motility factor in rhabdomyosarcomas (RMS). *In situ* hybridization analysis has demonstrated that over-expression of IGF-II occurs specifically in the tumor cells in virtually all RMS tumors examined. We have cloned regulatory regions of the IGF-II gene from a rhabdomyosarcoma and ongoing experiments are aimed at determining whether there are structural alterations in these *cis*-regulatory regions leading to the disordered regulation of IFG-II expression in these tumors.

29. We have demonstrated that suramin interrupts the IGF-II autocrine growth loop of RMS cells *in vitro* and have initiated a Phase II study of suramin in relapsed RMS patients.

30. Since IGF-II acts as a mitogen in rhabdomyosarcoma cells via the type I IGF receptor, we have created an IGF-I-PE40 oncotoxin

in collaboration with the Laboratory of Molecular Biology, DCBDC. This toxin specifically binds type I IGF receptors and is capable of killing cells bearing such receptors on the cell surface. This toxin molecule has been demonstrated to inhibit the growth of three separate RMS cell lines. We are currently working to improve the binding of the IGF-I-PE40 toxin molecule to type I receptors by structurally modifying this protein. In addition, we have fused the PE40 toxin molecule to a monoclonal antibody that alone is capable of inhibiting the growth of RMS cell lines.

31. We have demonstrated that all-trans retinoic acid inhibits the growth of RMS cells without any evidence of differentiation activity, and this activity appears to be stereo-specific in that 13-cis retinoic acid has no effect on these same cells.

32. We have demonstrated that low dose Ara-C reverses the transformed phenotype and differentiates a RMS cell line. These observations have led to a Phase II study of Ara-C in relapsed RMS patients.

33. We have begun to characterize the frequency and diversity of p53 mutations in RMS cell lines and tumors. To date, 4/5 cell lines have been shown to have homozygous mutations that lead to significant alterations of protein structure. In addition, 2/3 tumor specimens were also found to have significant alterations of p53 including a tumor with complete deletion of both alleles. These data indicate a high frequency of p53 mutations in RMS.

34. Invasive fungal infections are significant and increasing problems of morbidity and mortality in cancer patients and those with AIDS.

- We demonstrated that three potent antifungal triazole compounds (itraconazole, fluconazole, and SCH-39304) were most effective when administered as preventive or early antifungal chemotherapy and have the clinical potential for use in early empirical antifungal therapy.

- We have demonstrated that cilofungin, the model compound of the class 1,3-b-glucan synthase inhibitors, known as echinocandins, is highly fungicidal *in vitro*. As echinocandins are also lethal to *Pneumocystis carinii*, these properties may also impact upon treatment of this organism.

- We found that the novel combination of amphotericin B (AMB) plus fluconazole was more active than the combination of AMB plus flucytosine or the agents used alone in the rabbit model of chronic disseminated (hepatosplenic) candidiasis.

- We developed a novel method of continuous intravenous infusion and simultaneous monitoring of plasma levels of investigational compounds in ambulatory non-tethered rabbits. This method provided a safe, reliable, and well-tolerated method of studying the experimental pharmacokinetics of antimicrobial compounds, immunomodulators, and other compounds with short plasma half-lives in rabbits.

- In order to better understand the effects of corticosteroids on gastrointestinal immunity, we examined the immunological and histological changes in gut-associated lymphoid tissues after intravenous administration of dexamethasone to rabbits. In treated animals, lymphoid domes and follicles were considerably reduced in size, and the dome epithelial layer was markedly depleted of M cells and lymphocytes. There were numerous open lesions at the luminal surface of dome epithelium, consistent with necrosis of M cells, and a striking depletion of follicular B cells in treated animals. These immunologic and histologic effects of corticosteroids could have profound, deleterious effects on mucosal immune responses and host resistance to invasive fungal, bacterial and protozoal infections.

- Recombinant human G-CSF was found to be most effective in the prevention rather than the treatment of disseminated candidiasis in granulocytopenic rabbits. G-CSF was able to shorten duration but not depth of neutropenia. When the *ex vivo* effects of the administration of G-CSF on PMN function were assessed in rabbits in this study, G-CSF was found to enhance superoxide production in response to FMLP and opsonized *C. albicans* blastoconidia as well as the phagocytic and microbicidal activity of PMN against *S. aureus* but not against *C. albicans* blastoconidia.

- In order to further understand the pathogenesis, immunodiagnosis, and treatment of disseminated *Trichosporon beigelii* infection, we developed models of disseminated and gastrointestinal infection in persistently granulocytopenic rabbits. Antigenemia cross-reactive with cryptococcal polysaccharide (described in cases of disseminated trichosporon infection) were reproduced. We further demonstrated the immunohistological origin of cryptococcal antigenemia in invasive trichosporonosis as arising from cell wall and matrix of *Trichosporon beigelii*. Infection developed in rabbits with *T. beigelii* gastrointestinal colonization following cytotoxic chemotherapy.

- We developed a novel model of primary pulmonary aspergillosis in persistently granulocytopenic rabbits. This

model histologically, pathophysiologically, and immunologically closely resembles the human infection of primary pulmonary aspergillosis and permits the study of antifungal chemotherapeutic agents, recombinant cytokines, and markers of invasive disease.

- We characterized the plasma pharmacokinetics and demonstrated the efficacy of a unilamellar formulation of liposomal amphotericin B (LAMB) in our model of primary pulmonary aspergillosis. This system demonstrated that LAMB administered at 5 and 10 mg/kg/d was significantly more effective than conventional desoxycholate amphotericin B (AMB) in improving survival and in preventing pulmonary infarction and hemorrhage due to Aspergillus. The LAMB compound was also less nephrotoxic than AMB.

- We found that the Aspergillus metabolite, d-mannitol, as measured by mass spectroscopy and gas-liquid chromatography is present in serum and bronchoalveolar lavage specimens obtained from persistently granulocytopenic rabbits with primary pulmonary aspergillosis.

- We have developed a new method for measuring phagocytosis of fungi. Whereas conventional methods do not reliably distinguish between intracellular and extracellular but attached fungi, our fluorescent quenching method distinguishes between ingested and attached organisms.

- We assessed the effect of G-CSF and IFN-g on the oxidative metabolic burst (superoxide production) of normal PMNs in response to opsonized or nonopsonized hyphae of *C. albicans* and we compared it with that in response to FMLP and to blastoconidia of the same organism. Both G-CSF and IFN-g enhanced the responses to blastoconidia as well as to hyphae of *C. albicans*, although G-CSF showed some effect at higher only concentrations. Studies of the effects of these cytokines on the PMN- or elutriated monocyte-induced killing of *C. albicans* hyphae are underway.

- In experiments using PMNs from healthy adult donors and hyphae of *Aspergillus fumigatus*, we found that both G-CSF and IFN-g enhance the superoxide production in response to hyphae and the degree of damage caused by the PMNs to the hyphae. In other experiments, we found that both hydrocortisone and dexamethasone in higher concentrations inhibit the antihyphal capacity of normal PMNs but G-CSF and IFN-g appear to correct this steroid-induced defect. The combination of the two cytokines together shows greater effect than each of them separately. Similar studies with elutriated monocytes as effector cells are underway.

- In conjunction with our clinical AIDS trials, we have been evaluating the development of viral resistance to antiretroviral therapy in virus isolates from patients on Pediatric Branch treatment protocols who have received long-term therapy with AZT/ddC or with ddI. While the development of AZT resistance appears to be a common occurrence in this setting, we have not observed high level resistance to either ddC or ddI in HIV isolates.

- We have also been evaluating the activity of antiretroviral therapy in reducing viral load in blood as determined by quantitative viral culture of plasma and peripheral blood mononuclear cells (PBMC's) from patients receiving combination antiretroviral therapy with AZT and ddI in the Pediatric Branch protocol 90-C-09. Preliminary results indicate significant decreases in viral titer in both plasma and PBMC's relative to baseline after 12 to 20 weeks of therapy.

  PMN from HIV-infected children were demonstrated to have significant impairment in their bactericidal capacity against *S. aureus*. *In vitro* incubation of defective PMN with GM-CSF corrected the bactericidal impairment. These findings may help explain the increased incidence of bacterial infections in this population, and suggest a potential therapeutic role for GM-CSF.

- To better understand the humoral deficiency of HIV+ children, we measured serum levels of IgG subclasses in a number of patients and correlated them with the frequency of bacterial infections. No association was found between low levels of specific IgG subclasses and increased susceptibility to bacterial infections. We concluded that other functional parameters rather than quantities of antibodies are more important in the humoral deficiency found in these patients.

- In a retrospective study we analyzed the bacterial infections that occurred in a cohort of HIV+ pediatric patients. The central venous catheters were shown to contribute to increased number of bacterial infections especially in association with younger age and lower CD4 counts. Antiretroviral therapy may have an effect on reducing non-catheter related infections.

- Different patterns of unresponsiveness of T helper cells to recall and allogeneic antigens as well as to PHA were found, and there was a significant correlation between T helper cell dysfunction and the susceptibility to opportunistic and bacterial infections.

Follow-up of the T helper function of these patients during therapy with ddI showed that asymptomatic patients improved significantly more than symptomatic patients, and the improvement observed in the symptomatic patients was associated with fewer opportunistic and bacterial infections. T helper cell function may serve as a surrogate marker of HIV infection during antiretroviral treatment.

- In studies using mononuclear leukocyte cells of monozygotic twin pairs one of which was HIV-infected, we found that the cells of the HIV-infected sibling suppress the T helper function of the cells of the healthy sibling and the suppressive factor is released in the supernatant of the cells without being the virus itself.

RADIATION ONCOLOGY BRANCH

Description

The Radiation Oncology Branch (ROB) is dedicated to multidisciplinary care of patients with malignant diseases. The treatment emphasis is on gliomas, lymphomas, sarcomas, breast, gastric, bladder and lung carcinomas. Photon and electron treatment are used and interstitial techniques have been developed. A major study of interstitial radiation therapy combined with chemotherapy is underway. Our radiology laboratory is investigating basic mechanisms of radiation/drug-induced cell killing, evaluating and developing a completely new class of radiation protectors, and contributing quantitative/biochemical information from biopsies taken from patients on protocol. New methods for integrating CT scanning with simulation are being devised to optimize external beam and radiolabelled antibody treatment planning. Protocols have begun to utilize phototherapy in randomized studies, as well as Phase I studies in different body cavities. Laboratory efforts in phototherapy center around devising new methods of light delivery and evaluation of ultrasound as a source of excitation for phototherapy sensitizers.

Accomplishments

ROB continues to meet its three major goals: (1) major emphasis on clinical trials of a combined modality nature, collaborative with other clinical branches; (2) a strong laboratory program, with heavy emphasis on basic science, radiologic physics; and (3) a training program in Radiation Oncology, equivalent to the stature of the programs of training in the NCI Medicine, Surgery and Pediatric Branches.

Ongoing clinical work focuses on small cell carcinoma of the lung, mycosis fungoides, soft-tissue sarcomas, pediatric sarcomas, ovarian carcinoma, lymphomas and Hodgkin's disease. These will be

presented by other respective Branches, under whose aegis the protocols are carried out.

The ROB's primary clinical study on early-stage breast cancer has been completed. This randomized study with Stage I and II breast cancer patients randomized patients to receive either modified radical mastectomy or definitive radiation therapy, with preservation of the breast. Results suggest comparability between treatment arms. This study differed from the NSABP in that the surgical excision made no attempt to have the margins surgically negative, but simply remove the gross lump. Cosmesis is a major endpoint, in addition to survival and freedom from relapse. These studies were open to patients who have masses up to 5 cm with or without nodes, thereby making breast preservation techniques applicable to the vast majority of women in this country who present with breast cancer. Patients with positive nodes all receive adjuvant chemotherapy. The social aspects of these treatment approaches are being studied, and the follow-up papers are being prepared. From the study of stage III breast cancer patients we have learned that neoadjuvant sterilization of the diseased breast (as shown by biopsy) followed by radiation gives far inferior local control than trimodality therapy. There was no clear difference in overall survival between surgery/radiation versus radiation alone.

As a replacement study, we are beginning to accrue patients with bladder cancer. We have no track record in this population of patients, and it remains to be seen if we will be successful. Our priority will be to try to retain the bladder, dealing with phototherapy for the early stages of bladder cancer and studying combined modality treatment with emphasis on interstitial treatment for invasive carcinoma. As noted above, we have to see if we can recruit these patients effectively.

Halogenated pyrimidines have been investigated as radiosensitizers. Phase I studies have been completed. We were not able, within the context of our protocol, to show a role for high dose hyperfractionation and continuous IV infusion of IdUrd in brain tumors. Our laboratory has been able to give us some possible explanations for these results. Thymidine replacements in tumor cell DNA has been evaluated from 4 patients who received IdUrd infusion for 5-7 days prior to surgery. Replacement values ranged from 0-4%. In vitro studies would suggest that replacement values $\sim$6-10% are required to observe significant radiosensitization. High local control rates in large unresectable sarcoma treated with high dose radiation/IdUrd has been confirmed in a more extensive study. We are now randomizing patients with unresectable sarcomas without metastatic disease to receive the halogenated pyrimidine IdUrd as a radiosensitizer. IdUrd replacement data from this set of patients has thus far

revealed much higher replacement values (7.3-14.2%) than was seen for gliomas. Other patients such as gliomas and unresectable head and neck cancers are being treated on a non-randomized basis at this time, with clinical-laboratory interaction to obtain labeling and uptake information. In a small group of head/neck patients IdUrd replacement values have ranged from 2.9-0.1 perhaps explaining the dramatic tumor response observed in these patients.

The Experimental Phototherapy Section has continued to conduct both clinical and basic research in the application effecting the understanding and curing of cancer. To that end at the basic research there has been interest in extended the means in which phototherapy is conducted. Concentration of effort in energy source potentially, other than the traditionally used laser sources, may provide needed clinical assistance in two critical areas: 1) The treatment of large surface areas quickly and efficiently; 2) The treatment of tumors that are larger than 5-10 mm. Chemiluminescence, that is the use of chemical emitted light, is a potential sources of radiant energy that could activate a sensitizer dye. In particular, the use of chemiluminescent agents have the potential of being easily dispersed over a large surface, such as the peritoneal cavity, the thoracic cavity, or the bladder cavity. The dispersive properties of the chemiluminescent liquid allow for light emission in the intimate boundary of the potential tumor site. Previously only highly lipid soluble agents were available; however, our current interest only extends to water soluble chemiluminescent agents.

There has been continued interest in the laboratory regarding ultrasound and the induction of microcavitation phenomena which results in the formation of highly active free radicals species (hydroxyl and methyl radicals). As a natural consequence of the studies of ultrasound we have considered utilizing radiant energy created curing ultrasound: sonoluminescence. One of the major limitations of currently employed phototherapy is the lack of tissue penetrance; the consequence of which is the inability of efficaciously treating tumors which are greater than 10 mm in depth. If ultrasound were capable of creating sufficient sonoluminescence to activate photosensitizing agents, then the obstacle of tissue would be overcome. It is in this context that ultrasound is being studied and in which very early results appear to in indicate a biologic antitumor effect.

A trial conducted in collaboration with Dr. Harvey I. Pass (Surgical Oncology Branch) was done to demonstrate the efficacy of photoimmunotherapy for the treatment of a human lung tumor implanted into the flank of thymic mice. Chemical conditions were optimized for covalently attaching hematoporphyrin to a monoclonal antibody directed against the xenografted human lung tumor. It was shown that photoimmunotherapy can be an effective antitumor modality, as well as a safe means of site directing the sensitizer

dye and limiting dermal toxicity. Studies are ongoing to establish the efficacy of photoimmunotherapy in the treatment of a model mesothelioma in an athymic rat model.

Collaboration with Dr. Jose Raviv to realize non-classical energy transfer processes continues. The results of studies utilizing iodonaphthylazide (max=320mm) as the receiving agent from sensitizer dye activated at (~500 nm light) indicate that this avenue of study is worth continued exploration.

Studies in collaboration with Paul Smith (BEIB) and Leon Esterowitz (Naval Research Laboratories) continue toward development of ultrasensitive singlet oxygen detectors for use in the Clinical Center. Currently a liquid cooled silicon doped germanium diode with an avalanche response profile appears to be most promising for time-domain resolution of 1268 nm light (signature of singlet oxygen phosphorescence decay).

In the clinical theater PDT continues to be explored as an experimental treatment modality for bladder and ovarian cancer, and malignant mesothelioma. Both murine and canine studies indicated that the treatment of ovarian carcinomatosis with PDT could be an effective modality for minimal disease. A combined Radiation and Surgery Phase I trial has been completed which was designed to test the toxicity of 630 nm laser light in the peritoneum after intravenous administration of DHE and indicates that 7.5 J/M^2 can be administered--toxicity restrictions at this dose of light were associated with bowel tolerance (particularly atanastomosis sites) and item of light delivery.

Several things were learned form the Phase I studies: 1) the technique is feasible, 2) real time light-dosimetry is necessary, 3) 514 nm (green light) is most suitable and safe for the treatment of potential micrometastatic disease of the bowel and mesentery, 4) intralipid (lipid vesicles) are effective but must be free of all but traces of blood to avoid absorbance of 514 and 630 nm light, as well as to limit Tindel effect, 5) liver toxicity is not a limitation, 6) problems associated with dermatophototoxicty are easily manageable and 7) ovarian carcinoma appears to be sensitive to light.

After designing a phantom to study light dosimetry within the thoracic cavity, Dr. Harvey Pass conducted large-animal feasibility and toxicity studies. Once these studies were completed and showed that intrathoracic PDT could be conducted safely, seven patients were evaluated in a pilot feasibility study which led to a Phase I clinical trial (Radiation and Surgical Oncology Branches). To date twenty-six intrathoracic cases have been enrolled into the Phase I Intrathoracic PDT Trial. Of these 26 patients, 17 had pleural mesothelioma considered to be

unresectable by outside surgeons. The dose-limiting light toxicity has not been reached at 32.5 J/cm^2 630 nm laser light. The natural history of disease progression has been delayed and altered. Local recurrence has occurred, but to this point has not been a major clinical issue. Treatment at maximally tolerated 630 nm laser light dose has not been reached in this Phase I study and preliminary clinical results regarding tumor response have been encouraging. There has been no effort to escalate dose by administration of 514 nm laser light.

Studies in the Radiobiology Section have centered on mechanisms of sensitization or protection, resulting from radiation modifiers, and several different chemotherapy drugs. Studies from our laboratory and others have identified the intracellular thiol, glutathione (GSH), as being important in the cytotoxicity of certain chemotherapy drugs and hypoxic cell radiation sensitizers. This finding prompted the study of GSH levels from 27 lung tumor biopsies, comparing them to normal lung GSH. Our findings show that 1) precise GSH measurement of tumor cells are complicated by infiltration of leucocytes (infiltration in some tumors exceeded 40% of the total mass) in lung tumors; 2) normal lung GSH values were remarkably constant among the 27 samples evaluated; 3) several squamous lung cancer samples had populations of tumor cells in the biopsy with 3 to 5 fold higher GSH levels than found in normal lung. The techniques we have worked out should aid researchers who wish to measure GSH levels in tumors--this should be particularly helpful in clinical trials where BSO is being used to deplete tumor GSH prior to melphalan treatment.

We have made considerable progress toward tranfecting cells with inducible genes both in the sense and antisense direction for enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. These transfected cell lines will enable us to possibly delineate the importance of each enzyme when cells are exposed to oxidative stress such as radiation, selected chemotherapy drugs, and photodynamic therapy.

Our laboratory has continued to work on the issue of cross resistance between radiation and chemotherapy drugs. Studies in our lab indicate that cells that are pleotropically drug resistant are not necessarily resistant to radiation therapy. Moreover, such cells are definitely not resistant to photodynamic therapy. We will continue studies on this topic to establish whether this is a general finding among different drug resistant cells.

We have spent considerable effort toward the characterization of a class of stable free radical agents known as nitroxides. We have shown that nitroxides such as Tempol protect cells against superoxide, hydrogen peroxide, and ionizing radiation. Tempol has been shown to protect mice against whole-body radiation and

protect when topically applied against radiation-induced alopecia. The findings of our laboratory establish nitroxides as a completely new class of non-thiol radioprotectors. These agents may have utility in other areas, for example, based upon our findings other laboratories have shown that Tempol protects against ischemia/reperfusion injury in isolated rat heart. Apart from having possible clinical utility, nitroxides will be most useful in studying mechanisms of free radical reactions mediated by radiation and drugs in cells and tissues by virtue of the ability to follow free radical intermediates via electron paramagentic resonance (EPR).

Under the direction of Dr. Otto Gansow, the Inorganic and Radioimmune Chemistry Section has developed the chemistry necessary to produce clinical doses of Yttrium-90 and anti-Tac antibody for use in treatment of T-cell leukemia. In clinical trials led by Dr. Tom Waldmann, all patients treated to date have responded favorably to treatment. A dose escalation study is underway. In other experiments, the alpha-particle emitter Bi-212 has been linked to antibody 103A for use in treatment of erythroleukemia in mice. Complete remission of disease was accomplished with no toxicity as evaluated by histology. Prolonged survival of mice treated with labeled tumor-specific antibody was seen, but not for mice treated with labeled control antibody. This is the first treatment of a systemic cancer by an alpha-emitter.

On other studies, patients have been treated with radiolabeled antibody, but the only antibodies under active study at the present time are B72.3 labeled with Iodine, and T-101 labeled with Yttrium. Several protocols are close to starting, including a Col-1 antibody trial and a Lu-177CC49 pancarcinoma trial, both with the Medicine Branch. We have begun a study on B1 antibody imaging in the treatment of B-cell lymphomas and have accrued 4 patients with scanning data and bone marrow biopsy data, and plan to move to B1 monoclonal therapy in concert with the Medicine Branch. A range of studies are in progress at the bench and animal level to assess the utility of Bi-212 and Pb-212 as possible isotopes for clinical use. CTEP is aware of our efforts to get GMP grade antibodies derived from intramural work into production.

SURGERY BRANCH

Laboratory and clinical efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients.

Significant laboratory accomplishments of the Surgery Branch in the last year:

1. Techniques have been developed for inserting foreign genes into human tumor-infiltrating lymphocytes (TIL) using retroviral-mediated gene transduction. TIL have been successfully transduced with the gene coding for neomycin resistance, and these cells can grow in high concentrations of the neomycin analog G418 that is lethal to all other eukaryotic cells.

2. Genes coding for tumor necrosis factor (TNF) have been successfully introduced into human TIL. These TIL can produce over 100 times the normal level of tumor necrosis factor. Clinical trials utilizing these TNF-modified TIL for the treatment of human cancer are underway.

3. TIL have been isolated from patients with melanoma. These TIL exhibit unique lytic specificity for the tumor from which they were derived and not for other normal tissues or other allogeneic tumors. These TIL have now been characterized as oligoclonal populations of T lymphocytes exhibiting MHC restricted lysis of autologous tumor.

4. Progress has been made in isolating a melanoma-specific antigen recognized by human TIL. A subtracted cDNA library has been prepared from a cultured melanoma line killed by TIL and its variant subpopulation resistant to TIL lysis. This subtracted library has been transfected into the resistant tumor line. Transfection has restored lysability to a number of isolated tumor clones. Characterization of the transferred DNA conferring lysability is in progress.

5. Studies using human TIL derived from melanomas to kill allogeneic HLA-matched melanomas have shown that melanoma-specific antigens recognized by human TIL can be broadly expressed and recognized in an MHC restricted fashion among many patients. Multiple MHC class I determinants were capable of functioning in tumor antigen recognition, including HLA-A, B and C molecules.

6. The transfection of the HLA-A2 gene into allogeneic human melanomas has been shown to result in the lysis of these allogeneic melanomas by HLA-A2 specific TIL. This provides strong additional evidence that melanomas share MHC-restricted tumor antigens recognized by TIL.

7. TIL have potent antitumor reactivity *in vivo*. Non-cytolytic, $CD8^+$ TIL eradicate established lung tumors in mice. Many cytolytic and non-cytolytic $CD8^+$ TIL cultures specifically secrete interferon-gamma and tumor necrosis factor when stimulated with tumor cells *in vitro*. The effectiveness of TIL when adoptively transferred to mice bearing

micrometastases correlates better with their ability to specifically secrete cytokines in vitro than with their cytotoxicity in vitro.

8. Studies have shown that some melanoma and breast carcinoma TIL cultures, are capable of secreting the cytokines TNF-α, GM-CSF, and/or IFN-g specifically on contact with autologous or HLA-matched tumors. This has established cytokine release as a specific indicator of human T cell recognition of relevant tumor antigens.

9. Tumor cells may evade T cell immunosurveillance as well as T cell-based immunotherapy via defects in antigen processing or presentation. Defective antigen presentation of endogenous antigens by a murine sarcoma, MCA-101, has been identified by its failure to present endogenously synthesized influenza virus antigens to specific CTL.

10. We have found that enriched $CD4^+$ cell populations from MCA-203 tumor immune murine splenic T cells proliferate strongly and specifically to tumor pulsed Langerhans cells.

11. IL-7 has been shown to exhibit potent T-cell growth activity in vitro in the generation of allospecific as well as tumor specific CTL in mice. IL-7-generated tumor-specific CTL have potent antitumor effects in vivo upon adoptive transfer to mice with established pulmonary metastases.

12. Genes coding for IL-2 and TNF have been transduced into mouse and human tumors and lead to the secretion of large amounts of cytokine. Mouse tumors secreting IL-2 and TNF are more immunogenic than the corresponding unmodified tumors.

13. Substantial tumor regression can be mediated by the systemic administration of IL-6 in mice. This antitumor effect requires the participation of host-derived $CD4^+$ and $CD8^+$ T cells. IL-6 induces the generation of potent, specific antitumor cytotoxic T cells in vivo.

14. Soluble factors chemotactic for TIL have been demonstrated in fresh murine tumors. Those chemotactic factors are being characterized.

15. IL-6 in collagen matrix has been shown to be a beneficial adjuvant in the generation of murine TIL.

16. TIL cells have been shown to have substantial functional longevity in vivo and these long term persisting cells manifested memory T cell functions.

17. TIL have been shown to proliferate in vivo under the influence of exogenous interleukin-2 and this proliferation in vivo continues for as long as interleukin-2 can be administered to the animal. These studies have relevance to ongoing clinical trials of TIL in patients with cancer.

18. Specific increases in intracellular calcium have been demonstrated in TIL following stimulation by a cellular stimulus using flow cytometry. This allows the sorting of subpopulations of responsive T cells within bulk cultures of tumor-infiltrating lymphocytes and has potential relevance to TIL therapy as well as numerous other avenues of investigation in T-cell immunology.

19. Pretreatment with TNF-α or IL-2 protects rodents against the lethality, hypotension and hypothermia of gram negative sepsis.

20. The beneficial effects of either IL-1 or TNF-α are induced following a single iv dose of cytokine and protection is associated with induction of the protective enzyme manganous superoxide dismutase in tissue.

21. A specific receptor antagonist to IL-1 can block the lethality of endotoxin when given after a lethal dose of endotoxin (LPS).

22. Interferon-γ mediates part of the lethality of both TNF and LPS because specific antibodies to it block the lethality of TNF and LPS.

23. In order to identify the kidney cancer disease gene we have determined loss of heterozygosity on the short arm of chromosome 3 in 51/58 sporadic renal cell carcinomas and have localized the location of this disease gene to the 3p21-26 locus.

24. In order to evaluate loci of other tumor suppressor genes which might be involved in progression of this malignancy we evaluated loss of heterozygosity on chromosome 13 at the retinoblastoma locus, chromosome 17 at the p53 locus and nm23 and chromosome 18 at the DCC (colon cancer) locus. By RFLP analysis we determined loss of heterozygosity in 13/27 (48%) of the cell lines at the p53 locus, 3/17 (18%) at the nm23 locus, 45% at the retinoblastoma locus and 6/15 (40%) at the DCC locus. These studies suggest that abnormalities of tumor suppressor genes at other loci may be involved in progression of this malignancy.

25. In a study performed in order to clone the disease gene for familial kidney cancer we have evaluated over 500 members of

42 kindreds of patients affected with von Hippel-Lindau syndrome, 240 of whom underwent clinical evaluation at the NIH. We have localized the VHL disease gene between RAF-1, a protooncogene at 3p25, and D3S18, an anonymous marker at 3p26 and are currently sequencing a conserved disease gene candidate cDNA at 3p25.5.

26. In an evaluation of the effect of suramin, an antigrowth factor agent, we have demonstrated that suramin inhibits proliferation of human prostatic carcinoma and that the combination of suramin and TNF has more in vitro antitumor activity than either agent alone. In a study of the paracrine effect of tumor produced growth factors and lymphokines we have shown that prostate carcinoma inhibits the bone resorbing activity of these agents. We have participated in the clinical trials evaluating the antitumor effect of suramin in patients with advanced prostate carcinoma.

27. The increased efficacy of photodynamic therapy in vitro and in vivo by conjugation of the sensitizer to a monoclonal antibody for increased targeting has been demonstrated.

28. Free radical generating systems (hydrogen peroxide or superoxide) can cause peritoneal exudate cells to secrete tumor necrosis factor and certain chemotherapies, specifically cisplatin and mitomycin-c, can cause peritoneal exudate cells to amplify TNF production.

29. Spin traps acting as superoxide dismutase mimics, such as TEMPOL, can protect in vitro against tumor necrosis factor toxicity.

30. Pretreatment of lung cancer cells with TNF will make them more resistant to a subsequent challenge with a free radical generating stress (superoxide) or to adriamycin cytotoxicity, possibly partially through induction of MnSOD.

31. Techniques for laparoscopic delivery of intra-abdominal photodynamic therapy have been developed in experimental animals.

32. The long-term effects of intraoperative radiotherapy on abdominal and retroperitoneal tissues in experimental animals has been evaluated.

33. Demonstrated that TNF and IL-1 induce TNF gene expression and TNF production but do not act synergistically in growth inhibition of human breast cancer cells.

34. Demonstrated that TNF blocks estradiol stimulated growth, modulates steroid receptor metabolism, and increases insulin-like growth factor secretion in breast cancer cells.

35. Demonstrated that IL-1 antagonizes estradiol stimulation of growth and cell cycle progression but not estrogen regulated metabolism, and that this may be mediated by increased transforming growth factor-beta secretion in breast cancer cells.

Significant clinical accomplishments of the Surgery Branch include the following:

1. The first clinical trials of the gene therapy of cancer have begun using tumor infiltrating lymphocytes (TIL) transduced with the gene for tumor necrosis factor (TNF). Thus far four patients have been treated without toxicity.

2. The first gene transfer trials conducted in man have been completed. Ten patients with advanced melanoma have received treatment with autologous TIL modified with the bacterial gene coding for neomycin phospho-transferase, which confers resistance to the antibiotic neomycin. These studies have shown that gene-modified cells can survive up to 189 days in the circulation and up to 64 days at the tumor site. No toxicity has been seen in patients due to the gene transfer.

3. Clinical trials with lymphokine activated killer cells and high-dose interleukin-2 (IL-2) or the administration of high-dose IL-2 alone have demonstrated, in over 350 patients, that approximately 10% of patients with metastatic melanoma and renal cell cancer can undergo a complete regression of all cancer and approximately 1/4 to 1/3 of patients will undergo a 50% or greater regression of malignancy.

4. A prospective randomized study has entered 181 patients to compare the use of lymphokine activated killer cells plus IL-2 to treatment using IL-2 alone in patients with advanced cancer. This study has shown a tendency towards increased survival in melanoma patients receiving LAK cells and IL-2 ($p2 = .06$). IL-2 and LAK cells plus IL-2 appear to be equivalent in the treatment of patients with advanced renal cell cancer.

5. Pilot trials utilizing TIL plus IL-2 in 50 patients with advanced melanoma have shown that approximately 38% of all patients with advanced melanoma will show objective regression of malignancy. Equal response rates are also seen in patients that have previously failed other regimens utilizing high dose IL-2.

6. Extensive trials in humans with advanced cancer have been conducted using the administration of interleukin-4. Interleukin-4 causes a dose-related increase in vascular leak, nasal congestion and gastritis. In studies using interleukin-4 alone, antitumor effects have not been seen.

7. We have completed a phase I trial of the combination of 5-FU, leucovorin, and interleukin-2 for colorectal cancer, demonstrating safety and efficacy.

8. We have completed a phase I trial of PEG-IL2, and a randomized trial evaluating its efficacy in combination with IL-2 in the treatment of melanoma and renal cell cancer. Follow-up is in progress.

9. Phase I clinical trials evaluating the therapeutic efficacy of M-CSF have been initiated.

10. We are evaluating in a prospective, randomized protocol the benefits of radiation therapy in the local control of extremity high-grade sarcoma in patients receiving adjuvant chemotherapy.

11. A Phase I trial of the use of intrapleural-intraoperative photodynamic therapy for pleural malignancies has begun which, at present, includes 26 patients with light dose escalation from 15 Joules/cm^2 to 32.5 Joules/cm^2.

12. A Phase III trial of neoadjuvant therapy is being conducted in patients with non-small cell lung cancer comparing surgery and postoperative radiation therapy to preoperative chemotherapy followed by surgery and postoperative chemotherapy for Stage IIIA disease.

13. Investigations of the use of endobronchial photodynamic therapy for the management of endobronchial obstruction are underway.

14. A prospective trial was performed in order to evaluate if presymptomatic detection of VHL disease by DNA-polymorphism analysis could be performed. In 47/48 asymptomatic, at risk family members there was concordance between the DNA polymorphism analysis and the clinical evaluation. This is the first study demonstrating the effectiveness of presymptomatic molecular diagnosis of this familial cancer syndrome.

15. Demonstrated that patients who present with metastatic gastrinoma in the context of sporadic Zollinger-Ellison syndrome (ZES) (approximately 25% of the population of patients with ZES) have a 20% 5 year survival rate. Patients who present with localized disease and undergo surgery to resect it have a 59% chance of being cured of ZES at initial evaluation and a 38% chance of long-term cure.

16. A prospective randomized clinical trial evaluating the efficacy of intensive preoperative chemotherapy for the treatment of patients with stage II breast cancer is underway.

17. Demonstrated that the level of axillary lymph node metastases from stage I-II breast cancer is not an independent predictor of survival.

18. Completed Phase I trial of intra-abdominal photodynamic therapy for patients with disseminated intraperitoneal malignancies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07209-03 CO

PERIOD COVERED
October 1, 1990 through September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Administration of 2',3'-dideoxyinosine (ddI) for severe HIV infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Robert Yarchoan, M.D., Senior Investigator, Clinical Oncology Program, NCI
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Jill Lietsau, R.N., Medicine Branch, NCI

COOPERATING UNITS (if any)

DCT, DTP: Dr. Neil Hartman, Dr. David G. Johns

LAB/BRANCH
Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A Phase I trial of 2',3'-dideoxyadenoxine (ddA) was initiated in February of 1988. ddA is a pro-drug of 2'3'-dideoxyinosine (ddI). Whereas ddA is metabolized in the stomach adenine (which can cause renal toxicity). In contrast, ddI is metabolized to hypoxanthine. Thus, ddI appeared to be the preferred form for oral use. With this background, a Phase I trial of ddI was initiated in July of 1988. By July of 1989, it was apparent that: 1) the maximum tolerated dose for long-term therapy was approximately 10 mg/kg/day; 2) doses of 3 to 10 mg/kg were associated with anti-HIV activity; 3) dose-limiting toxicities were painful peripheral neuropathy, pancreatitis, and hepatitis; 4) doses of 3 to 10 mg/kg/day were well-tolerated in the majority of patients with AIDS or AIDS-related complex and were associated with long-term clinical and laboratory improvement. Based primarily on the results of this study (with supportive evidence from 2 other Phase I studies), 3 Phase II/III trials of ddI, sponsored by the NIAID and Bristol-Myers Squibb Company were launched in October of 1989 in medical centers around the country. In addition, the FDA enabled patients who could not tolerate AZT or had failed AZT to receive ddI under the mechanisms of a Treatment IND or open label protocol, respectively. At present, more than 10,000 patients have received ddI throughout the United States under these protocols. We are continuing to follow our Phase I patients receiving ddI. We have learned that survival can be excellent with this drug - 80% of AIDS patients entered on the study are alive at 20 months. In addition, we have observed that patients with AIDS dementia can have improvement on ddI. Finally, we have observed that patients with extensive prior AZT use have limited CD4 rises on ddI, whereas they do respond with decreases in HIV p24 antigen. We are now exploring the combination of ddI and DHPG (a drug used for retinitis) and ddI used with interferon.

INTRODUCTION

The purine analogue 2′,3′-dideoxyinosine (ddI) was found to have activity against HIV *in vitro*. Upon being activated to dideoxyadenosine-5′-triphosphate, it is believed to act at the level of reverse transcriptase. Several properties of ddI made it suitable for clinical testing: it had a comparatively low toxicity for T cells and bone marrow progenitor cells; it had potent anti-HIV activity in monocytes; and once activated to the active triphosphate moiety, it could remain in cells for a long time (half life over 12 hr). With this background, we instituted a Phase I study of ddI in July 1988 in patients with AIDS or ARC who had less than 4 months of prior therapy with AZT. ddI was found to be well absorbed when given orally with antacids and was well-tolerated for short-term therapy. In addition, most patients receiving doses of 3.2 mg/kg/day or greater had short-term clinical, immunologic and virologic improvement. However, therapy of patients with AIDS or earlier HIV infections will probably have to be administered for months or years. We have thus extended this Phase I trial to evaluate the long term activity and toxicity of ddI in patients with AIDS or ARC. We have also examined its effect on patients with prior long-term AZT therapy or who had HIV-induced cognitive impairment.

MATERIALS AND METHODS

Patients

Three groups of patients with AIDS or ARC (total 75 patients) were studied: (1) 37 patients who entered the original dose-escalating Phase I study of ddI starting in July 1988. All but 2 had 4 or less months of prior AZT therapy, (2) 5 patients originally treated with intravenous 2′,3′-dideoxyadenosine (ddA), a pro-drug of ddI, starting in March 1988, and switched to oral ddI in August 1988, (3) 33 additional patients entered on an extension of the Phase I study starting in July of 1989. Preference was given in this third group to patients who had had more than 4 months of AZT therapy or who had HIV-induced cognitive dysfunction.

The median number of $CD4^+$ cells at entry was $47/mm^3$ (range 4 to 267). All patients had a hemoglobin of $\geq$8.5 g/dl and $\geq$600 neutrophils/mm^3. Most patients received no antiretroviral therapy during the 4 weeks prior to entry.

Administration of ddI

The protocol was approved by the Institutional Review Board of the National Cancer Institute, and all patients gave informed consent. ddI was provided by the Developmental Therapeutics Program of the National Cancer Institute and Bristol-Myers Squibb Company. After a 2-week period of intravenous dosing, the 37 patients who comprised the original dose-escalating Phase I trial received ddI at oral doses ranging from 0.8 to 51.2 mg/kg/day, divided into 2 or 3 daily doses. Since ddI is acid labile, oral drug was administered to fasting patients either with antacids or in a citrate/phosphate/sucrose ddI formulation. The pharmacokinetic profile of this latter formulation was similar to that of ddI given with antacids. As

the trial proceeded, patients initially entered on the lower dose groups were escalated up to a maximum of 9.6 mg/kg/day (except patients who started at 6.4 mg/kg/day were maintained at that dose). All patients on higher doses had their dose reduced to 19.2 and later to 6.4 - 9.6 mg/kg/day when it became apparent that higher doses were associated with a high incidence of toxicity. Patients generally received suppressive therapy for P. carinii pneumonia when appropriate.

Monitoring of patients

Patients were followed for up to 3 years on ddA and ddI. The patients were closely monitored for clinical and laboratory changes. Most had more than one determination of lymphocyte subsets and HIV p24 antigen during the 3 weeks prior to therapy; in such cases, mean values were used as the time zero determination. To assess aspects of neuropsychological function known to be affected by HIV, we used the Wechsler Memory Scales to assess memory, the Trail Making Test to evaluate psychomotor speed and attention, and, in one impaired patient, the Mattis Dementia Rating Scale. Mood was assessed with the Beck Depression Inventory.

Statistics

The significance of changes was assessed by the two-sided Wilcoxon signed rank test for paired observations, $p<0.05$ being regarded as significant. The influence of prior AZT therapy on the changes in CD4 cells was assessed using the two-sided Wilcoxon rank sum test, and the influence of initial CD4 cells and the influence of ddI dose on this effect was analyzed using linear multivariate analysis. The method of Kaplan and Meier was used to analyze survival and the relationship of ddI toxicity to time on drug.

RESULTS

Anti-HIV Activity of ddI

In the initial dose-escalating Phase I study, we found that patients receiving 3.2 to 51.2 mg/kg/day of ddI by the oral route had an increase in the total lymphocytes; enhanced delayed type hypersensitivity; and where evaluable, a decrease in HIV p24 antigen during the first 10 weeks of therapy. As will be discussed below, side effects were most frequent in patients receiving more than 9.6 mg/kg/day of ddI. Thus, doses of 3.2 to 9.6 mg/kg/day appeared to be tolerated and have short-term activity.

As experience was gained with higher doses of ddI, patients originally entered on low doses were escalated up to a maximum of 6.4 or 9.6 mg/kg/day. Thus, the 13 patients who entered at 3.2 to 9.6 mg/kg/day can provide data on the long-term activity of ddI at doses which may be clinically feasible. These 13 patients have received a median of 17 months of ddI (range 8 to 20 months), and all but three are still on drug. The changes in $CD4^+$ lymphocytes and total lymphocytes in these patients is shown in Table 1. They had a mean entry CD4 count of 157 $\pm$ 26 (mean $\pm$ SEM). Upon receiving ddI, they had a maximum mean increase in $CD4^+$ lymphocytes of 78 $\pm$ 31 entry. Statistically significant increases

persisted for at least 9 months. Of the 13 patients, 6 had detectable serum p24 antigenemia at entry (mean 220 pg/ml). Four of these 6 patients were still receiving ddI 15 months after entry, and 3 of these 4 patients had undetectable p24 antigen at that time. None became p24 antigenemic on ddI. Thus, the immunologic and virologic improvements in patients receiving ddI can persist for at least 9 months, and in some patients at least 15 months.

In the initial escalating dose study, we preferentially entered patients who had received AZT for 4 or else months. We subsequently entered additional patients at doses of 6.4, 9.6 and 19.2 mg/kg/day, in part to evaluate the effect of prior AZT therapy. Pooling these with the patients from the original Phase I study who started at those doses, the evaluable patients at these doses overall had an increase in $CD4^+$ lymphocytes during the first 10 weeks of study from 115 $\pm$ 20 cells/mm^3 (mean $\pm$ SEM) to 165 $\pm$ 29 cells/mm^3 (P=0.0096). However, the patients who had previously received AZT for less than 4 months had a significantly greater percentage increase in CD4 cells than those with >4 months prior AZT therapy (P=0.00270). This effect of prior AZT therapy was still discernable if the absolute increase was examined or if we corrected for the effect of initial CD4 cell number and the dose of ddI using multivariate analysis. In the patients who had antigenemia, the serum p24 antigen fell from 154 x/: SEM) at entry to 52 x/: 1.27 pg/ml at week 10 (P=0.0049). Substantial decreases in HIV p24 antigen were observed both in patients who had little prior AZT therapy and in those with prior long-term therapy.

In three patients where this was examined, the mean CSF:plasma ratio of ddI concentration 1 hr after an intravenous dose was 0.21. With this background, we examined the effect of ddI on 4 patients who had cognitive dysfunction compared to what one would expect based on their educational and professional backgrounds. Each of these patients, who generally received 9.6 or 19.2 mg/kg/day of ddI, had improvement during 6 to 12 weeks on ddI. The mean Memory Quotient increased from 97 (range 80 - 113) at entry to 110 (range 94 - 121) after 6 to 12 weeks of therapy. Also, mean performance on the Trailmaking B test, a measure of attention and psychomotor speed, improved from 106 seconds (range 152 - 78) at entry to 90 seconds (range 117 - 68). A fifth patient, who was too impaired to utilize these tests, had improved performance on the Mattis dementia scale from -4.83 SD below the mean of a normal elderly population to -1.64 below the mean after 6 weeks on ddI. These improvements could not be attributed to changes in mood.

Toxicities and Other Clinical Observations

At high daily doses of ddI, painful peripheral neuropathy, sporadic pancreatitis, and sporadic hepatitis were dose-limiting toxicities. In all, 12 patients developed peripheral neuropathy, 3 developed frank pancreatitis, 2 developed mild pancreatitis, and 2 (both with hepatitis B infection) developed hepatitis. In addition, 3 patients seized; however, each was found to have an underlying cerebral disorder, and the relationship to ddI therapy is unclear. No patient died of toxicity. Patients receiving more than 9.6 mg/kg/day had a relatively high

probability of developing neuropathy, pancreatitis, or hepatitis during the first 6 months of therapy (Figure 1). By contrast, only 3 of 35 patients developed toxicity on doses of 3.2 to 9.6 mg/kg/day of ddI for up to 21 months of therapy (median 10 months) ($P<0.00001$ for dose effect). This differential toxicity rate is even more striking if one considers that the 35 patients in the latter group received 6.4 or 9.6 mg/kg/day most of the time.

Ten of the 12 patients who developed ddI-associated neuropathy had received high doses of ddI (at least 12.8 mg/kg/day) and had a cumulative total dose of at least 1.5 gm/kg of ddI. By contrast, only 2 of 35 patients receiving lower daily doses developed neuropathy in spite of receiving up to 4.4 gm/kg. Thus, dose intensity is a crucial determinant of ddI neuropathy. The neuropathy generally appeared as a painful or burning sensation in the feet, intermittent at first but then becoming more constant. We found that if ddI was withdrawn when the pain became mild to moderate in intensity and lasted for several hours, the neuropathy generally subsided within several weeks. In 6 patients, ddI was restarted without recurrence.

Frank pancreatitis was observed in 3 patients: 2 receiving 19.2 mg/kg/day, and 1 on 25.6 mg/kg/day. One patient required admission to the intensive care unit. In each case, the pancreatitis resolved rapidly without sequelae. Two of the patients had histories of ethanol abuse. In one, pancreatitis was preceded by marked triglyceride elevation (> 800 mg/dl) which may have been a harbinger of the pancreatic damage. Two patients were subsequently restarted on 6.4 mg/kg/day of ddI without pancreatitis recurring. Two additional patients taking 9.6 mg/kg/day developed what may have been mild pancreatitis. Six patients developed transient asymptomatic amylase evaluations (>1.5 X upper limit of normal); drug was transiently held in two of these patients. In addition, several patients had more persistent amylase elevations; in two cases this was associated with xerostomia. Fractionation revealed predominantly salivary isoamylase. The significance of this finding is unclear at this point.
Several milder reactions were observed including anxiety, insomnia, increased irritability, and headaches; none of these effects required drug discontinuation. Two patients became confused after taking triazolam (Halcion) with ddI; this cleared upon temporarily stopping both drugs and did not recur upon readministration of ddI alone. Two patients developed mild erythematous macular eruptions. Most patients receiving more than 9.6 mg/kg/day had asymptomatic increases of 0.5 to 5 mg/dl of uric acid, almost certainly from ddI catabolism. Several patients reported transient abdominal pain or vomiting not associated with hyperamylasemia and not requiring drug discontinuation. Several patients developed diarrhea which appeared to be from the buffer formulation (magnesium-based antacids or citrate/phosphate/sucrose vehicle) rather than from the ddI itself, and patients on average had a slight decrease in serum potassium (mean 0.11 meq/L) upon being switched to the citrate/phosphate/sucrose ddI formulation. Finally, about a third of the patients had elevations of their serum triglycerides above 500 mg/dl at some point.

More than half the patients reported increased energy, reduced sleep or nap requirements, and improved appetite on ddI. On average, they gained 1.4 kg when started on therapy.

In all, 19 patients died as of June of 1991. The median survival of the AIDS patients was approximately 27 months, while that of the ARC patients is over 30 months.

Earlier work with ddI suggested that ddI can improve immunologic and virologic parameters during short-term therapy of patients with AIDS or ARC. We now extend those results and show that whereas high doses can be associated with serious toxicities, many patients with AIDS or ARC can tolerate up to 9.6 mg/kg/day for at least 30 months without developing such toxicity. In addition, the data suggest that doses of 3.2 to 9.6 mg/kg/day of ddI are associated with virologic and immunologic improvements which can be sustained for at least 9 months. However, while patients with extensive prior AZT therapy may have a decreased viral load on ddI, these patients may have only a slight or absent rise in CD4 cells. Finally, the results demonstrate that ddI can improve cognitive dysfunction in certain patients.

The relationship of ddI toxicity to dose intensity is noteworthy. While toxicity often developed within 6 months in patients receiving more than 9.6 mg/kg/day, lower doses could be tolerated for long periods of time in most patients. Even at the highest doses tested, bone marrow suppression was not prominent, and in fact, hematologic parameters have been observed to improve during ddI administration. It is worth noting that we do not understand the mechanism(s) for ddI toxicities, and one cannot necessarily extrapolate from other drugs causing similar reactions. Azathioprine (Imuran) or 6-mercaptopurine, for example, causes acute pancreatitis in 5% of patients in certain settings. Once pancreatitis occurs with these drugs, rechallenge with lower doses will nearly always induce a recurrence. By contrast, the two patients with ddI-induced pancreatitis who were rechallenged did not have a recrudescence of the pancreatitis. Clinicians should bear in mind that the principal toxicities of ddI (neuropathy, pancreatitis, or hepatitis) may be found in AIDS patients as a result of underlying HIV infection, secondary complications or other medications. (Indeed, ddI has been reported to reverse HIV-induced neuropathy in certain patients.) In particular, cytomegalovirus infection can cause both painful peripheral neuropathy and pancreatitis, and other drugs commonly used in AIDS (such as pentamidine, even given by aerosol, or trimethoprim/sulfamethoxazole) can cause pancreatitis.

It will be important to learn how to best prevent and manage ddI toxicities. Pancreatitis in particular can be a life-threatening complication; as of March, 1990, 7 of approximately 8300 patients with AIDS or severe ARC receiving ddI in clinical trials or in an "expanded access" program died of pancreatitis. Many of these patients were AZT-intolerant and had advanced AIDS, and more needs to be learned about the factors contributing to pancreatitis in this setting. It is clear from the data in this study, however, that attention to dose intensity is a crucial parameter in reducing the risk of toxicities from ddI. Also, until more is know, patients on ddI should avoid alcohol and other drugs associated with

pancreatitis or neuropathy. For example, ddI should be temporarily stopped if patients receive intravenous or intramuscular pentamidine. (However, it would be impractical to avoid using ddI along with aerosolized pentamidine, particularly as little drug is absorbed from the lungs.) We now measure amylase and triglyceride levels and temporarily hold ddI when the amylase rises to 1.5x the upper limit of normal or when the triglyceride levels rise above 700 ug/dl in patients starting with normal triglycerides; ddI is then reinstituted when the levels approach normal. Transient asymptomatic elevations of triglycerides or amylase are often seen in HIV-infected patients, but do not constitute clinical pancreatitis. As noted, two patients on ddI had xerostomia and elevated salivary isoamylase, and this finding will bear further study. In regard to neuropathy, we now stop ddI when patients develop foot pain of mild to moderate severity and several hours duration. Finally, until more data emerge, we would avoid the use of cimetidine or ranitidine (which could enhance ddI absorption or cause pancreatitis in their own right) and triazolam (which may have caused confusion in two patients). The specific formulation is also important. As noted, the buffers used to effect absorption of this acid-labile drug can cause symptoms independent from ddI. The citrate/phosphate/sucrose vehicle may cause substantial diarrhea in certain patients (and even decreased potassium) which is probably unrelated to the drug itself. Additional formulations are now being examined.

Overall, the results of this trial suggest that certain doses of ddI (3.2 to 9.6 mg/kg/day) may be tolerated in most patients for long-term therapy and are associated with evidence of anti-HIV activity. However, the optimal dose of ddI will not be known without further study. Indeed, the optimal dose of AZT is still under discussion 3 years after its approval as an anti-retroviral drug. Finally, while relatively few patients died on this study, it should be stressed that this extended Phase I study cannot conclusively address the questions of whether ddI reduces disease progression or mortality in patients with HIV infection, or whether it is superior, equal, or inferior to AZT as an anti-AIDS drug. The resolution of these questions will require carefully controlled clinical trials.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07210-02 CO

PERIOD COVERED
October 1, 1990 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of NHL in the setting of severe HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lymphoma is a well known complication of HIV infection. Previous studies have identified several mechanisms of B cell activation in patients with HIV infection which may lead to lymphoma: 1) increased numbers of Epstein-Barr virus infected B cells; 2) T cell dependent polyclonal B cell activation induced by HIV; 3) antigen-specific B cell activation by HIV; 4) increased IL-6 production induced by HIV. We have recently observed that 8 of 55 patients (14.5%) with AIDS or severe ARC entered onto three long-term phase I trials of azidothymidine (AZT) or AZT-based regimens at the NCI between 1985 and 1987 developed a high-grade non-Hodgkin's lymphoma (NHL), B cell type, after initiating antiretroviral therapy. The NHL occurred a median of 23.6 months after initiating antiretroviral treatment. The estimated probability of developing lymphoma by 24 months of therapy was 12%, increasing to 29% after 36 months. The patients who developed NHL had a median of 6 CD4 cells/mm^3 at the time of NHL diagnosis. There was a statistically significant difference in the rate of development of NHL during the time the patients had 50 CD4 cells compared to the time they had 50 CD4 cells, independent of the time they received antiretroviral therapy. Patients with symptomatic HIV infection who survive for prolonged periods while severely immunosuppressed have a relatively high probability of developing lymphoma. We are currently examining a number of parameters to determine which factors are predictive for the subsequent development of NHL. As improved therapies for the treatment of HIV and its complications result in prolonged survival, NHL may become an increasingly significant problem. In collaboration with Dwight Kaufman, M.D. in the Radiation Oncology Branch, we are conducting a trial of combination chemotherapy with AZT and GM-CSF. Preliminary results suggest that some patients may respond to this regimen but that toxicity can be a problem.

Introduction

In 1981, acquired immune deficiency syndrome (AIDS), a disorder now know to be caused by infection with human immunodeficiency virus (HIV), was first recognized as a new illness occurring in individuals in certain risk groups. Soon after their recognition of AIDS as a new disease, the clustering of high-grade non-Hodgkin B cell lymphomas in individuals in these same risk groups and infected with HIV was noted. These lymphomas frequently occurred in extranodal sites, particularly the central nervous system. In 1985, the definition of AIDs was expanded by the Centers for Disease Control (CDC) to include high grade non-Hodgkin lymphoma as an AIDS-defining illness in certain clinical settings. At present, approximately 3% of new AIDS cases have non-Hodgkin lymphoma as their AIDS-defining illness. The incidence of HIV-associated non-Hodgkin lymphoma continues to rise.

The occurrence of non-Hodgkin lymphoma in other settings of immunosuppression has been recognized for years. Indeed, the inter-relationship between immunodeficiency and cancer has been a major focus of research for several decades. In 1973, the Immunodeficiency Cancer Registry was established to maintain a central registry of malignancies that develop in patients with genetically determined immunodeficiency diseases. As of 1987, nearly 500 such cases have been reported, of which 50.7% are non-Hodgkin lymphoma. In addition, an increased incidence of neoplasms has been documented in patients iatrogenically immunosuppressed following organ transplantation. Thirty-sic percent of such neoplasms are non-Hodgkin lymphoma. Many of these tumors have unusual sites of presentation, including the central nervous system. Thus, it would appear that non-Hodgkin lymphoma occurs with substantially increased frequency in the setting of immunosuppression, particularly in patients with dwefects in T cell function.

The course of HIV infection is changing as a result of therapeutic advances. In particular, the life expectancy of patients with HIV infection is presently increasing as a result of improved therapies both for the treatment of HIV-associated infectious complications and of HIV infection itself. Relatively little information now exists on the incidence of lymphoma in AIDS patients who are followed longitudinally, particularly since the advent of antiretroviral therapy.

The National Cancer Institute (NCI) has a cohort of patients that has received anti-HIV therapy for an extended period of time. These patients represent some of the earliest recipients of zidovudine (azidothymidine, AZT) and zidovudine-containing regimens, and as such may provide a database of what may be observed with the widespread use of such therapies. We have observed the development of non-Hodgkin lymphoma in an unexpectedly high number of these patients, particularly those who were long-term survivors with decreased CD4 lymphocytes. It is possible that an increased cumulative incidence of such lymphomas may be an ironic by-product of prolongation of survival by effective antiretroviral therapy.

Materials and Methods

Patients: We examined the charts of 55 HIV-seropositive patients receiving long-term dideoxynucleoside antiretroviral therapy entered into three Phase I studies in the Clinical Oncology Program (COP) of the NCI from 1985 to 1987. The studies were (1) the Phase I study of zidovudine alone (29 patients); (2) a pilot study of zidovudine with simultaneously administered acyclovir (8 patients); and (3) a pilot study of zidovudine alternating with 2',3'-dideoxycytidien (18 patients). These were the only studies initiated during that period in which patients administered antiretroviral therapy were followed for more than six months. All of the patients in these three trials had either AIDS or AIDS-related complex, the latter having either oral candidiasis or greater than 10% weight loss. All patients had less than 350 T4 cells/mm^3 at the time of entry. Patients with a Karnofsky Performance score less than 70, active opportunistic infections, or an expected survival of less than three months were not included. In general, these were patients with AIDS or poor-prognosis AIDS-related complex who were clinically stable at the time of entry.

Of these 55 patients, 8 developed non-Hodgkin lymphoma. In seven of the patient, the diagnosis was made antemortem, while one patient was diagnosed postmortem. Each of those patients diagnosed antemortem underwent staging evaluation and treatment at the NCI with combination chemotherapy, radiation therapy or a combination of these modalities.

Patient evaluations: All patients on these studies were followed at the Warren G. Magnuson Clinical Center of the National Institutes of Health (NIH), Bethesda, Maryland. Clinical and laboratory evaluation were performed every two to four weeks, and patients were admitted for evaluations and treatment when medically indicated. In all patients, lymphocyte subsets reacting to Leu 3 (CD4+, T4+, helper-inducer T cells) or to Leu 2 (CD8+, T8+, suppressor-cytotoxic T cells) were periodically analyzed by flow cytometry. The 1987 revised CDC criteria for AIDS was used in defining the onset of each patient's AID-defining illness.

Immunophenotyping: The immunologic phenotype of the lymphoma cells was determined by immunostaining with monoclonal antibodies to antigens expressed on B cells, T cells, and mononuclear phagocytes. In six cases (patients 2, 4-8) immunostaining was performed on paraffin sections using the avidin-biotin-complex immunoperoxidase technique as previously described. Cells were stained for the B cell associated antigen, L-26, the T cell associate antigen, UCHL-1, and lysozyme. In patients 1 and 3, immunostaining was performed on air-dried cytocentrifuge preparations from the lung aspirate and peritoneal fluid respectively. In patient 3, cells derived from the pleural fluid were stained with monoclonal antibodies and subjected to phenotypic analysis using a flow cytometer and the avidin-biotin-complex immunoperoxidase technique applied to cytocentrifuged preparations. An extensive battery of monoclonal antibodies directed against T cells, B cells, mononuclear phagocytes, and myeloid cells was employed. Both a skin biopsy of recurrent large cell immunoblastic lymphoma and a brain biopsy showing small non-cleaved cell lymphoma from

patient 3 were also subjected to immunoperoxidase staining on paraffin sections.

Statistical Analysis: The method of Kaplan and Meier was used to estimate the probability of lymphoma developing in this group of patients as a function of time on antiretroviral therapy. Follow-up was available to the time of death or to the present in 52 of the 55 patients; three patients lost to follow-up were censored at the most recent point at which data was available. Twenty-nine patients who died without developing NHL were censored at their times of death, and 15 other patients who are alive and have not developed NHL were censored at the time last known alive. Confidence intervals for the Kaplan-Meier analysis were determined using the method of Rothman.

The 95% confidence intervals for the overall proportion of patients developing lymphoma were calculated by an exact method. For each of the eight patients who developed non-Hodgkin lymphoma, the time elapsed from the initial fall of their CD4 cells below 100 or 50 cells/mm^3 to the development of lymphoma was calculated. In this analysis, two consecutive determinations of CD4 values below 100 or 50 cells/mm3 were required, and the time was calculated from the first of these determinations.

Survival of patients with non-Hodgkin lymphoma was determined from the time the diagnostic biopsy was performed until the time of death. Median values, with the appropriate ranges, were then determined for each time period.

Results

Non-Hodgkin lymphoma developed in 8 of the 55 patients (14.5%, 95% confidence interval of 6.5% to 26.7%). As of May, 1991, analyzed by the method of Kaplan and Meier, the estimated probability of developing lymphoma in the patients within 24 months of starting antiretroviral therapy was 12% (95% confidence interval of 5% to 27%), increasing to 29% (95% confidence interval of 15% to 49%) after 36 months. One patient developed a second distinct lymphoma 16 months after his initial occurrence of non-Hodgkin lymphoma; however, only the first occurrence of lymphoma in this patient was considered in the above calculations.

The median CD4 cell count at the initiation of antiretroviral therapy for all 55 patients was 71 cells/mm^3 (range 0 to 953 cells/mm^3). Median survival for the 55 patients overall was 19.8 months.

The 8 patients who developed lymphoma had received antiretroviral therapy for a median of 23.6 months (range 15.4 to 34.8 months) before the onset of lymphoma. The median time from the diagnosis of their AIDS-defining illness to the development of non-Hodgkin lymphoma was 21.8 months (range 2.7 to 76.6 months). Median CD4 cell counts at the occurrence of non-Hodgkin lymphoma was 6 cells/mm^3 (range 4 to 30 cells/mm^3). Patients who developed non-Hodgkin lymphoma had had less than 50 CD4 cells/mm^3 for a median of 15.4 months (range 3.2 to 34.8 months) prior to the occurrence of

lymphoma. There was a statistically significant difference in the rate of development of NHL during the time that the patients had more than 50 CD4 cells/mm^3 (0/57.3 patient-years of follow-up) compared to the time they had less than 50 CD4 cells/mm^3 (8/46.3 patient-years of follow-up) (p=0.002) by the test for a difference in two Poisson rates. This relationship was independent of the time that they received antiretroviral therapy. Thus, it appears that prolonged survival in a severely immunosuppressed state, in particular with less than 50 CD4 cells/mm^3, may result in a significantly increased incidence of developing NHL.

All of the lymphomas reported here histologically were of high-grade, four being large cell immunoblastic lymphoma and four small non-cleaved cell lymphoma. Seven of the lymphomas were B cell type and one was null cell type. Each of the patients had serological evidence of infection with Epstein-Barr virus. All occurred in extranodal sites. Five patients had primary involvement of the central nervous system: four had mass lesions within the brain and one had leptomeningeal disease only. The other three patients presented with visceral involvement of the lung (patient 1), esophagus, liver and spleen (patient 2), and ascites and pleural fluid (patient 3).

Median survival for the four patients with primary central nervous system involvement diagnosed antemortem was 1.8 months (range 0.6 to 3.2 months). The three patients who presented with visceral involvement had survivals of 0.4, 7 and 18 months.

Discussion

The occurrence of non-Hodgkin lymphoma in the setting of HIV infection is well established, and high-grade non-Hodgkin lymphomas account for approximately 3% of the initial AIDS-defining illnesses in reported adult and adolescent cases of AIDS. However, there is presently little available literature on the temporal development of NHL in cohorts of patients with AIDS or severe AIDS-related complex, particularly since the development of effective antiretroviral therapy. We selected these three protocols for analysis because they represent the first three protocols of our group in which patients were followed for long periods of time on antiretroviral therapy. Included in this cohort are the first patients to have ever received zidovudine. It is worth noting, however, that we have also observed the development of lymphoma in HIV-infected patients on other antiretroviral protocols. In particular, one patient developed stage IE primary small non-cleaved cell lymphoma of the liver while studied on a Phase I trial of dideoxyadenosine, while a second patient initially entered on a study of recombinant soluble CD4 developed stage IVB large cell immunoblastic lymphoma. A third patient initially entered on the Phase I study of 2′,3′-dideoxycytidine subsequently developed stage IVB Hodgkin disease.

The patients reported here were followed for up to 48 months while receiving continuous antiretroviral therapy with zidovudine or zidovudine-based regimens. It is possible that because of the screening process

(e.g., patients had to be free of active opportunistic infections), the study patients may not be representative of AIDS or AIDS-related complex patients in the general population. Nevertheless, the estimated probability of lymphoma developing in 29% of patients by 36 months after starting antiretroviral therapy is striking. Further evaluations of larger populations will be needed to more accurately define the probability of patients with severe HIV infection on antiretroviral therapy developing a non-Hodgkin lymphoma. The data presented here indicate that non-Hodgkin lymphoma may well become a limiting factor in the survival of patients with HIV infection as improved antiretroviral therapies are developed. It will be important to learn how to prevent this complication. For example, earlier intervention with antiretroviral therapy may delay the decline of the CD4 cells below $50/mm^3$, and this may result in a lower incidence of lymphoma. This possibility will require further study.

Lymphomas can be difficult to diagnose in patients with HIV infection, and this may result in an under reporting of non-Hodgkin lymphoma in these patients. For example, two of the cases of lymphoma in this study (patients 5 and 7) were diagnosed in patients being treated for cerebral toxoplasmosis who developed new or enlarging brain lesions. Also, none of the eight cases presented here had the diagnosis established by biopsy of a lymph node. Patients with lymphoma in the setting of AIDS pose substantial therapeutic challenges. In spite of the interventions used, the overall survival in the patients described here following the development of lymphoma was poor. Interestingly, one patient developed a dramatic flare of his Kaposi's sarcoma when administered a chemotherapeutic regimen for the lymphoma which contained steroids, consistent with previous reports. Improved therapeutic strategies for these conditions are needed, and this will be an important area for future research.

Although the mechanism of AIDS-related non-Hodgkin lymphoma development is not known, many inter-related factors have been postulated to be involved. It has been found that patients with AIDS or AIDS-related complex have a polyclonal B cell proliferative lymph node expansion Several factors may contribute to this process. Polyclonal B cell activation can be a direct response to HIV infection, either through mitogenic or antigenic stimulation.

HIV-infected patients have increased numbers of circulating Epstein-Barr virus infected cells, which may in part be due to their profound defect in T cell immunity. However, direct Epstein-Barr virus involvement of tumor has not been documented in the majority of patients with HIV-associated lymphoma, and this issue bears further study. Whatever the mechanism, polyclonal B cell proliferation may provide a milieu for the development of transforming events to occur. While these transforming events have not yet been delineated, there is evidence that in many cases of AIDS-related Burkitt lymphoma, a c-myc oncogene rearrangement similar to that seen in endemic and sporadic Burkitt lymphoma occurs. Unregulated oncogenic expression could then become the proximal cause of the transformed state in patients. Alternately, it is possible that the replication of an as yet unidentified oncogenic virus may be enhanced in patients infected with HIV.

Although the cellular events involved in the pathogenesis of AIDS-related non-Hodgkin lymphoma have not been elucidated, it is likely that immunosuppression plays a substantial role. The occurrence of high-grade, B cell lymphomas (particularly at extranodal sites) developing in patients with other forms of immunodeficiency, either primary (e.g., Wiskott-Aldrich syndrome, ataxia telangiectasia) or from immunosuppressive therapy, has been well-documented. Prolongation of survival in patients with primary immunodeficiency has been felt to increase the cumulative risk for the development of a non-Hodgkin lymphoma. In patients with Wiskott-Aldrich syndrome, for example, the overall risk of developing a malignancy has been calculated to be 126 times that of the general population, with a cumulative risk of 2% per year for the first 25 years of life. The majority of these malignancies are lymphoreticular in origin (74.5%), and high-grade, B cell lymphomas, frequently occurring in extranodal sites, particularly the central nervous system, predominate. Thus, with enhance control of infection and other therapeutic advances, the cumulative probability of such patients developing a non-Hodgkin lymphoma has increased along with the prolongation of life expectancy.

Recent evidence suggests that as a result of clinical advances in the therapy of HIV infection, patients with this disease are experiencing an improvement in survival. For example, the median survival of patients diagnosed with AIDS reported to the San Francisco Department of Public Health has increased from 10.8 months for those diagnosed in 1985 to 15.6 months for those diagnosed in 1987. This improvement has been particularly striking for those who present with Pneumocystis carinii pneumonia as their AIDS-defining illness; in these patients, the median survival during the same period has increased from 10.5 to 17.9 months. While earlier diagnosis and improved methods of treatment and prophylaxis of P. carinii pneumonia may have contributed to this phenomenon, there is evidence that the use of zidovudine has resulted in prolonged survival over and above any affect of PCP prophylaxis. The median survival of 22 months in this study population lends further support to this impression. Therefore, patients with profound immunodeficiency are living longer, theoretically allowing more time for the development of non-Hodgkin lymphoma or other malignancies.

It is noteworthy that patients in our series had had AIDS for a median period of 21.8 months (range 2.7 to 76.6 months) and had less than 50 CD4 lymphocytes/mm^3 for a median of 15.4 months (range 4.2 to 34.8 months) prior to the development of lymphoma. Prior to the development of antiretroviral therapy, it would have been relatively unusual to have such prolonged survival after the development of AIDS or profound CD4 depletion. Lymphomas may develop in HIV-infected patients at any point in the course of their illness. However, while the numbers are small, the findings here suggest that patients who survive for long periods with profound immunodeficiency manifested by less than 50 T4 cells/mm3 may have a particularly high likelihood of developing high-grade lymphomas. Indeed, assuming a 10 year incubation period from the initial infection with HIV to the development of AIDS, and that non-Hodgkin lymphoma developing in early stages of HIV infection is approximately 0.3%. If we were to assume that

the development of non-Hodgkin lymphoma is constant over the period of follow-up, then the estimated incidence of non-Hodgkin lymphoma in our cohort of patients with AIDS and severe AIDS-related complex is nearly 8% per year of follow-up. This wide disparity again indicates that non-Hodgkin lymphoma is much more likely to develop in the setting of severe immunodeficiency and thus may be considered an "opportunistic" tumor.

Finally, one must wonder if zidovudine or other antiretroviral drugs might directly contribute to the development of lymphoma in these patients. In this regard, zidovudine can act as a mutagen, and vaginal malignancies have been reported to develop with increased frequency in mice and rats receiving lifelong high dose zidovudine. Further research will be required to determine if the same effects occur in humans. It is worth noting that the lymphomas in our patients are of the same type as those which typically develop in the setting of HIV infection, suggesting that zidovudine therapy was less likely to be a direct cause of these tumors. There is no question that zidovudine has improved rates of the morbidity and mortality of HIV infection. Nevertheless, the direct oncogenic potential of zidovudine and related drugs cannot be discounted. This serves as an incentive to find the lowest effective doses for such agents. It will be important to sort out the relative contribution of immunosuppression, prolonged survival, a possible effect of antiretroviral therapy, and perhaps other unrecognized factors in the development of lymphomas in order to learn how to minimize the occurrence of this condition.

In the period between 1973 and 1983, the Surveillance, Epidemiology and End Results (SEER) Program of the NCI documented an increase of greater than 50% in the incidence of NHL. This trend includes a dramatic increase in NHL in never-married males ages 20-49 beginning in 1983, presumably due to HIV-associated lymphomas. The SEER database includes both AIDS-defining lymphomas as well as those arising in patients with a prior AIDS diagnosis. A projection of this trend, taking into account the regional variation in the incidence of HIV infection, would predict that there will be about 4700 cases of AIDS-associated NHL in 1982. However, projections can also be made based on estimates of future AIDS incidence, as well as on estimates of the incidence lymphomas that are AIDS-defining and that occur in patients with a prior AIDS diagnosis. Using the results of the NCI cohort, projects made by Gail and colleagues suggest that there may be between 2900 and 9800 AIDS-related NHL in 1992 (with an intermediate estimate of about 5000 cases). This would involve 8% to 27% of all NHL occurring in the United States during that year. Nearly 80% of these lymphomas are expected to develop in patients who were diagnosed with AIDS prior to the development of their lymphoma. Thus, it would appear that HIV-related NHL will have a significant impact on the overall incidence of NHL in the very near future.

PUBLICATIONS

1. Pluda JM, Yarchoan R, Jaffe ES, et al. Development of non-Hodgkin lymphoma in a cohort of patients with severe human immunodeficiency virus (HIV) infection on long-term antiretroviral therapy. Ann. Intern. MEd. 1990; 113:276-282.

2. Pluda JM, Tosado G, Steinberg S, et al. Parameters predictive of opportunistic non-Hodgkin's lymphomas (NHL) developing in patients with severe HIV infection on long-term antiretroviral therapy. Blood 1990 (abstract); 76:492a.

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7. Pluda JM, Yarchoan R, Venzon D, et al. Non-Hodgkin's lymphomas in severely immunocompromised HIV-infected patients surviving for prolonged periods on antiretroviral therapy. MMWR 1991 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07211-02 CO

PERIOD COVERED
October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibition and modulation of HIV infection in monocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the infection of human peripheral blood monocyte/macrophages by HIV and ways of inhibiting this process. In contrast to other reports which had appeared in the literature, we found that dideoxynucleosides (including AZT, ddC, ddI and ddA) were potent inhibitors of do novo HIV infection in monocyte/macrophages. In regard to AZT, this was surprising, as monocytes have very low levels of thymidine kinase (responsible for catalyzing the first step of AZT phosphorylation) and there were very low levels of AZT-5'-triphosphate in monocytes exposed to AZT. We further found that monocytes have very low levels of thymidine-5'-triphosphate. Thus, the ration of AZT-triphosphate to thymidine-triphosphate is actually higher in monocytes than in T cells, and this can account for its activity. In further experiments, we found that granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) both enhanced the replication of HIV in monocytes. GM-CSF, however, also stimulates the acti-HIV activity of AZT and other thymidine analogues such as 2',3'-didehydro-2', 3'-dideoxythymidine (D4T). In the case of AZT, the increased activity appears to occur because of a combination of increased entry and increased phosphorylation. GM-CSF does not enhance the anti-HIV activity of other dideoxynucleosides such as ddC and ddI. Interestingly, M-CSF does not appear to enhance the anti-HIV activity of AZT or the other dideoxynucleosides. We further explored whether CD4 binding was a necessary component of the entry of HIV into monocytes. Infection of monocytes was inhibited by agents which block gp120 binding to CD4 such Leu 3, CD4, or CD4-IgG. We further asked whether this would apply in the presence of enhancing antibodies. Very low concentrations of anti-HIV antibodies were found to enhance infection of monocytes by HIV. However, even under those conditions, infection was blocked by Leu 3 or soluble CD4. We are now examining lymphokine production by HIV-infected monocytes and exploring the activity of other drugs and cytokines.

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While CD4+ T cells were the first identified target cell for HIV infection, it has been found that monocyte/macrophages (M/M) can be infected by HIV and are important in the pathogenesis of AIDS. In particular, the infection of M/M-derived cells in the brain are believed to be important in the pathogenesis of AIDS dementia. In addition, M/M may act as reservoirs for HIV infection and can efficiently transmit the virus to T cells. For the last several years, we have studied the infection of M/M by HIV and the means of controlling this process.

Our first experiments were directed at addressing the question of whether dideoxynucleosides could inhibit the infection of M/M by HIV. Dideoxynucleosides, including azidothymidine (AZT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-dideoxyinosine (ddI) must be phosphorylated to a 5′-triphosphate moiety by enzymes in target cells. Non-proliferating cells, such as M/M, have very low levels of such enzymes (kinases), and concerns were raised that dideoxynucleosides would not protect such cells from infection by HIV. If this were the case, it would mean that specific strategies to inhibit HIV replication in M/M would have to be developed. We observed in 1986 that certain patients with HIV-induced dementia had reversal of the dementia upon being administered AZT. This suggested to us that AZT might in fact inhibit the infection of M/M by HIV. Examining this in the laboratory, we observed that dideoxynucleosides would not protect such cells from infection by HIV. If this were the case, it would mean that specific strategies to inhibit HIV replication in M/M would have to be developed. We observed in 1986 that certain patients with HIV-induced dementia had reversal of the dementia upon being administered AZT. This suggested to us that AZT might in fact inhibit the infection of M/M of HIV. Examining this in the laboratory, we observed that AZT, ddC and ddI are potent inhibitors of HIV replication in M/M. Furthermore, we found that this occurs even though the levels of the 5′-triphosphates of these drugs are much lower than they are in proliferating T cells. M/M, however, also have very low levels of the competing deoxynucleoside-5′-triphosphates, and thus the ratio of the dideoxynucleoside-5′-triphosphates:deoxynucleoside-5′-triphosphates is actually higher in M/M than in proliferating cells.

In subsequent experiments, we explored the effects of stimulatory cytokines of HIV replication in M/M. Some of these cytokines have potential utility in the treatment of HIV-associated or AZT-associated bone marrow suppression. We found that several of these cytokines, including GM-CSF, M-CSF, and insulin-like growth factor type I (G-CSF) do not. Interestingly, GM-CSF appeared to enhance the anti-HIV activity of aZT and other thymidine congeners in M/M, in part by enhancing entry and in part by enhancing its phosphorylation. However, the activity of other dideoxynucleosides, including ddC and ddI, was not affected. In addition, the activity of AZT as well as other dideoxynucleosides was not enhanced by M-CSF. These findings have potential utility in designing treatment protocols. For example, we have that HIV-infected patients given GM-CSF (without AZT) actually have an increase in their serum HIV p24 antigen. We are now conducting a small pilot study to evaluate the use of AZT plus GM-CSF in patients with HIV infection.

We next explored the mechanism by which HIV entered M/M. We found that while not commonly regarded as CD4+ cells, M/M did express low levels of CD4 on their surface, and in addition that the infection of such cells was inhibited by either recombinant CD4 (rCD4) or anti-CD4 antibodies. In further studies, we evaluated the enhancement of infection of M/M by low concentration of anti-HIV antibody. We found that enhancement of 4 to 10 fold was reproducibly observed in M/M exposed to very low concentrations of anti-HIV antibody. We further found that even under conditions of enhancement, the infection of M/M could be inhibited by either rCD4 or anti-CD4 antibodies. This suggested that enhancing antibodies did not permit infection by a new cellular receptor, but rather served to bring the viral gp120 into closer contact with CD4 on the surface of M/M.

We are now exploring the cytokines produced by M/M and their potential role in the pathogenesis of HIV-related conditions. In particular, we have found evidence suggesting that patients with high serum interleukin-6 (IL-6) levels have a relatively greater chance of developing non-Hodgkin's lymphoma (NHL). We are now exploring the production of IL-6 by M/M in response to HIV, and exploring means of modulating this process. Such studies may have implications in devising methods to reduce the incidence of HIV-associated lymphomas.

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2. Perno C-F, Yarchoan R, Cooney DA, et al. Inhibition of human immunodeficiency virus (HIV-1/HTLV-III$_{Ba-L}$) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2′,3′-dideoxynucleosides. J. Exp. Med. 1988; 168:1111-1125.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07216-01 CO

PERIOD COVERED

June 1, 1990 through June 31, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Treatment of AIDS-related Kaposi's sarcoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Robert Yarchoan, M.D., Senior Investigator, Clinical Oncology Program, NCI
James M. Pluda, M.D., Senior Investigator, Clinical Oncology Program, NCI
Laura Shay, R.N., Medicine Branch, NCI
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Samuel Broder, Director, NCI

COOPERATING UNITS *(if any)*

Medicine Branch, NCI Clinical Pharmacology Branch, NCI

LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Kaposi's sarcoma (KS) was one of the first AIDS-defining illnesses identified, and continues to be a source of considerable morbidity and mortality. The most current information regarding the pathogenesis and biology indicate that a variety of cellular cytokines and products, including basic fibroblast growth factor (FGF) are involved in the growth of KS, particularly in stimulating angiogenesis and neovascularization. Pentosan polysulfate is a sulfated polysaccharide (MW 6000 Daltons) that has been used in Europe as an anticoagulant for many years. Recently, pentosan has been shown to block cell surface receptor binding of FGF and to possess activity against certain FGF-dependent tumor cell lines. We thus instituted a feasibility (pilot) study administering pentosan to patients with AIDS-KS. Sixteen patients were enrolled in the trial. While no patient had either a complete or partial response, 7 patients had stable disease which might indicate a stabilization resulting from an inhibition of growth factors. The dose-limiting toxicities identified were thrombocytopenia and anticoagulation. There were no significant alterations in CD4 cells in HIV p24 antigen levels. We have developed a KS cell line and are now attempting to develop other therapeutic strategies.

Pentosan polysulphate is a sulphated polysaccharide, molecular weight ~6000 Daltons, that has been used as an anticoagulant in Europe for many years. Pentosan has been shown to possess anti-HIV activity in vitro, presumably via inhibition of HIV binding to the surface of susceptible cells, in a manner similar to dextran sulphate. There is also recent evidence that at very low concentratons, pentosan may increase in vitro HIV replication by stimulating lymphoproliferation. However, pentosan also has in vitro synergistic activity with AZT even at these same low pentosan concentrations.

AIDS-associated Kaposi's sarcoma (KS) cell lines have been found to respond in vitro to and produce a number of cytokines including basic fibroblast growth factor (FGF). It is currently felt that angiogenesis and neovascularization, mediated in part by FGF, are important in the pathogenesis of KS. Recently, pentosan has been shown to possess activity against FGF-dependent tumor cell lines in vitro, and is felt to inhibit FGF cell surface receptor binding. Pentosan has also been shown to be active against KS cell lines in vitro.

With this background, we instituted a feasibility (pilot) trial administering pentosan polysulphate to patients with HIV-associated KS. A total of 16 patients were enrolled in the study. Patients received pentosan by continuous intravenous infusion for 3 weeks followed by subcutaneousssss dosing. The dose-limiting toxicities identified were thrombocytopenia and anticoagulation, which were reversible upon discontinuation of drug. Additional toxicities seen included reversible liver function abnormalities and localized discomfort at the site of the pentosan injections. No patient achieved a complete or partial response. Seven patients had stable disease, and 9 patients had progression of their KS while receiving pentosan. There were no significant alterations in CD4 counts or in HIV p24 antigen levels associated with pentosan administration. In the laboratory, we have developed a KS cell line. This line grows in response to HTLV-II supernatant and steroids. Its growth is inhibited by pentosan polysulfate. We are using this line to develop other therapeutic strategies.

PUBLICATIONS

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07217-01 CO

PERIOD COVERED
October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Combination therapy of HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Robert Yarchoan, M.D., Senior Investigator, Clinical Oncology Program, NCI
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Kathleen M. Wyvill, R.N., Medicine Branch, NCI
Laura Shay, R.N., Medicine Branch, NCI
Jill Lietsau, R.N., Medicine Branch, NCI
Hiroaki Mitsuya, M.D., Senior Investigator, Clinical Oncology Program, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since the discovery that acquired immunodeficiency syndrome (AIDS) was caused by a retrovirus, now called human immunodeficiency virus (HIV), a number of anti-retroviral agents have been identified in this laboratory and elsewhere. Several of these agents, including azidothymidine (AZT), 2;,3'-dideoxycytidine (ddC), and 2',3'-dideoxyinosine (ddI) which have potent anti-HIV activity in vitro have been shown to induce laboratory and clinical improvements in patients with AIDS or related disorders. However, these agents are not cures, and their long term use is limited by the development of toxicity and/or resistance in a substantial proportion of patients. We are now exploring whether a combination regimen combining anti-HIV agents may provide a better therapeutic index in patients than single agents. In certain other areas, for example, the treatment of childhood leukemia, combination therapy has been far more useful than single agent therapy; in fact, cures are rarely possible in childhood leukemia with single-agent therapy but are not infrequently obtained with combination therapy. In the setting of HIV infection, rationales for combination therapy include the avoidance of drug toxicities, the delayed development of resistance, drug synergy, the suppression of opportunistic infections which may be acting as co-factors, and the reversal of drug toxicities by counteracting agents. We have initiated several combination drug trials including AZT with acyclovir; AZT and ddC; AZT with granulocyte-macrophage-colony stimulating factor (GM-CSF); AZT with acyclovir, ddC and ddI; and AZT and ddI. Preliminary results indicate that these regimens can be tolerated and may result in reduced toxicity compared with single agents.

The first combination regimen we tested was a combination of AZT and acyclovir. The rational for this was that acyclovir was found to be synergistic with AZT. In addition, acyclovir could potentially suppress certain herpes viruses which caused morbidity on their own and could act as co-factors for HIV. The results of a small initial pilot trail involving 8 patients showed that the combination of AZT 200 mg and acyclovir 800 mg every 4 hours could be well tolerated and that the pharmacokinetics of the drugs were not affected by their simultaneous administration.

The next combination trial that we undertook was AZT and ddC. The Phase I trial of ddC showed that the drug had anti-HIV activity, but that its use was limited by the development of painful peripheral neuropathy. We undertook an alternating study of AZT and ddC, with each drug being given for a one week period of time. We found that at least initial clinical and laboratory improvement was observed and that the toxicity from AZT and ddC were both reduced by the regimen. In particular, the total cumulative dose of ddC that could be tolerated was substantially larger than that found with continuous therapy with ddC. This trial is still ongoing.

We next undertook a study of AZT and granulocyte macrophage-colony stimulating factor (GM-CSF). CGm-CSF can stimulate the bone marrow to increase production of both granulocytes and monocytes. It thus had the potential of counteracting the neutropenia induced by AZT. We initially embarked on a trial of AZT alternating with GM-CSF. (An alternating regimen was chosen because of concerns that the simultaneous use of the compounds might induce greater AZT toxicity). During the initial conduct of this trial, we found in the laboratory that GM-CSF could enhance HIV replication by human peripheral blood monocytes. Interestingly, GM-CSF also enhanced the anti-HIV activity of AZT. Indeed, clinical results from the trial showed that while the regimen could be tolerated in neutropenic patients, the use of GM-CSF alone induced increases in serum HIV p24 antigen, suggesting that it might be enhancing HIV replication. Based on this observation and the laboratory observation that GM-CSF potentiated the anti-HIV activity of AZT, we initiated a regimen of AZT used simultaneously with GM-CSF. Preliminary results suggest that the use of GM-CSF can in fact reduce the toxicity induced by AZT. This trial is still ongoing.

With the completion of the Phase I trial of ddI, and the finding that it was well tolerated and had activity against HIV, we decided to attempt to combine AZT, ddC and ddI in one trial. Drawing on our experience with these three drugs (as well ad acyclovir), in December of 1989, we initiated a trial of AZT with acyclovir (for 1 week) alternating with ddC (for 1 week) alternating with ddI (for 1 weeks). So far, 21 patients have been entered on the study. Preliminary results indicate that the regimen is well tolerated with minimal toxicity over up to 18 months, that an anti-HIV effect can be observed, and CD4 cells can remain elevated for approximately one year. The study is closed to new accrual, but we are continuing to follow the patients.

Finally, we have started during the past year to examine the combination of AZT and ddI. Several factors suggested that it might be beneficial to design a combination regimen utilizing these two drugs. First, the two

drugs both have anti-HIV activity in patients. In addition, they have different toxicity profiles: AZT causes bone marrow suppression, while ddI causes painful peripheral neuropathy and sporadic pancreatitis. Finally, there is evidence that patients whose HIV has become resistant to AZT remains sensitive to ddI. It was not clear, however, how to best combine the two. For example, it was unclear if it was better to utilize alternating therapy or simultaneous therapy. In addition, it was unclear if it was better to utilize a half-dose of each drug, or to use a full dose of both drugs. To help address these questions, we initially embarked on a two arm randomized study: patients would receive either AZT alternating with ddI or AZT simultaneously with ddI. Both of these two arms involved patients averaging a half-dose of AZT (i.e. 300mg/day) and a half-dose of ddI (e.g. 250 mg/day) over the course of a six week cycle. We are in the process of adding a third arm employing full dose AZT (600 mg/day) given simultaneously with full dose ddi (500 mg/day). The study is designed as a pilot study. In addition, it may provide some information as to whether one of these regimens is superior to another.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CM 07218-01 CO

PERIOD COVERED

October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

The effect of DNA demethylation HIV-1 expression in vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Hiroaki Mitsuya, M.D., Senior Investigator, Clinical Oncology Program, NCI
Mary O'Brien, Biologist, Clinical Oncology Program, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The effect of DNA demethylation on human immunodeficiency virus type-1 (H-1)IV expression was investigated in several cell lines infected with HIV-1 in vitro. In order to demethylate proviral DNA, 5-azacytidine (AZC) and its analog were employed. A persistently HIV-1-infected T-cell line, ACH2, produced a low level of HIV-1 in vitro. However, following exposure to AZC or its analog, a profound potentiation of viral expression occurred as assessed by syncytia formation and production of reverse transcriptase and p24 Gag protein. Alternation in the methylation pattern of proviral DNA in ACH2 was confirmed by Southern blot hybridization using methylation sensitive/insensitive isoschizomer restriction enzymes.

AZC-induced potentiation of viral expression did not occur in any of high HIV-1-producers, chronically HIV-1 infected H9 cells, subacutely HIV-1-infected normal CD4+ T-cells, or subacutely infected lectin-activated peripheral blood mononuclear cells. Southern blot hybridization revealed that proviral DNA in these highly HIV-1-producing cells had already been extensively demethylated, and no altered DNA methylation pattern had occurred following AZC treatment. We also found that the inherent toxicity of AZC per se was, at least in part, associated with the observed enhancement of HIV-1 expression in ACH2 cells. The present data suggest that DNA methylation may play a role in regulating HIV-1 expression at least under certain circumstances. Further study is also warranted to test the possibility that certain therapeutics which exert toxicity to cells such as anticancer agents may affect the expression of HIV-1 in patients with HIV-1 infection and neoplasms.

INTRODUCTION

The enzymatic transfer of a methyl group from S-adenosylmethionine to the 5-carbon atom of cytosine shortly after DNA replication results in the formation of 5-methylcytosine in 1-7% of all cytosine residues of mammalian cellular DNA. Although retroviral RNA and linear double-stranded proviral DNA are not methylated, it has been shown that integrated proviral DNA sequences can be methylated.

Infection with HIV-1 is characterized by a period of clinical latency associated with low to undetectable expression of the virus. The mechanisms responsible for maintenance of the viral latency have been as yet poorly understood. Several factors have been implicated in the activation of HIV expression. These include immune activation, cytokine-mediated viral induction, trans-activation by certain DNA viruses, heat shock, and induction by ultraviolet or x-ray irradiation.

In this study, we asked whether the state of methylation of proviral DNA (or cellular DNA) could affect the expression of HIV-1 in infected cells. We describe here that a "latently" HIV-1 infected cell line, which produced only a barely detectable level of HIV-1, began producing a significant level of HIV-1 in the presence of AZC. We also found that the inherent toxicity of AZC per se was, at least in part, associated with the observed enhancement of HIV-1 expression in ACH2 cells.

MATERIALS AND METHODS

Cells:

ACH2, which is a clone of LAV-infected CD4+ CEM cells and contains a single proviral DNA copy per cell, was used in this study. Another cloned LAV-infected cell line, U1; a tetanus-toxoid specific human T-cell clone, TM11; and a CD4+ T-cell line which is permissive for HIV-1 replication, H9, were also employed. A CD4+ human T-cell leukemia virus type 1 (HTLV-1)-carrying cell line, MT2, was also used. H9 cells infected with HIV-1 derived from ACH2 cells (HIV-1_{ACH2}) were generated as follows. ACH2 cells were stimulated with 0.5 ng/ml phorbol-12-myristate-13-acetate (PMA) for 48 hr, y-irradiated (10,000 rads), and cocultured with an equal number of H9 cells. H9 cells were thus permissively infected with HIV-1_{ACH2} (designated as H9/HIV-1_{ACH2}). Phytohemagglutinin-stimulated peripheral blood mononuclear cells (PHA-PBM) from two HIV-1-negative volunteers were exposed to HIV-1_{IIIB}, cultured for 3 days (PHA-PBM/HIV-1_{IIIB}), and were also subjected to AZC treatment.

All cells except TM11 and PHA-PBM were cultured in complete medial [RPMI 1640 supplemented with 4 mM l-glutamine, 15% (v/v) undialyzed and heat-inactivated fetal bovine serum, and 50 units/ml penicillin and 50 ug/ml streptomycin]. TM11 and PHA-PBM were cultured in complete media containing 25 units/ml recombinant IL-2 (Amgen Biological, Thousand Oaks, CA).

Agents:

5-Azacytidine (AZC) and cytosine arabinoside (Ara-C) were purchased from Sigma Chemical Co. Dihydro-5-azacytidine (DHAC), cyclopentenyl cytosine (CPE-C), and 5-fluorouracil (5-FU) were kindly provided by Drs. V.E. Marquez and J.S. Driscoll. 2'-deoxycytidine (dC) was purchased from PL-Biochemicals. Working solution of AZC (10 ug/ml) did not contain any detectable amount of endotoxin (less than 0.08 endotoxin units/ml) as assessed by Limulus test.

Syncytia Formation:

ACH2 cells ($6x10^6$ in 40 ml culture medium) were exposed to various concentrations of AZC for 24 hr, washed, resuspended, and cultured for an additional 72 hrs without AZC. These ACH2 cells ($11x10^6$) were then washed, resuspended, and cocultured with an equal number of MT2 cells in 20 ml culture medium in a Costar 25 cm flask. In 24 hr of coculture, the level of syncytia formation was examined under the inverted microscope.

p24 Gag Protein Production:

Approximately six million cells that were logarithymically growing in 40 ml culture media were exposed to AZC for 24 hr, and further cultured in 40 ml drug-free media for an additional 72 hr. Otherwise stated, all drugs were left in culture media throughout the 96-hour study period. The amounts of p24 Gag protein were determined by radioimmunoassay (Dupont). Results were all analyzed by the Perkin-Elmer ELISA program. Values thus obtained were adjusted to represent the p24 Gag amount per 10^6 cells/ml based on the viable cell counts at the time culture was terminated.

Reverse Transcriptase Activity:

ACH2 cells ($6x10^6$ in 40 ml culture medium) were exposed to various concentrations of AZC for 24 hours, washed, resuspended in 40 ml fresh culture medium, and cultured for an additional 72 hours without AZC. Cultures were terminated by centrifugation and 5 ml of the supernatant were subjected to reverse transcriptase assay as previously described by Sarngadharan et al. Results are expressed as counts per minute of [methyl-^{3}H]deoxythymidine triphosphate (40-60 Ci/mmol; 1 Ci = 37 GBq) incorporated. Values are shown after adjusting to counts per 10^6 viable cells/ml based on the viable cell counts when culture was terminated.

DNA analyses:

Total DNA was isolated from cells by two cycles of phenol/chloroform extraction followed by two cycles of chloroform/isoamyl alcohol extraction, precipitated in ethanol, dissolved in Tris/EDTA buffer, and stored at 4 C as previously described by Sambrook et al. DNA samples were digested with Kpn I and Msp I or Hpa II (BRL). Equal amounts of the digested DNA samples were electrophoresed on a 1% agarose gel and transferred overnight to nitrocellulose in 10X SSC. Blots were than hybridized overnight at 42 C to a ^{32}P-radiolabelled 1.3 kb Bgl II fragment containing the env region of

BH10. Nitrocellulose membranes were washed 3 times for 30 min each with 0.1X SSC, 0.1% SDS at 65 C and exposed to x-ray film with an intensifying screen at -70 C.

RNA Analyses:

Cytoplasmic RNA was isolated from cells by the vanadyl-ribonucleoside complex method. Seven ug RNA per each lane were electrophoresed on a 1% agarose/formaldehyde gel, blotted overnight on to nitrocellulose in 20X SSC, and probed overnight at 42 C with the ^{32}P-radiolabelled 1.3 kb Bgl II fragment. Filters were then washed 3 times for 30 min in 0.1X SSC containing 0.1% SDS at 65 C, and exposed to x-ray film with an intensifying screen at -70 C. The intensities of the HIV-1-specific bands were measured by densitometry (X-Rite 301, X-Rite, Inc, Grand Rapids, MI). The dose-response difference was assessed by using the arithmetic mean of three independent intensity readings.

RESULTS

AZC Induces Expression of HIV-envelope Glycoprotein ACH2 Cells.

A human T-cell line, ACH2, is known to harbor HIV-1 (LAV) virus in its genome and produce a minimum level of the virus in vitro. We first asked if 5-azacytidine (AZC), an agent known to cause DNA demethylation, could affect the level of HIV replication in this cell line. The magnitude of syncytia formation between HIV-1 infected cells and CD4+ cells is thought to depend on the level of env-encoded glycoproteins produced by HIV-1-infected cells. ACH2 cells were exposed to various concentrations of AZC for 24 hr, washed, resuspended in fresh culture medium, cultured for an additional 71 hours, and then cocultured with CD4+ MT2 cells. When similarly treated, but AZC-unexposed control ACH2 cells were cocultured with MT2 cells, only a small number of syncytia was detected. However, when ACH2 cells treated with 5 uM AZC and were cocultured with MT2 cells, an extensive level of syncytia was detected. When ACH2 cells were treated with 20 uM AZC, a profound level of syncytia formation was identified. This observation strongly suggested that the AZC treatment enhanced the expression of HIV envelope glycoproteins.

AZC Also Increases the Production of Reverse Transcriptase by ACH2 Cells.

To corroborate and extend the observations described above, we asked if ACH2 cells treated with AZC secreted more reverse transcriptase in the culture supernatant than AZC-unexposed control ACH2 cells. We found a substantial increase in the activity of reverse transcriptase in a dose response manner. For example, when the cells were treated with 10 uM AZC, there was a 13.5 fold increase in the activity (Table 1). When treated with 20 uM AZC, ACH2 cells produced 35-fold increase in reverse transcriptase activity as compared to the control cells.

Enhancement of p24 Gag Protein by ACH2 Cells by AZC and Its Analogs.

As reported elsewhere by others, ACH2 cells produce only a low level of p24 Gag protein (0.13 - 0.85 ng/10^6 cells in the culture supernatants without AZC treatment (Table 2). When ACH2 cells were exposed to various concentrations of AZC for 24 hours and further cultured without AZC for an additional 72 hours, 16- and 47-fold increases in p24, Gag protein production were seen following 10 and 20 uM AZC treatment, respectively (Table 2). Treatment of cells with dihydro-5-azacytidine (DHAC), a hydrolytically stable analogue of AZC which also causes DNA demethylation, induced a comparable level of potentiation of p24 Gag protein production, 20-fold and 41-fold at 100 and 500 uM, respectively (Table 2).

AZC exerts toxicity to cells at high concentrations in vitro. Therefore, the cell lysate data may be more reliable since such data would be much less affected by the toxicity if adjusted based on the number of viable cells. When the p24 Gag content in the cell lysates of viable ACH2 cells after exposure to 2.5 to 10 uM AZC was compared, a dose-related increase in the amounts of p24 Gag protein in the cell lysates was detected (Table 3), which confirmed the observation of an increase in p24 Gag protein amount in culture supernatants described above.

Another chronically HIV-1-infected cloned T cell line, U1, was also employed in this study. U1 has been shown to contain two copies of LAV in its genome, but produces a barely detectable amount of the virus in vitro. This cell line, however, unlike ACH2, did not have a significant increase in Gag protein production following exposure to AZC.

The Effect of 5-Azacytidine on Methylation of HIV Genome in ACH2 Cells.

To determine whether the potentiated production of three HIV-encoded products, envelope glycoproteins, Gag protein, and reverse transcriptase, correlated with any changes in the state of methylation of HIV-1 proviral DNA, total DNA was extracted from the same cells in which AZC-induced potentiation was observed, and the methylation patterns was assessed by using Southern blot hybridization technique. High molecular weight DNA was digested with Kpn I and either of restriction endonuclease isoschizomers, Msp I and Hpa II, and the DNA samples were hybridized with a probe containing a 1.3 kilobase Bgl II fragment of an HIV-1 strain BH10. Both MspI and Hpa II recognize 5′-CCGG-3′, but Hpa II does not cleave when the CG pair is methylated. Co-digestion of DNA from untreated ACH2 cells with Kpn I and Hpa II generated only a 2.7 kb band while co-digestion with Kpn I plus Msp I generated two dominant bands, 1.5 kb and 0.3 kb bands, indicating that the Hpa II site had been essentially all methylated. However, following AZC treatment, co-digestion with Kpn I/Hpa II gave an additional three bands (2.1, 1.5, and 0.3 kb), indicating that a substantial level of demethylation had occurred in this region.

No Alteration in p24 Gag Production and DNA Methylation Profile was seen Following AZC Treatment in Cells Productively Infected with HIV-1

We then asked whether AZC could potentiate p24 Gag protein production in cells that were productively infected with HIV-1. H9/III_B cells, long-term cultured CD4+ T-cells chronically infected with HIV-1 $_{IIIB}$, produced a substantially high level of p24 Gag protein without AZC treatment. With the AZC treatment, these H9 cells did not increase their production of p24 Gag protein. TM11/III_B cells, normal tetanus-toxoid-specific clonal CD4+ T-cells infected with HIV-1_{IIIB}, also produced a substantial amount of p24 Gag protein, and the AZC treatment did not affect the Gag protein production. PHA-PBM from two volunteers were exposed to HIV-1 $_{IIIB}$ and cultured for 7 days (PHA-PBM/III_B), did not show a significant difference in the production of Gag protein before and after AZC treatment.

In order to ask whether the DNA methylation patterns in H9/III_B, TM11/III_B, and PHA-PBM/IIIB were altered after AZC treatment, co-digestion of DNA from each population with Kpn I and Msp I or Hpa II were performed. Southern blot analysis showed that the env-nef region of the proviral DNA in these cells had also been extensively demethylated without AZC treatment, while a small portion of DNA remained methylated even after 10 uM AZC treatment. These data suggest that proviral DNA, which might be related to HIV-1 expression had been demethylated to a full extent, and therefore, in spite of AZC treatment, no further enhancement in p24 Gag protein production occurred.

Cytotoxicity is Involved in Potentiation of HIV Expression in ACH2 Cells

AZC treatment potentiated the expression of HIV-I in ACH2 cells (Tables 2 and 3). However, AZC also exerted a substantial toxicity to ACH2 population (Table 2). For example, when ACH2 cells were treated at 20 uM for 24 hours, only 10% of the ACH2 cells (as compared to AZC-unexposed controls) survived, and the enhancement of Gag protein production by ACH2 cells was by 47-fold in one representative experiment (Table 2). It was thought that the observed potentiation of p24 Gag protein production might be related to the cytotoxicity of AZC rather than demethylation of proviral DNA. In fact, a variety of treatments which damage cells including ultraviolet light or x-ray irradiation have been reported to lead to enhanced HIV-1 expression in chronically infected cells. We, therefore, treated ACH2 cells with toxic concentrations of five pyrimidine nucleoside analogues other than AZC, 5-fluorouracil (5-FU), cyclopentenylcytosine (CPE-C), 2′-deoxycytidine (dC), 2′-azido-2′,3′-dideoxythymidine (AZT), and arabinosyl adenosine (Ara-C) (Table 2). To achieve a level of cytotoxicity comparable to that with 24-hour exposure to AZC (or its analog DHAC), cells were continuously exposed to four drugs (5-FU, CPE-C, dC and AZT) throughout the 96-hour study period. Ara-C was kept in culture for just 24 hours and the cells were further cultured without the drug for an additional 72 hours. Interestingly, these compounds showed a considerable potentiating effect on the Gag protein production by ACH2 cells although the amount was less compared to that obtained with AZC. 5-FU an anticancer agent, exerted a considerable potentiation in Gag production by

19 times with 29% survivability. Dc also enhanced Gag production by 11 times at an extremely high concentration, 20 mM, giving 17% survivability. Ara-C, well-known as an anticancer or antiherpetic agent, exerted a considerable toxicity to ACH2 cells at 0.05 uM and as few as 20% ACH2 cells could survive. Under this condition, Gag protein production was enhanced by 9.8 fold as compared to no drug control population. AZT, the only antiviral drug against HIV approved for prescription, also enhanced the Gag protein production by 3 times with 48% survivability. In this regard, if the AZC potentiation of HIV-1 expression was associated with de novo infection of ACH2 cells by newly produced HIV-1 virions, it was possible that AZT had inhibited some of the de novo infection of ACH2 cells, which could have resulted in an apparent lower level of potentiation. However, this is unlikely, since a comparable level of enhancement of Gag protein production by AZC was seen in the presence of maximally high concentration of ddI.

To ask whether the DNA methylation pattern was altered in ACH2 cells following exposure to a toxic concentration of dC, Southern blot analyses using the isoschizomer restriction enzymes, Msp I and HpaII, were performed. Resulting data revealed no altered profiles in DNA methylation pattern in spite of the considerable toxicity-related enhanced production of Gag protein, suggesting that the mechanism of toxicity-related changes in HIT-1 expression in ACH2 cells does not involve appreciable level of DNA demethylation.

Taken together, the present data suggest that the potentiating effect of AZC on HIV expression is associated with its capacity to demethylate HIV proviral DNA, while the inherent cytotoxicty of AZC can contribute at least in part to the Gag protein production in these cells.

DISCUSSIONS

Infection with HIV-1 is characterized by a period of clinical latency associated with low to undetectable levels of expression of the virus. There is an early acute phase of infection with viremia; however, after the first months of the infection, a stage of low level viremia with few infected cells detected in peripheral blood follows. The mechanisms responsible for the maintenance of this viral latency have been as yet poorly understood. If specific events in the infected cells causes conversion of the latent state to a productive one are identified, it might be possible to intervene the progression from an asymptomatic stage to the state of immune deficiency in patients with HIV-1 infection. Several factors have been implicated, which can cause the activation of HIV-1 expression. These include immune activation, cytokine-mediated viral induction, trans-activation of HIV-1 genomes by certain DNA viruses, heat shock, and irradiation of infected cells with ultraviolet or x-ray irradiation. Another possible mechanism of activation of HIV expression could be demethylation of proviral DNA sequence. In the present study, we showed that persistently HIV-1-infected T-cell line, ACH2, which produced a low level of HIV-1 in vitro, upon exposure to two DNA-demethylating agents, AZC and its analog, began forming syncytia or producing HIV-1-encoded products. Alteration in the DNA methylation pattern in proviral DNA in

ACH2 cells was confirmed by Southern blot hybridization using the isoschizomer restriction enzymes, MspI and Hpa II, and an HIV-1-specific probe.

It is possible that the HIV-1 strain which was harbored in ACH2 cells was defective or aberrant and the changes brought about by AZC treatment might have complemented the defect or aberration in the HIV-1 strain of ACH2. However, this does not appear to be the case. When ACH2 cells were stimulated with 0.5 ng/ml PMA for 2 days, irradiated, and cocultured with H9 cells, an extensive level of syncytia formation was observed within 2 days of culture, and subsequently an essentially complete cell death occurred. No significant change was seen when H9 cells were cocultured with PMA-unexposed ACH2 cells, indicating that, only after AZC treatment, ACH2 cells began to express envelope glycoprotein which was enough to cause an extensive syncytia formation. Then, in 5 days after coculture, some H9 cells surviving the contact with PMA-stimulated ACH2 cells started to grow. When these H9 cells (referred to as H9/LAV_{ACH2}) were washed and cultured for 3 days, the supernatant was found to contain a substantial amount of p24 Gag protein. These data suggest that the HIV-1 genome integrated in ACH2 cells was fully functional but was in check inside ACH2 cells, rather than HIV-1ACH2 had a genetic defect(s). In this regard, the latent state of ACH2 cells, would be related to certain cis-acting HIV-1 gene suppression (although not proven) or cellular events(s) that could cause failure of HIV-1 expression.

AZC-induced potentiation of viral expression did not occur in any of other chronically infected cells examined in the present study. Monocytoind U1 cells, which otherwise produced low to undetectable levels of HIV-1-encoded components, upon activation with PHA or other stimuli, did not show detectable enhancement of HIV-1 expression when exposed to AZC. It is possible that ACH2 cells undergo different processes for HIV-1 expression than U1 cells do.

AZC, is, by its own right, a toxic agent. We found that the toxicity of AZC per se was, at least in part, associated with the observed enhancement of HIV-1 expression in ACH2 cells. Indeed, it was subsequently found that certain other toxic substances including methotrexate or vinka alkaloids could also potentiate the expression of HIV-1 in ACH2 cells. The present observation that toxic nucleoside analogues can stimulate the expression of HIV at least under certain circumstances might be of practical importance, in particular when nucleoside analogues are administered at toxic levels as anticancer agents in HIV-infected individuals with neoplasms. Such toxic agents may stimulate HIV replication in vivo and aggravate the status of immunodeficiency. The issue of toxicity-related potentiation of HIV expression requires further research.

The present data suggest that DNA methylation/demethylation may play a role in regulating HIV expression at least under certain circumstances, although its relevance to in vivo events remains to be further investigated. The current data also warrant further study to test the possibility if certain therapeutics that exert toxicity such as anticancer agents may affect the expression of HIV.

PRESENTATIONS

1. O'Brien MC and Mitsuya H. The effect of DNA demethylation and HIV expression in vitro. American Federation of Clinical Research, Seattle, Washington, May 3-6, 1991.

2. O'Brien MC and Mitsuya H. The effect of DNA demethylation and HIV expression in vitro. Seventh International Conference on AIDS, Florence, Italy, June 16-21, 1991.

Table 1 Reverse Transcriptase Activity in Culture Supernatant of ACH2 Cells

	Concentration (μM)	RT Activity (cpm/10^6 cells/ml)	% Change
Exp. 1.	0	509 ± 417	100
	2.5	2,312 ± 1,043	454
	10.0	6,868 ± 794	1349
	20.0	17,590 ± 474	3456
Exp. 2	0	3,741 ± 2,251	100
	10.0	8,983 ± 1,089	240
	20.0	42,277 ± 1,543	1130

ACH2 cells were exposed to various concentrations of AZC for 24 hours, washed, resuspended in 40 ml culture medium, and cultured for an additional 72 hours without AZC. The culture was terminated by centrifugation, the supernatants were collected, the virus precipitated by PEG, and the reverse transcriptase activitydetermined as described in Materials and Methods. Values represent the amount of incorporated radioactivity. The background cpm were 1290 ± 327 and 1714 ± 677 for experiments 1 and 2, respectively. Values shown represent radioactivity subtracted with the mean background cpm.

Table 2. Potentiation of HIV Expression in ACH2 Cells May Be Associated with Toxicity of Agents Including AZC

Agent	Concentration (μM)	% Survivability	Gag protein produced ($ng/10^6$ cells/ml)	% Change
AZC	0	100	0.85	100
	10	26	13.61	1601
	20	10	40.33	4745
DHAC	0	100	0.33	100
	100	14	6.48	1964
	500	7	13.42	4067
5-FU	0	100	0.84	100
	10	39	7.7	917
	20	29	16.6	1924
CPE-C	0	100	0.54	100
	0.25	23	2.78	611
	10	12	5.93	1219
dC	0	100	0.70	100
	5000	92	0.94	130
	2000	17	9.0	1100
ARA-C	0	100	0.54	100
	0.01	67	0.85	157
	0.05	20	5.3	981
AZT	0	100	0.75	100
	50	57	1.8	230
	200	48	2.6	340

ACH2 cells cultured with AZC, DHAC, or ara-C were exposed to various concentrations of these drugs for 24 h, washed, resuspended in drug-free media, and cultured for an additional 72 hours. Cells cultured with 5-FU, CPE-C, dC, or AZT were continuously exposed to drugs throughout the 96-hour study period. Culture supernatants were subjected to p24 radioimmunoassay (Dupont).

Table 3. Amounts of p24 Gag Protein in Lysates and Supernatants of ACH2 Cells Cultured with Azacytidine.

Concentration (μM)	Cell Lysate (ng/10^6 cells/ml)	Supernatant (adjusted to ng/10^6 cells/ml)	Cell Survivability (%)
0	1.02 (100)	ND	100
2.5	2.12 (212)	.70	44
5.0	3.57 (356)	1.40	29
10.0	9.75 (972)	2.61	12

ACH2 cells were cultured with various concentrations of AZC for 24 hours, washed, resuspended, and further cultured without the drug. On day 4, the cultures were terminated by centrifugation, and the pelleted cells lysed in 500 μl of lysing buffer. The amounts of p24 Gag protein in the cell lysates and supernatants were then determined by RIA. Values shown represent ng p24 Gag protein/10^6 cells/ml. Values in parentheses represent % change. ND, not detected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07219-01 CO

PERIOD COVERED

October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Development of resistance of HIV in patients receiving AZT, ddC or ddI

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We asked whether patients with AIDS or ARC receiving long-term therapy with AZT, ddC, or ddI developed resistance to these drugs, and if so, to explore its biochemical basis. Primary HIV strains were obtained from peripheral blood mononuclear cells (PBM) from adult patients with AIDS or ARC who received an alternating regimen of AZT and ddC (AZT/ddC) for 15-41 months or who received ddI for 12-16 months. Isolates were obtained before and during antiviral therapy. All virions were isolated by coculturing patient's PBM and phytohemagglutinin(PHA)-stimulated PBM from healthy volunteers. The sensitivity of primary HIV-1 isolates to AZT, ddC, or ddI was assessed by drug concentrations (uM) that brought about 50% p24 Gag negative wells (CN_{50}) when HIV p24 Gag protein production by PHA-PBM exposed to a fixed dose of each viral isolate was determined in 10-12 days culture 4-8 replicates.

We found that AZT therapy can induce AZT-resistant HIV variants as early as 2 months; and once acquired, AZT resistance may remain after switching to ddI therapy for 1 year in some patinets. HIV appears to develop resistance to ddC and ddI less easily than to AZT, while these data do not provide basis for concluding that AZT/ddC or ddI are inferior, equivalent, or superior to AZT as therapy of AIDS. In addition, more research is needed to make clinical correlations between in vitro drug sensitivities of HIV and clinical outcome in HIV infection.

INTRODUCTION

Human immunodeficiency virus (HIV) is a pathogenic retrovirus which is the causative agent of acquired immunodeficiency syndrome (AIDS) and its related disorders. One of the central questions after HIV was discovered was whether antiretroviral therapy would ever be feasible. One drug, 3′-azido-2, 3′-dideoxythymidine (AZT or zidovudine) has now been proven to confer prolonged survival and improved quality of life in patients with advanced HIV infection. More recently, the administration of AZT was shown to delay clinical progression in certain asymptomatic patients with HIV infection.

However, we have faced several new challenges in the antiretroviral therapy of AIDS. These include long-term drug-related toxicities; development of various cancer, particularly as effective therapies bring about a prolongation of survival, and emergence of drug-resistant HIV variants. In 1989, Larder and his colleagues first reported that AZT-resistant HIV strains were isolated from patient with HIV infection who had been under AZT therapy for more than 6 months. Comparative nucleotide sequence analysis of reverse transcriptase-coding region from five paris of sensitive and resistant isolates revealed three amino acid substitutions common to all the resistant strains (Asp^{67} -> Asn, Lys^{70} -> Arg, Thr^{215} -> Phe or Tyr) plus a fourth substitution in three isolates (Lys^{219} -> Gln). Partially resistant isolates had combinations of these four changes. The mutations described would likely affect the charge and/or tertiary structure of the probably catalytic site for the reverse transcriptase. It is worth noting that reverse transcriptase purified from these AZT-resistant HIV variants have shown on significant difference in sensitivity to AZT-TP.

Two other analogs of the dideoxynucleoside family 2′,3′-dideoxycytidine (ddC) and 2′,3′-dideoxyinosine (ddI or didanosine) have demonstrated activity against HIV-1 in some patients with AIDS and ARC in several short-term Phase I clinical trials. As of July of 1991, at least 13,000 patients with AIDS or ARC have received ddC. One of the salient features of HIV is its extreme hypermutability. Thus, from a clinical point of view, the emergence of drug-resistant HIV variants must always be considered in virtually any therapy.

MATERIALS AND METHODS

Virus Isolation

Primary HIV strains were isolated from peripheral blood mononuclear cells (PBM) from adult patients with AIDS or ARC who received an alternating regimen of AZT and ddC (AZT/ddC) for 15-41 months or who received ddI for 12-26 months in the National Cancer Institute. HIV strains were isolated before and during antiviral therapy. PBM ($5x10^5$) purified by Lymphocyte Separation Medium gradient centrifugation were cocultured with $5x10^5$ phytohemagglutinin (PHA)-stimulated PBM (PHA-PBM) from healthy volunteers

in 2 ml RPMI 1640 medium consisting of 15% heat-activated fetal calf serum, 10% (vol/vol) lectin-depleted interleukin-2 (Advanced Biotechnologies, Inc., Silver Spring, MD), recombinant interleukin-2 (10 U/ml, Amgen, CA) supplemented with 4 mM L-glutamine, 50 U/ml of penicillin, and 50 ug/ml streptomycin (IL-2 containing complete medium) in 24-well microtiter culture plates in quadruplicate. One milliliter of culture medium was changed with an equal amount of fresh medium twice a week. On days 8-10 in culture, supernatants were collected and the amount of p24 Gag protein was assessed by radioimmunoassay (DuPont, NEN Research Products, Boston, MA). A culture was considered positive when the concentration of p24 Gag protein in the supernatant exceeded 1 ng per ml.

Titration of Infectivity of Virus Preparation

Various amounts of culture supernatants containing infectious HIV-1 virions were added to $5x10^5$ PHA/PBM in 2 ml in 8 replicates, and cultured at 37 C for 8-10 days. The 50% tissue culture infectious dose ($TCID_{50}$) per ml of culture supernatants was determined by an endpoint titration method by Leland and French, based on Gag protein production by PHA-PBM.

Determination of Sensitivity of Virus against Drugs

Target PHA-stimulated PBMC ($5x10^5$) were exposed to a 20 $TCID_{50}$ viral dose followed by the addition of drugs, and cultured for 8-10 days in 24-well microtiter culture plates in quadruplicates. Control cells were similarly treated but not exposed to the virus. A half of culture medium was changed with an equal amount of fresh medium twice a week and the drug concentrations were kept the same. On day 8 of 10 in culture, supernatants were harvested and the amount of p24 Gag was determined by radioimmunoassay. Sensitivity of HIV-strains to drugs was expressed as drug concentrations (uM) that brought about 50% p24 negative wells (CN_{50}). All experiments were performed in 4-8 replicates.

Amplification of pol Gene by Polymerase Chain Reaction

Cells from a culture of PHA-PBM and patient's PBM were harvested on day 8=10 days of culture, washed twice with PBS, lysed in 50 mM KCI, 10 mM tris-HCI, ph 8.3, 10 mM $MgCl_2$, 0.045% (vol/vol) Nonidet-P 40, and proteinase K (1 mg/ml) for 1 hours at 50 C, and heated at 95 C for 12 min. Twenty microliters of such cell lysate were added to PCR reaction mixture (100 ul) that contained 50 mM KCI, 2 mM $MgCl_1$, 10 mM tris -HCI (ph 8.3), 0.91% gelatin, 02 mM deoxyadenosine-5'-triphosphate (dCTP), thymidine-5'-triphosphate (dTTP), and 50 pmol of each primer. The reaction mixture was boiled for 5 min, added with Taq DNA polymerase (2.5 units, Perkin-Elmer Cetus), overlaid with 60 ul of light mineral oil, and subjected to PCR. PCR was carried out with 40 cycles with denaturation at 94C for 1.5 minutes, annealing at 50 C for 3 minutes, and extension at 72 C for 10 minutes. The oligonucleotide primers used (Lofstrand Labs Ltd., Gaithersburg, MD) were as follows: at the 5' end of pol region, 5'-TTGCACTTTGAATTCTCCCATTAG-3' and at the 3'end, 5'-CTTATCTATTCCATCTAGAAATAGT-3'.

Determination of Nucleotide Sequences

Following PCR, the pol fragment (1.7 Kb) amplified in reaction mixture was extracted with phenol/chloroform, precipitated with ethanol, and resuspended in distilled water. The pol fragment was then digested with Eco RI and Xba I (Bethesda Research Laboratories), subjected to electrophoresis in 0.8% agarose gel, and collected at the 1.7 kb level of the gel. The digested pol fragment was retrieved from agarose gels using glass beads (Geneclean, BIO 101, San Diego, CA), and inserted to pTZ19R vector (United States Biochemical Corp., Cleveland, OH). Competent E.coli (strain DH5) was transformed with the pol-inserted pTZ19R, followed by cloning using conventional methods. Clone E. coli was expanded, and pol-inserted pTZ19R as double-stranded DNA was purified using alkaline lysis method and DNA affinity column (Qiagen, GmbH, Germany). The pol-inserted pTZ19R was then subjected to sequencing using the dideoxy chain termination method. Nucleotide sequences of at least ten different clones per one isolate were determined.

RESULTS

HIV isolated from patients receiving AZT/ddC is resistant to AZT but remains sensitive to ddC

We first noted a substantial level of variability in the infectivity of primary HIV-1-isolate preparations when their tissue culture infectious doses ($TCID_{50}$) were determined by variability was by up to 6-fold depending PHA-PBM preparations used for culture. Therefore, each pair of isolates (pre- and post-therapy) to be compared was simultaneously titrated and subsequently assessed simultaneously for drug sensitivity.

We found that all patients examined who received AZT/ddC developed resistance to AZT but not to ddC. For example, patient 100's pre-therapy HIV-1 ($HIV\text{-}1_{100pre}$) was fairly sensitive to AZT; and its CN_{50} was as low as 0.2 uM; however, after 15 months of AZT/ddC therapy, CN_{50} of post-therapy HIV-1 ($HIV\text{-}1_{100post}$) was unusually high (16 uM), indicating that patient 100 developed a high level of AZT resistance. However, the sensitivity of the post-therapy HIV-1 to ddC and ddI was found to be virtually the same as that of pre-therapy HIV-1. Interestingly, patient 104, who received AZT/ddC for 3.5 years, appeared to have developed AZT resistance together with relative resistance to ddC, although it should be pointed out that CN_{50} of $HIV\text{-}1_{104post}$ for ddC was just 2 uM, and further follow-up is required before one makes a conclusion in this case.

HIV-1 strains had no mutations common to patients who received AZT/ddC other than AZT-related mutations

The pol region of the genome of HIV-1 isolates from 4 patients receiving AZT/ddC was amplified by polymerase chain reaction by using a pair of pol-specific primers. The 1.7 kb pol-specific PCR products were then cloned in a pTZ19R vector, and the nucleotide sequences of ~700 base pairs in the 5′ end pol region were determined. Three isolates ($HIV\text{-}1_{100post}$, $HIV\text{-}1_{101post}$, and $HIV\text{-}1_{102post}$) had at least one of the 4 previously reported AZT-related mutations. However, one AZT-resistant strain

($HIV\text{-}1_{103post}$) did not have any reported AZT-related mutations. Further research is underway to identify mutations which may be responsible for the observed AZT resistance in $HIV\text{-}1_{103post}$. In the present study, we did not identify any obvious resistance to ddC in our assay system using PBM as target cells. It was still possible that the assay system employed in this study was not sensitive enough to detect resistance to ddC (although sensitive enough to detect AZT resistance) and we may have failed to detect it. However, there were no mutations common in HIV-1 strains from patients who received ddC. Assuming a finite number of mutations, presumably in combinations, can render HIV-1 resistant to ddC, the present nucleotide sequence data would support that HIV-1 strains we examined had no obvious resistance to ddC, although more research is required.

HIV Isolated from Patients Receiving ddI Remains Sensitive to ddI

Six patients who received ddI for up to 26 months were also examined for emergence of ddI-resistant HIV-1 variants. However, no obvious ddI-resistant HIV-1 strains were identified. The sensitivities of post-therapy HIV-1 strains to AZT, ddC and ddI did not differ from those of pre-therapy HIV-1 strains in all patients who had received ddI.

Among 6 ddI patients, 4 had received AZT for 2-12 months prior to ddI therapy. An HIV-1 strain from patient 204 who received AZT for just 2-3 months ($HIV\text{-}1_{204post}$), was found to be highly resistant to AZT; and this aZT resistance persisted on ddI for about 23 months. However, patient 205's HIV-1 strain isolated after 29 months of ddI therapy without AZT ($HIV\text{-}1_{205post}$), resumed sensitivity to AZT. It should be noted that no significant changes in the sensitivity to ddC or ddI was observed in these patients. Patient 203 received AZT for I year and developed a high level of AZT resistance; however, when HIV-1 was isolated 2 years after switching to ddI therapy, the level of AZT resistance was found to be substantially decreased with CN_{50} changing from 32 uM down to 2 uM. However, upon short-term AZT therapy, AZT resistance developed again relatively quickly. No change in the sensitivity to ddI and ddC was identified throughout this study. It should be noted that any HIV-1 strains isolated from patients who received ddI, no common mutations were identified. It is worth stressing that while all patients who received AZT developed resistance to AZT ($p \leq 0.04$, Wilcoxon's signed rank test), those who received ddI for up to 3 years remained sensitive to ddI.

DISCUSSIONS

In this study, we examined 5 patients receiving an alternating regimen of AZT for 1 week and ddC for 1 week for 15-41 months. We also examined 6 patients receiving ddI for 12-26 months. HIV-I strains were isolated by culturing patient's peripheral blood mononuclear cells (PBM) and normal PHA-stimulated PBM. The success rate of isolating HIV-1 strains was ~90%. Sensitivities of HIV-1 strains were assessed by drug concentrations that gave 50% negative wells (CN_{50}). In spite of requirements of multiple medium replacement in long-term PBM culture, employing CN_{50} for comparison of HIV-1 sensitivity to drugs mitigated the inherent fluctuations when p24 Gag amounts were used as an endpoint.

In 1989, Larder el al reported that AZT-resistant HIV-1 variants were isolated from patients with AIDS who had received AZT $\geq$ 6 months. However, in our study, AZT-resistant HIV-1 strains were isolated as early as 2-3 months of therapy with AZT. Once acquired, AZT resistance may remain after switching to ddI therapy for >1 year, although in some patients, AZT resistance may decrease off AZT within a year. We also observed that upon resumption of AZT therapy after 1 year's ddI therapy off AZT, AZT resistance was relatively quickly (after 2 months' AZT therapy) identified in one patient. In theory, once integrated, a genome of HIV-1 remains unchanged and persists in an infected cell unitl the cell terminally differentiates or gets destroyed by the virus. If ddI is present, the infection and replication of AZT-resistant HIV-1 variants would be kept suppressed; however, such HIV-1 variants will remain in cells for a long period of time as long as those cells survive. Presumably, cells that harbor AZT-resistant HIV-1 persistently produce a low level of HIV-1, which in the presence of ddI fail to infect and propagate in target cells. But if ddI is stopped and AZT is resumed, AZT-resistant HIV-1 variants can start infecting cells and propagating itself. It is not known whether AZT-resistant HIV-1 gets detected earlier in patients upon resumption of AZT than in patients who receive AZT for the first time. This is one of the major topics for future investigation.

The previously reported mutations would likely change the charge or tertiary structure of the probably catalytic site of reverse transcriptase. Such changes might affect the access of AZT-5'-triphosphate (TP) to the catalytic site or to other functionally critical sites such as the nucleotide binding site or the primer binding groove. Richman et al have described that AZT-resistant HIV-1 variants do not show cross-resistance to ddC, ddI or d4T. Our present data suggest that HIV-1 develops resistance to AZT more readily than to ddI or ddC. Taken together, it is conceivable that HIV-1 takes a strategy to develop resistance to AZT by mutating itself to exclude AZT-5'-TP during proviral DNA synthesis while perserving the functional integrity of reverse transcriptase; however, it is rather difficult for the virus to mutate itself to exclude ddATP or ddCTP and preserve the function of reverse transcriptase since both dideoxynucleotides closely resemble their corresponding normal nucleotides, dATP or dCTP, in their structures.

It is worth noting that although the present data suggest taht HIV may develop resistance to AZT more easily than to ddC or to ddI in patients receiving antiviral therapy, such data do not provide basis for concluding that AZT/ddC or ddI are inferior, equivalent, or superior to AZT as therapy of AIDS. More research is needed to make clincial correlations between <u>in vitro</u> drug sensitivities of HIV and clincial outcome in HIV infection. Study of drug-resistant viral strains may make it possible to identify novel viral targets and to develop more effective therapeutic strategies.

PUBLICATIONS

1. Mitsuya H, Yarchoan R and Broder S. Molecular targets for AIDS therapy. 1990; Science 24:1533-1544.

PRESENTATION

1. Shirasaka T, Yarchoan R, Husson R, Shimada T, Wyvill KM, Broder S and Mitsuya H. HIV may develop resistance preferentially to azidothymidine (AZT) as compared to dideoxycytidine (ddC) and dideoxyinosine (ddI) in patients receiving antiviral therapy. 1991; Seventh International Conference on AIDS, Florence, Italy, June 16-21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07220-01 CO

PERIOD COVERED
October 1, 1990 through September 20, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

In vitro inhibition of HIV-1 replication by C_2 symmetric HIV protease inhibitors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Hiroaki Mitsuya, M.D., Senior Investigator, Clinical Oncology Program, NCI
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Office of the Associate Director, Clinical Oncology Program

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

HIV strains used in this project included a laboratory strain (HIV-1_{IIIB}), a monocytotropic strain (HIV-1_{Ba-L}), and primary HIV strains isolated from AIDS patients in phytohemagglutinin-stimulated peripheral blood mononuclear cells (PHA- PBM). Target cells used included CD4+ ATH8 cells, purified monocytes/ macrophages (M/M), and PHA/PBM. Endpoints used included the inhibition of the cytopathic effect (CPE) of HIV-1 and p24 Gag protein production by target cells, following HIV exposure. Analysis of drug interactions was performed using the COMBO program. We found that all protease inhibitors tested exhibited a significant inhibition of the CPE against ATH8 cells with a range of 50% inhibitory concentrations (IC_{50}) being 0.06 - 1.8 um. In addition, they completely inhibited the replication of HIV-1_{Ba-L} in M/M at 0.75 - 2 uM throughout 26 days of culture. A potent inhibitory activity against primary HIV isolates was also observed with IC_{50} ranging from 0.09 to 0.22 uM for all three compounds when PHA/PBM were employed as target cells. Cellular toxicity was neglible at the highest concentreations used (up to 10 uM). When the protease inhibitors and dideoxynucleosides were used in dombination against primary HIV isolates, their antiviral activities appeared additive in some cases and synergistic in others.

We conclude that C_2 symmetric HIV protease inhibitors tested in this study had a potent antiviral activity against a wide range of HIV isolates including monocytotropic strains and primary HIV isolates, and they represent promising experimental antiviral agents for the therapy of HIV infection.

INTRODUCTION

The HIV protease represents a crucial virus-specific target for new therapies of AIDS. Such an approach is one way of inhibiting the production of mature, infectious virions in chronically HIV-infected cells. Recently, antiretroviral peptide analogs have been synthesized based on the knowledge of physiology and structure of HIV-1 protease. One class of such protease inhibitors is C_2 symmetric protease inhibitor.

Logical extension of current approaches for therapy of HIV infection would be the use of combinations of multiple antiviral agents which have different antiretroviral mechanism(s). Thus, we have now explored whether symmetric HIV protease inhibitors are active against a variety of HIV strains and synergize with dideoxynucleosides such as AZT or ddI in vitro. We have also asked whether such inhibitors cause irreversible changes to HIV protease. In the present report, we describe that C_2 symmetric HIV protease inhibitors tested in this study had a potent antiviral activity against a wide range of HIV isolates including monocytotropic strains and primary HIV isolates. We also demonstrate and discuss effect of combinations of such C_2 symmetric HIV protease inhibitors and AZT or ddI, dideoxynucleoside analogues which are already in a clinical domain.

MATERIAL AND METHODS

Reagents

All C_2 symmetric HIV protease inhibitors used in this study were synthesized as previously described by J. Erickson et al. 2;,3′-dideoxyinosine (ddI) was provided by the Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, while 3′-azido-2′,3′-dideoxythymidine (AZT) was purchased from Sigma Chemical Co.

Viruses and Cells

HIV-1 was pelleted by ultracentrifugation from the culture supernatants of HIV-$1/III_B$-producing H9 cells and was prepared to contain 7.39×10^{10} virus particles per ml. The 50%-tissue culture infective dose ($TCID_{50}$) per ml of the cell-free HIV-1 preparation was determined by an endpoint titration method using CD4+ T-cells (ATH8) as previously described by Leland and French.

The supernatant of monocyte/macrophage (M/M) culture following exposure to HIV-1_{Ba-L} was collected and used as a source of infectious monocytotropic virus as previously described.

Cloned CD4+ T-cells, ATH8, were used as target cells for infection by HIV-1 in this study.

HIV-1 Cytopathic Effect Inhibition Assay

HIV cytopathic effect inhibition assay was performed as previously described. Briefly, target CD4+ T cells (ATH8) were exposed to a 3162 or 1581 $TCID_{50}$ dose of HIV-1 for 1 hr, resuspended in 2 ml of fresh complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/ml of penicillin, and 50 ug/ml of streptomycin) containing 15% (vol/vol) interleukin-2 (IL-2, lectin-depleted; Advanced Biotechnologies Inc., Silver Spring, MD) and 50 U/ml of recombinant IL-2 (Amgen, Thousand Oaks, CA), and the cells were incubated at 37 C in 5% CO_2-containing humidified air. Control cells were treated similarly but were not exposed to the virus. At various time points, viable cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method.

Determination of HIV-1 Gag Protein Production by PHA-PBM or Monocytes/Macrophages

HIV-1 Gag protein production by PHA-PBM or M/M were determined as previously described. Briefly, target PBM or M/M (10^6) were preincubated with drugs for 2 hours or 24 hours respectively, exposed to 1581 or 3162 $TCID_{50}$ viral dose of clinical isolate of HIV-1 or a 21 $TCID_{50}$ viral dose of $HIV\text{-}1_{Ba\text{-}L}$ preparation. PHA-PBM were cultured in 2 ml and M/M were cultured in ml in the presence or absence of the drug. Every 4-5 days, a half of the culture medium for PHA-PBM or 90% of the culture media for M/M were replaced with an equal amount of fresh medium. The amounts of Gag protein in M/M and PBM cultures were determined by radioimmunoassay (DuPont, NEN Research Products, Boston, MA) on day 6 and beyond and on day 10, respectively.

RESULTS

IN VITRO ANTIRETROVIRAL ACTIVITY OF C_2 SYMMETRIC HIV PROTEASE INHIBITORS AGAINST HIV

All of C_2 symmetric HIV protease inhibitors tested in this study were active against $HIV\text{-}1_{IIIB}$ when assessed in the HIV-1 cytopathic effect inhibition assay using ATH8 cells as target cells (Table 1). Three compounds, A75925, 77212 and 76890, were most potent based on the molarity. ED_{50} of these compounds ranged between 0.055 and 0.072 uM. Other compounds were also active and their ED_{50S} were between 0.49 and 1.8 uM. The toxicity of these compounds was negligible or marginal even at the highest concentrations tested.

Antiviral activity of three selected protease inhibitors, the most potent compound (A75925) and two moderately potent compounds (A77003 and A76928) based on the molarity, was confirmed in culture assays employing monocyte/macrophages (M/M) as target cells.

In the absence of drugs, by day 12 in culture, M/M following the exposure to $HIV\text{-}1_{Ba}$ began to produce a detectable amount of HIV-1; and by day 26, they produced as much as 25 ng/ml p24 Gag protein as assessed by

radioimmunoassay. However, when M/M were cultured in the presence of 0.75 uM A75925, A77003, or A76928 replication of the virus was virtually completely inhibited.

Three Compounds A75925, A77003, and A76928 Can Suppress the Replication of Clinical Isolates of HIV-1 In Vitro

It has been shown that clinical isolates of HIV-1 behave differently from laboratory HIV-1 strains that have been maintained for a long period of time in vitro in terms of sensitivities to certain antiviral agents including soluble CD4 preparations. We then asked whether three of C_2 symmetric HIV protease inhibitors could suppress the replication of clinical isolates of HIV-1 (HIV-1_{ESR205}) in PHA-activated PBM when these cells were exposed to the virus. PBM were preincubated with the drugs for 2 hours, exposed to 100 $TCID_{50}$ dose of the virus, and cultured in the presence or absence of the drugs. In a representative experiment, 0.05-0.1 uM A75925 always gave a complete suppression of HIV replication. This precipitous nature of dose response was observed when A77003 and A76928 were tested against both HIV-1_{ESR205} and HIV-1_{ESR105}. In these experiments cellular toxicity of protease inhibitors was negligible at highest concentrations used (up to 10 uM).

Antiviral Activity of A75925, A77003 and A76928 Combined with AZT or ddI

We finally asked whether two drugs, A75925, A77003, and A76928 could synergize against clinical isolates of HIV-1 when combined with AZT or ddI. When PBM was cultured in the presence of 0.09 uM A75925, HIV-1 replication was suppressed by about 50% in a representative experiment. At 0.32 uM, AZT gave a virtually complete suppression. On the other hand, A75925, at 0.05 uM, exerted only a marginal antiviral effect. However, when 0.08 uM AZt and 0.05 uM A75925 were combined, a virtually complete suppression was observed. As assessed by using COMBO analysis, AZT was found to be synergistic with A75925 ($p < 0.001$). This was the case when another C_2 symmetric HIV protease inhibitor, A76928 was combined with AZT ($p = 0.009$). When A77003 was combined with AZT, there was also a trend to synergy ($p = 0.009$). When A77003 was combined with AZT, there was also a trend to synergy ($p = 0.06 - 0.07$). However, under the same conditions, ddI was found to be additive with A75925 or A76928; and when ddI was combined with A77003, the observed antiviral activity turned out to be additive (even antagonistic in one experiment) (Table 2).

DISCUSSIONS

In 1990, Erickson and his colleagues developed C_2 symmetric inhibitors of HIV-1 protease based on the three-dimensional symmetry of the enzyme active site. These C_2 symmetric compounds exert a potent activity against several strains of HIV-1 in vitro. In the present study, we asked whether these C2 symmetric inhibitors could suppress the replication of a wide range of HIV-1 in various cell culture system and whether the C_2 symmetric inhibitors had synergistic antiviral activities when combined with DNA-chain terminating antiviral nucleoside analogs, AZT and ddI.

All C_2 symmetric HIV-1 protease inhibitor tested in this study exerted potent activity against HIV-1 in several different culture systems.

Some selected C_2 symmetric HIV-1 protease inhibitors completely inhibited the replication of HIV-1_{Ba-L} in macrophages-monocytes at 0.75-2 uM throughout 26 days of culture. A potent inhibitory activity against primary HIV isoaltes was observed with IC_{50} ranging from 0.09-0.22 uM for three selected C_2 symmetric HIV-1 protease inhibitors (A77003, A76928, and A75925). In these assay systems employing various kinds of target cells, no significant cellular toxicity was observed even at highest concentrations used (up to 10 uM). These data appear to encourage further investigations directed for clinical application. However, all compounds tested in the present study were all highly hydrophobic and could be dissolved in only dimethyl sulfoxide (DMSO). Because of this insolubility in the present study, only up to 10 uM could be tested for toxicity. This general insolubility may be a potential problem in applying this class of antiviral agents and further study is necessary.

ANtiviral activities of protease inhibitors tested were found to be synergistic or toward synergism when combined with AZT. However, only a trend toward additivism was identified when combined with ddI. These data may suggest that protease inhibitors affect differently the anabolism of these dideoxynucleosides to their corresponding active moieties or the normal pool of deoxynucleotides. More studies are warranted.

Taken together, at least several selected C_2 symmetric HIV-1 protease inhibitors tested appeared to be promising experimental antiviral agents for the therapy of HIV-1 infection and further investigation is warranted.

PRESENTATION

1. Kageyama S, Erickson J, Norbeck D, Weinstein J, Kempf D, Broder S, Plattner, J and Mitsuya H. In vitro inhibition of HIV-1 replication by C_2 symmetric HIV protease inhibitors as single agents or in combinations with azidothymidine (AZT) or dideoxyinosine (ddI). Seventh International Conference on AIDS, Florence, Italy. June 16-21, 1991.

PUBLICATIONS

1. Mitsuya H, Yarchoan R, Kageyama S and Broder S. Targeted therapy of human immunodeficiency virus-related disease. FASEB (in press).

2. Mitsuya H, Yarchoan R and Broder S. Molecular targets for therapy of AIDS. 1990; Science 249:1533-1544.

Table 1. In Vitro Anti-HIV Activity of C_2 symmetric Protease Inhibitors

Compound	TCID50	Concentration (μM)	% Protective effect	ED50 (μM)	% Toxicity
A75925	3,162	0.01, 0.05, 0.1	0, 45, 94,	0.055	12, 12, 6
	3,162	0.1, 1, 10	72, 83, 89	<0.1	17, 17, 17
	1,581	0.1, 1, 10	89, 71, 89	<0.1	8, 0, 0
A77212	3,162	0.01, 0.05, 0.1	4, 33, 91	0.065	3, 0, 9
	1,581	0.1, 1, 10	100, 100, 100 <	0.1	0, 8, 0
A76890	3,162	0.01, 0.05, 0.1	0, 18, 91	0.072	0, 9, 9
	1,581	0.1, 1, 10	100, 100, 100	<0.1	0, 0, 0
A76928	3,162	0.01, 0.05, 0.1	0, 17, 20	0.6	12, 0, 6
	1,581	0.1, 1, 10	100, 100, 100	0.1	8, 0, 0
A76264	3,162	0.1, 1, 10	5, 89, 72	0.58	0, 11, 6
A77002	3,162	0.1, 1, 10	2, 83, 94	0.63	11, 0, 17
A75912	1,581	0.1, 1, 10	0, 45, 100	1.8	0, 0, 0
A77003	1,581	0.1, 1, 10	41, 81, 100	0.3	8, 17, 0
A76792	1,581	0.1, 1, 10	48, 100, 100	0.13	0, 0, 0
A76889	1,581	0.1, 1, 10	8, 41, 100	1.2	0, 0, 0

SUMMARY OF DATA ON COMBINATIONS OF ddN's AND PROTEASE INHIBITORS (COMBO analysis)

ddN	Protease Inhibitor	Prot. Inhib. IC_{50} (μM)	Potentiation Param. PC_{50} (μM)*	p-value**	% error per point***	Conclusion
AZT	**A77003**	**0.21** (±0.01)	**0.32** (0.19 to 1.04)	**0.07**	6.9	**Trend to synergy**
AZT	**A77003**	**0.13** (±0.01)	**0.10** (0.06 to 0.28)	**0.06**	16.3	**Trend to synergy**
AZT	**A75925**	**0.09** (±0.003)	**0.04** (0.03 to 0.05)	**<0.001**	9.0	**Synergy**
AZT	**A76928**	**0.18** (±0.02)	**0.13** (0.09 to 0.23)	**0.009**	7.9	**Synergy**
ddI	**A77003**	**0.22** (±0.01)	**2.17** (>0.61)	**0.35**	7.0	**Additivity**
ddI	**A76928**	**0.18** (±0.01)	**-0.56** (<-9.58)	**0.83**	8.8	**Additivity**
ddI	**A75925**	**0.09** (±0.005)	**-0.40** (<-0.83)	**0.75**	9.7	**Additivity**

* The PC_{50} (potentiation parameter) is defined as the concentration of protease inhibitor required to increase the apparent potency of the ddN by two-fold (after subtracting any intrinsic effect of the protease inhibitor).

- Low PC_{50} indicates strong potentiation;
- PC_{50} approaching ∞ indicates additivity;
- PC_{50} < 0 indicates antagonism.

Limits shown are asymmetric (distribution of parameter estimates is non-gaussian)** One-tail t-test for null hypothesis of additivity (vs synergy)

** One-tail test; null hypothesis of additivity (vs. potentiation).

*** Mean weighted % deviation from model per data point

For explanation of COMBO analysis of drug combinations, see Weinstein, et al., Annals N.Y. Acad. Sci. 616: 367, 1990; Bunow and Weinstein, *ibid*, 490, 1990; Ashorn, et al., PNAS 87: 8889, 1990)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 CM 07221-01 CO

PERIOD COVERED

October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Plasma HIV-1 viremia in HIV-1 infected individuals assessed by RNA-polymerase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Hiroaki Mitsuya, M.D., Senior Investigator, Clinical Oncology Program, NCI
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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

A rapid and sensitive method to determine the levels of human immunodeficiency virus type 1 (HIV-1) in plasma from patients with HIV-1 infection was established by using RNA polymerase chain reaction (PCR). Our plasma RNA-PCR technique quantitatively detected HIV-1 particles in plasma from 76 of 77 (98.7%) HIV-1 infected individuals examined at varying clinical stages with a range of more than 4 orders of magnitude, while p24 antigen capture enzyme-linked immunosorbent assay (ELISA) failed to detect circulating p24 antigen in 43 of 72 (59.7%) seropositive individuals. Acid treatment prior to ELISA increased the positivity for p24 antigen, but 21 of 60 (35.0%) seropositive individuals still remained negative. The numbers of HIV-1 particles in plasma from patients with AIDS or ARC were markedly higher than those in plasma from asymptomatic seropositive individuals ($p<0,0001$). When the changes of viral load following the antiviral therapy were monitored by using this plasma RNA PCR method, 10 of 10 patients who received oral 2',3'-dideoxyinosine (ddI) at $\geq$ 6.4 mg/kg/day doses had a significant decrease in plasma HIV-1 particle numbers following 8 to 14 weeks of therapy ($p=0.0051$).

These data suggest that plasma HIV-1 virion levels determined by the current RNA PCR technique represent the actual plasma HIV-1 viremia status in patients with varying stages of HIV-1 infection, and may provide clinically useful information, especially in patients with negative serum p24 antigen. However, more research is required to evaluate the usefulness of this technique in assessing the disease status and monitoring the effect of anti-retroviral therapy.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is a causative agent of acquired immunodeficiency syndrome (AIDS) and its related disorders. HIV-1 has been shown to infect and replicate in a variety of cells in vivo including CD4+ T cells, certain B cells, monocytes/macrophages, Langerhans cells and brain glial cells. HIV-1 genomes have been identified in such cells by molecular biology technologies and a number of virus strains have been isolated from various tissues using a variety of culture techniques. There are considerable amounts of data to suggest that virus load and immunosuppression are major determinants of the severity of HIV-1 related diseases. Moreover, it has been suggested that the degree of viral load is related to the progress in immunological deteriorations. Thus, the measurement of virus load in patients is probably crucial for prognostic assessment. However, there is no sensitive and specific assay system to estimate virus load at the present time. HIV-1-related antigens such as p24 Gag protein are often undetectable in blood of many seropositive individuals as assessed by the conventional radioimmunoassay or enzyme-linked immunosorbent assay (ELISA). The positivity of detectable circulating p24 antigen ranges from 15 to 86% in HIV-1 seropositive individuals depending upon patient populations or methods employed. These findings and a study using in situ hybridization showing that only 1 in 10^5 peripheral blood mononuclear cells (PBM) from patients with HIV-1 infection expresses HIV-1 messenger RNA (mRNA) in vivo have led to the general notion that the level of HIV expression is low and the HIV-1 viremia status is often hard to be monitored. In this regard, Ho et al have recently developed end-point-dilution culture technique and demonstrated that the levels of HIV-1 in plasma and PBM are much higher than previously thought. If the levels of viral load are directly and quantitatively determined, such technologies might provide more accurate information to asses the disease processes. Furthermore, while a number of experimental antiretroviral therapies are in advanced or early phases of clinical testing, no precise clinical markers to determine the efficacy of such drugs are as yet available. We report here a methodology to quantitate HIV-1 particles in plasma of HIV-1 infected individuals and its potential to monitor the changes of viral load in patients with AIDS or ARC during antiretrovial therapy against HIV-1 infection.

METHODS

Patients

Plasma or serum samples were obtained from 77 patients with HIV-1 infection, including 28 asymptomatic HIV-1 seropositive carriers (CDC class II & III[18]), 38 with ARC (CDC class VI_A) and 11 with AIDS (CDC class $VI_{C/D}$). Among 49 patients with AIDS or ARC, 11 enrolled in the Phase I clinical trial with ddI conducted at the National Cancer Institute. Plasma or serum samples were collected from these randomly selected 11 patients before, during and after ddI administration. Other HIV-1 infected individuals had not received any antiretroviral drugs when plasma samples were obtained. Samples were also collected from 23 HIV-1 seronegative individuals (7 with adult T-cell leukemia, 3 with asymptomatic human T-cell

leukemia virus type 1 (HTLV-1)-carriers, 2 with common variable immunodeficiency, 2 with Wiskott-Aldrich syndrome, 1 with severe combined immunodeficiency, 4 with Non-Hodgkin's or Burkitt's lymphoma, 3 with solid tumors and 1 with prolymphocytic leukemia). Ten HIV-1-seronegative healthy volunteers served as controls.

RNA Extraction from Pelleted Virus

Heparinized blood samples were centrifuged at 1,000 g for 25 minutes to remove all cellular components and plasma in the top phase was carefully removed and stored at -70 C until use. Otherwise stated, 1-2 ml of plasma samples were ultracentrifuged at 44,000 g for 1 hour at 4 C. The virus pellet suspension (50 ul) was mixed with 400 ul lysis buffer containing 0.5% SDS, 0.3 M NaCI, 10 mM EDTA, 15 mM Tris/HCl (pH 7.4), 10 mM vanadyl ribonucleoside complex (Bethesda Research Laboratories; BRL, Gaithersburg, MD) and 50 ug/ml yeast tRNA (BRL). Viral RNA was extracted from the mixture with an equal volume of phenol/chloroform, then with chloroform, and was precipitated with ethanol. Rabbit globin mRNA served as an exogenously added internal control for reverse transcription and subsequent PCR.

Oligonucleotides

Two oligonucleotide primer pairs were employed to amplify HIV-1 sequences: SK 38/39 from a 115-bp region of gag (1551-1578 and 1638-1665) and SK 68/69 from a 142-bp region of env (7801-7820 and 7922-7942). An oligonucleotide primer pari, RAG 3/4 (5′-TACTCTCAGCGACCTGCACGCGCACAA-3′ AND 5′-ACGATATTTGGAGGTCAGCACGGTGC-TCACGT-3′) was selected to amplify a 181-bp region of rabbit -globin-encoding gene (243-269 and 392-423, respectively).[21] For hybridization of amplified products, ^{32}P-labeled probes, SK 19 (1595-1635), SK 70 (7841-7875), and RAG 2 (5′-TGAATTCACTGGGGTGGT-GGTTGGCCAGGGGTCA-3′) (320-352) were used for SK 38/39, SK 68/69, and RAG 3/4, respectively.

Reverse Transcription and PCR

A portion (15 ul) of the resuspended RNA solution was treated with 10 units RNase-free DNase I in a final volume of 20 ul containing 2 mM $MgCl_2$ at 37 C for 10 minutes, then at 56 C for 10 minutes to inactivate DNase I. Reverse transcription (RT) was performed as described previously [22-25] with some modifications. Briefly, a portion (5 ul) of DNase-treated RNA sample was mixed with 15 ul of RT reaction mixture to contain 15 mM DTT, 40 units of RNasin ribonuclease inhibitor (Promega, Madison, WI), 500 units Moloney Murine Leukemia Virus reverse transcriptase (BRL), 12.5 pmol of two downstream primers, SK 39 or SK 69 and RAG 4. The mixture was incubated at 37 C for one hour followed by an incubation at 95 C for 5 minutes to inactivate the reverse transcriptase. The addition of RAG 4 in RT reaction mixture and RAG 3/4 in PCR mixture was confirmed not to affect the efficiency of reverse transcription of HIV-1 sequences and the subsequent PCR, respectively.

A portion (13.3 ul) of thus obtained cDNA-containing RT reaction mixture was subjected to PCR as previously described. This 13.3 ul portion represented a RT solution containing HIV-1 cDNA derived from 1/20 of HIV-1 RNA originally isolated from a plasma sample. The 13.3 ul portion was mixed with PCR working buffer (86.7 ul) to contain 62 mM KCl, 20 mM Tris HCl, 12 m?M $MgCl_2$, 0.02% gelatin, 50 pmol of SK 38/39 or SK 68/69 and RAG 3/4, 200 uM dATP, dGTP, dTTP, dCTP, and 5 units of Thermus aquaticus (Taq) polymerase, and the mixture was subjected to 30 cycles of denaturation at 94 C for 1 min, primer annealing at 55 C for 2 min, and polymerization at 72 C for 3 min using DNA thermal cycle. The amplified DNA was extracted by chloroform; hybridized in solution with a ^{32}P-labeled probe, SK 19, SK 70 and RAG 2 subjected to electrophoresis on an 8% polyacrylamide gel; and the dried gel was exposed to Kodak X-OMAT film at a room temperature for 1-12 hours. The density of three bands (see below) was measured by desitometer (S-Rite 301; X-Rite Inc, Grand Rapids, MI). The radioactivity of the area encompassing these bands was also directly measured by a radioisotope scanner (AMBIS System Inc., San Diego, CA).

Standardization of Plasm RNA PCR

For standardization, various amounts of a full-length, double stranded HIV-1 DNA-containing plasmid, pHXB2; 17 Kb in length, were combined with 1 ug DNA extracted from CD4+T cells (CEM). THe numbers of HIV-1 DNA molecules in certain amounts of pHXB2 were determined by the following formula: numbers of HIV-1, DNA molecules = $[X \times 6.0225 \times 10^{23}]/[17{,}000 \times 650]$, where X represents the amounts of pHSB2 (gram); 6.0225×10^{23}, Avogadro's number; 17,000, the number of base paris of pHXB2; and 650, molecular weight per one base pair of double stranded DNA. One ug of each standard DNA containing 3.3 to 10^5 HIV-1 DNA copies was subjected to PCR using SK 38/39 followed by a liquid hybridization with ^{32}P-labeled probe, SK 19, as described above. Liquid gel, which represented different forms of heteromultiples. The predominant band, with a mobility of 193 bp, represents a heteroquadriplex [(115 + 115 + 115 + 41)/2]. A second band, with a mobility of 78 bp, represents a heteroduplex. Similarly, a third branch, with a mobility of 596 bp, presumably represents heteroelevenplex. The density of these three bands was measured by densitometry. The combined densities were plotted against 2 x [the numbers of HIV-1 DNA copies]. The numbers of HIV-1 DNA molecules have been multiplied by 2 assuming both plus and minus strands of an HIV-1 DNA copy served as templates. To ensure that the bands seen on the gel represented HIV-1 specific sequences, PCR products were hybridized with ^{32}P-labeled SK 19, digested with a restriction enzyme BstN I, and analyzed on a 20% polyacrylamide gel. BstN I digestion produced radiolabeled 4-mer as a result of cleavage at a known site in HIV-1-specific hybridized products which served as a "diagnostic" fragment[20]. Densities of these "diagnostic" fragments yielded a standard curve virtually identical to the one obtained using densities of three major bands. Standardization was also performed using an env primer pair, SK 68/69, which produced density and radioactivity curves virtually identical to those obtained by using SK 38/39. Thus, in the subsequent studies, we employed the standard density curves constructed by PCR using SK 38/39 and combining the densities of

three major bands. Only when rabbit globin mRNA PCR products were comparably formed in each sample, HIV-1 data were analyzed.

The numbers of HIV-1 particles were obtained from the following formula (assuming one HIV RNA molecule produced one HIV cDNA copy by RT with 100% efficiency): HIV-1 particles (vp/ml)=(HIV [DNA copy number determined from the standard curve) x 20]2/plasma volume (ml), where the factor 20 is to adjust values since 1/20 HIV RNA originally isolated from plasma was used for RNA PCR, and the factor 2 is to convert number of RNA molecules to number of viral particles whose genomes exist as a dimer of identical RNA molecules.

Assay for p24 Gag Protein in Serum or Plasma

Plasma or serum p24 antigen levels were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kit (DuPont). In some assays, the acid pretreatment (pH 2.5) was performed to dissociate antigen-antibody complex prior to ELISA as previously described.[29,30] Briefly, each phosphate-buffered saline, 89.2 ul glycine-HCI buffer (1 M, ph 2.2), thus achieving ph 2.5; incubated for one hour; neutralized to pH 7.5 with 21.6 ul Tris buffer (5 M pH 11) in a final volume of 200 ul; and subjected to a standard ELISA. Normal plasma specimens mixed with known amounts of p24 antigen were also subjected to the acid-treatment prior to ELISA and served as reference.

Statistical Analysis

Wilcoxon's rank sum test and signed-rank test were employed for nonparametric statistical comparison. McNemar's test was utilized to compare the sensitivity of plasm RNA PCR assay and ELISA with or without acid-pretreatment to detect circulating HIV-1 virion and circulating p24 antigen, respectively. Spearman's correlation coefficient was obtained to assess the association between plasma HIV-1 virion numbers and p24 antigen levels determined by ELISA with or without acid-pretreatment.

RESULTS

Assay Condition of Plasma RNA-PCR and Validity of Results

In order to determine plasma volume required for RNA-PCR assay, various volumes (0.1 to 5 ml) of plasma from 5 patients with AIDS or ARC were tested for HIV-1 particle numbers. When 0.1 to 2 ml of plasma were used, the number of HIV-1 particles detected showed a linear correlation with plasma volume. Thus, 1 to 2 ml of plasma samples were used for the majority of patients in the current study. For asymptomatic carriers, increased plasma volumes up to 5 ml were used if 2 ml plasma was not sufficient for the assay in quantitating the HIV-1 virions.

We then asked if the numbers of HIV-1 particles detected in plasma differed from those detected in serum. To this end, HIV-1 was pelleted from paired plasma/serum samples (2.0 ml) obtained from 5 different seropositive

individuals and subjected to RNA-PCR. We found that the number of HIV-1 particles in plasma was significantly higher (mean 8-fold, range 1.2 to 15.3-fold) than in serum in all 5 paired specimens ($p=0.0431$). In addition, since we often kept samples frozen until assay, we asked whether one cycle of freezing and thawing affected the results of the assay. However, the numbers of HIV-1 particles using fresh plasma samples were not significantly different from those using the same sample after freezing and thawing. We then examined variability of the plasma RNA-PCR results. When HIV-1 particle numbers (vp/ml) were determined tin 10 replicate in one assay for 3 different patients, values for each sample were proximate to one another. When 3 different fresh plasma samples were aliquotted, kept frozen and tested on 6 different occasions during up to 4 months period of time, values for each sample also did not differ from one another. Thus, we concluded that, in view that the current plasma RNA-PCR assay can quantitate HIV-1 in a range of more than 4 orders of magnitude, both intra- and inter-experimental variabilities were reasonably small in the amounts of detectable HIV-1 RNA. These quality control experiments were periodically performed to ensure the reliability of the current assay system.

All HIV-1 Seropositive individuals tested had Detectable HIV-1 Particles in Plasma

Plasma samples from 77 untreated HIV-1 seropositive individuals (49 patients with AIDS or ARC and 28 asymptomatic HIV-1 carriers) and 23 HIV-1 seronegative patients with various illnesses were subjected to RNA-PCR. Ten seronegative normal volunteers were also tested as controls. Figure 3 depicts representative autoradiography profiles when PCR products were hybridized with a gag specific probe (SK 19) or rabbit globin mRNA specific probe (RAG 2). Data were analyzed only when the amounts of RAG RNA-PCR products were comparable among samples. Seventy-six of 77 (98.7%) HIV-1 seropositive individuals had detectable HIV-1 virions in plasma ranging from 10 to $\geq 10^6$ (vp/ml) (Figure 4). In contrast, among such seropositive individuals, only 29 of 72 (40.3%) were positive for p24 antigen in serum or plasm ($p<0.00001$). With acid-pretreatment of serum or plasma samples, 21 or 60 (35.0%) tested still remained negative for p24 antigen ($p<0.00001$). None of 23 HIV-1 seronegative individuals including 10 HTLV-1 seropositive subjects were tested positive for plasma HIV-1 virion. All 10 normal volunteers also gave negative results.

We then asked whether plasma RNA-PCR data correlated with the clinical stages of HIV-1 infection or HIV disease progression as indicated by CD4+ T cell counts. The numbers of HIV-1 particles detected in plasma from patients with AIDS or ARC were significantly higher than those from asymptomatic HIV-1 carriers ($p<0.0001$). Moreover, the individuals with CD4+ T cell counts less than 200 had the significantly higher plasma HIV-1 particle numbers than those with CD4+ T cell counts between 200 and 500 ($p<0.0025$), and these with CD4+ T cell counts between 200 and 500 had the significantly higher plasma HIV-1 particle levels than those with CD4+ T cell counts more than 500 ($p<0.002$).

Decreased Plasma HIV-1 Particle Levels during ddI Therapy

In recent ddI phase I studies conducted at the three major centers and a phase I/II study in pediatric patients, individuals receiving ddI showed improvement in immunological functions and evidence of a decrease in viral load as assessed by serum p24 antigen levels.[31-35] We, therefore, asked whether numbers of plasma HIV-1 particle in patients with AIDS or ARC enrolled in NCI's ddI phase I trail changes after the therapy; and, if so, such changes correlated with two existing clinical markers, CD4+ T cell counts and serum p24 antigen levels. Plasma samples were collected at entry and different time points during the study from randomly selected total of 11 patients. In order to minimize the variability between different RNA-PCR assays, the numbers of HIV-1 particles in a series of plasma samples from a given patient were determined in one RNA-PCR assay using an equal volume of plasma, which had been kept frozen until the assay. A patient (Panel A) who was positive for p24 antigen at entry became negative after 12 weeks on ddI therapy and remained negative throughout the study. In this patient, plasma HIV-1 particle numbers markedly decreased down to 23 (vp/ml) at week 12 and remained marginally detectable (10-20 vp/ml) to undetectable thereafter, while the absolute counts of circulating CD4+ T cells remained above the entry value. In another patient who was also positive for p24 antigen at entry, there was a substantial decrease in serum p24 antigen and plasma HIV-1 particle numbers along with an increase in CD4+ T cell counts as assessed at week 6 on ddI. When ddI was withheld due to ddI-related neuropathy in this patient, serum p24 antigen returned to the initial level with a substantial increase in plasma HIV-1 particle numbers and a decrease in CD4+ T cell counts. However, after AZT was begun and subsequently decreased dose of ddI therapy was resumed, decrease in both serum p24 antigen and plasma HIV-1 particle levels was again noted in this patient. Panel C and D (Figure 5) depict profiles of two other patients who were negative for serum p24 antigen throughout the study. In both patients, substantial numbers of HIV-1 particles were detected in plasma at entry. It appeared that a decrease in panel C and D (Figure 5) depict profiles of two other patients who were negative for serum p24 antigen throughout the study. In both patients, substantial numbers of HIV-1 particles were detected in plasma at entry. It appeared that a decrease in panel C and A decrease followed by a subsequent increase in panel D in HIV-1 particle numbers reflected antiretroviral effect and interruption of ddI therapy, respectively. Patterns similar to pane C or D were observed as well in the other 6 patients treated with ddI, who had negative serum p24 antigen throughout the study.

Of 11 patients who were tested for plasma HIV-1 particle levels before and after ddI treatment, ten patients received ddI for 8-14 weeks, while one patient experienced an early interruption of the treatment at week 6 due to ddI-related neurotoxicity. In these 10 patients, a significant decrease in plasma HIV-1 particle levels was demonstrated when assessed at 8-14 weeks ($p=0.0051$). Figure 6-A shows changes of plasma HIV-1 particle numbers in 7 patients who continuously received $\geq$ 6.4 mg/kg/day doses of ddI for up to 70 weeks. All these patients had a significant decrease in plasma HIV-1

particle numbers when assessed at 45-71 weeks (p=0.018) and the levels were sustained low in all patients as compared to the entry levels throughout the study. Figure 6-B shows results in 4 patients who received $\geq$ 6.4 mg/kg/day doses of ddI, but had various periods of interruption of the treatment due to the adverse effects of ddI. All 4 patients had a substantial decrease in plasma HIV-1 particle numbers for the first several weeks on ddI. However, plasma HIV-1 particle numbers returned to the initial levels following the interruption of ddI therapy in all these 4 patients.

Association between Plasma RNA-PCR Results and p24 Antigen Levels

We lastly asked whether p24 antigen levels correlated with plasma HIV-1 particle numbers determined by the current plasma RNA-PCR assay in 72 untreated patients with HIV-1 infection. As shown in Figure 7-A, plasma HIV-1 particle numbers showed a vastly scattered pattern and had no significant correlation with serum p24 antigen levels (r^2=0.26). Acid-treatment (pH 2.5-3.0) prior to standard ELISA has been reported to improve the detectability of p24 antigen. Pretreatment of plasma or serum samples at pH 2.5 significantly increased the positivity of p24 antigen from 29 of 72 (40.3%) by standard ELISA to 39 of 60 (65.0%) individuals (p=0.000015). This acid pretreatment also resulted in 5 fold increased of the p24 values on the average in those who had detectable circulating p24 antigen as assessed by standard ELISA. Nevertheless, no significant correlation was seen between plasma HIV-1 particle numbers and circulating p24 antigen levels detected by standard ELISA following an acid-treatment (r^2=0.199).

DISCUSSION

In this study, we established a rapid and sensitive method to quantitate the amounts of HIV-1 particles in plasma. The current method using RNA-PCR can be performed with relatively small volumes of plasma (1-2 ml), and can detect as few as 10 to 30 HIV-1 particles per ml plasma. Reliability of quantitation using PCR technique is still controversial since efficiency of PCR rarely equals the theoretical two-fold amplification per cycle and substantial variabilities in the amounts of PCR products could exist even when the same amounts of templates and primers are used. However, in the current assay system, one standard deviation of 6 and 10 replicate determinations were within half an order when inter- and intra-experimental variabilities were examined using multiple plasma samples with various levels of HIV particle numbers. This is notable in view that the current method can quantitate HIV-1 in a range of more than 4 orders of magnitude. It should also be noted that this quantitative assay is completed in 2 days if the given laboratory is equipped with an ultra-centrifuge, a DNA thermal cycler, and a desitometer, as compared with other quantitative culture techniques such as end-point-dilution method which requires 2-3 weeks to complete.

By using this technique, HIV-1 particles were detected in 76 of 77 (98.7%) HIV-1 seropositive individuals with various clinical stages, while none of seronegative controls were found to be positive, verifying specificity of the current assay system. It is known that circulating HIV-1 specific

neutralizing antibodies can abolish the infectivity of the virus. Thus, different levels of such neutralizing antibodies in different plasma samples may generate substantial variabilities in quantitation of viral titer obtained by end-point-dilution culture technique. Indeed, while Ho et al reported that plasma viremia was detected in 100% of HIV-1 seropositive individuals, in the report by Coombs et al plasma viremia was detected in only 56% of HIV-1 infected individuals even though both groups employed the similar quantitiation methods and tested the similar populations. This difference is probably not only due to different culture techniques used in two different laboratories but also due to various levels of neutralizing antibodies contained in plasma samples tested. It is also noteworthy that end-point-dilution method may not be suitable for monitoring antiviral agents in patients plasma may affect the replication of HIV-1 in culture. In addition, HIV-1 envelope glycoprotein is known to be relatively unstable, and therefore, its function may be damaged upon freezing and thawing, or during the storage. This may also affect the infectivity of the virus in end-point-dilution culture method. In contrast, the current RNA-PCR method uses viral RNA extracted from pelleted virus directly from plasma, and thus, the HIV-1 particle levels determined by this method should not be affected by neutralizing antibodies or antiretroviral agents in plasma. Moreover, it was confirmed that the numbers of plasma HIV-1 particle determined by the current RNA-PCR were not affected by a cycle of freezing and thawing. Such dependability of the current assay and its ease in handling samples may stem from the fact that viral RNA molecules are protected by capsid proteins and are much more stable than outer envelope glycoproteins whose entirety is essential for the infectivity of virions.

In the current method, we used radioautography and optical densitometry to quantitate the amounts of PCR products on polyacrylamide gels. The signal responses of x-ray film exposed to gels were not linear in particular when the reading went beyond 2.5 O.D. by the densitometer we employed in this study. However, we could substantially broaden the range of linear portion of the standard curve by combining densities of three dominant bands on the gel, and we were able to quantitate HIV-1 particles with a range of more than 4 orders of magnitude. The observed linearity was validated when the standard curve generated by densitometry was shown to be virtually identical to the standard curve obtained by direct radioisotope scanning of the radioactivity on the same gel. The densitometer employed in the present study is widely available and does not require any extensive training on the side of researchers. We wish to stress that we developed the current method so that quantitation of HIV-1 can be relatively easily performed in average laboratories without acquiring as yet substantially costly radioisotope scanners or an apparatus using photostimulable storage phosphor imaging plates (phosphorimager). However, it should be noted that if a phosphorimager or related device is employed for the current method, the range of the linear portion of the standard curve will be further extended and its sensitivity will also be markedly improved.

In the present study, we demonstrated that there was a significant reduction of plasma HIV-1 numbers in all 10 patients who received ddI for 8-14 weeks of ddI therapy. This study was carried out in the same patients' group as in the present study, and it appeared the changes in plasma HIV particle levels and HIV DNA levels in PBM from each individual were virtually in agreement although the timings were not completely synchronized in some cases (data not shown). Moreover, all 7 patients receiving ddI for 45-71 weeks with no less than 4 weeks of interruption had also a significant decrease in plasma HIV-1 particle numbers ($p=0.018$). In contrast, when ddI therapy was interrupted for more than 4 weeks because of ddI-related adverse effects, the numbers of plasma HIV-1 particles subsequently returned to the initial levels in 4 of 4 patients. In 3 patients who had detectable serum p24 antigen at entry, the changes of plasma HIV-1 particle levels were found to correlate well with the changes of serum p24 antigen during the study. Although we did not examine plasma samples from patients who had comparable stages of the disease and did not receive antiviral therapy for extensive periods of time (it is unethical to do so), we concluded that the changes of plasma HIV-1 particle levels we observed in the present study were most likely due to ddI treatment rather than natural history of HIV-1 infection in advanced stages.

Our study demonstrated no significant correlation between the plasma HIV-1 particle levels and circulating p24 antigen levels in untreated patients with HIV-1 infection. It is generally thought that measurement of circulating p24 antigen levels does not merit estimation of the number of virions present in the sample. Assays such as ELISA and RIA to measure p24 antigen levels rely on binding of antibodies to p24 antigen used in the assay kit. Binding of the kit antibodies is interfered with circulating antibodies in patients plasma or serum up to a variable extent, and thereby, accurate quantitation of circulating p24 antigen is difficult. Although acid-treatment prior to ELISA significantly increased p24 antigen levels detected and its positivity in our study, no correlation between plasma particle levels and p24 antigen levels was identified regardless of the stages of HIV-1 infection. This may be due to (i) failure of acid treatment to denature immune complexes, and/or (ii) different ratios of virion particle numbers and p24 antigen molecule numbers in each individual's plasma, although other as yet unknown factor(s) may be responsible for the lack of correlation.

Taken together, the present data suggest that plasma HIV-1 particle numbers determined by the current method reflect plasma viremia status in HIV-1 infected individuals and may also serve as a new potential clinical marker to monitor the disease process and to assess the effect of antiretroviral therapy. However, it should be stressed that one interpret these data with caution and the further longitudinal studies be carried out to determine the reliability and usefulness of the current plasma RNA-PCR method.

PUBLICATIONS

1. Aoki S, Yarchoan R, Thomas RV, Pluda JM, Marczyk K, Broder S and Mitsuya H. Quantitative analysis of HIV-1 proviral DNA in peripheral blood mononuclear cells from patients with AIDS or ARC: Decrease of proviral DNA content following treatment with 2′,3′-dideoxyinosine (ddI) AIDS Res. Hum. Retrov. 1990; 6:1331-1339.

2. Aoki-Sei S and Mitsuya H. Quantitative analysis of HIV-1 in clinical specimens from patients with HIV-1 infection by polymerase chain reaction (PCR). In: "Implications for Prognosis and Drug Monitoring" (ed. JM Andrieu and P Cramer). John Libbey Eurotext, 1991 (in press).

PRESENTATIONS

1. Sei-Aoki S, YArchoan R, Kageyama S, Hoekzema D, Pluda J, Broder S and Mitsuya H. Quantitation of HIV-1 virus particles in plasma from patients with HIV-1 infection by rna polymerase chain reaction: decrease in viral load in plasma following treatment with 2′,3′-dideoxyinosine (ddI). Seventh International Conference on AIDS, Florence, Italy. June 16-21, 1991.

2. Sei-Aoki S, Kleiner DE, Chandra R, Yarchoan R, Husson R, Pizzo PA, Broder S and Mitsuya H. Quantitative analysis of HIV-1 DNA and mRNA in various tissues from patients with AIDS or ARC by polymerase chain reaction (PCR). National Meeting of American Federation of Clinical Research, Seattle, WA May 3-6, 1991.

3. Mitsuya H and Sei-Aoki S. Quantitation of HIV-1 in clinical specimens from patients with HIV-1 infection by polymerase chain reaction. Workshop on Viral quantitation in HIV Infection: Implications for Prognosis and Drug Monitoring. Paris June 13-14, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07222-01 CO

PERIOD COVERED

October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

In vitro inhibition of hepatitis B virus replication by dideoxynucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH

Office of the Associate Director, Clinical Oncology Program

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

HepG2-derived hepatoblastoma cells (2.2.15), which actively produce hepatitis B virus (HBV), were cultured in the presence of 2',3'-dideoxyguanosine (ddG), 2',3'-dideoxyinosine (ddI), or 3'-azido-2',3'-dideoxythymidine (AZT). ddG was the most potent agent diminishing viral replication by as much as 95% as assessed by the amount of episomal HBV DNA without impairing cellular growth. AZT was least effective against HBV. Northern blot analysis revealed no apparent difference in the pregenomic viral RNA profile, suggesting that these dideoxynucleosides suppress reverse transcription in the replicative cycle of HBV. The effect of varying the time of drug exposure showed that these agents can suppress HBV replication even when added late in culture. HBV replication in another 2.2.15 cell population of the same lineage was affected by ddG differently, which may present an opportunity to investigate phenotypic and/or genetic alterations during culture. The present data suggest that some 2',3'-dideoxynucleosides can exert a potent antiviral activity against HBV in vitro at least under certain circumstances.

INTRODUCTION

During the last decade, a great deal has been learned about the replicative cycle of human hepatitis B virus (HBV), a DNA virus that causes acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The latter complication causes over one million deaths worldwide every year. HBV infection is an increasingly serious problem among gay men and intravenous drug abusers, many of whom are co-infected with human immunodeficiency virus type 1 (HBV-1), and RNA virus (retrovirus) that causes the acquired immunodeficiency syndrome (AIDS) and its related diseases. Although there are a number of distinct differences between HBV and HIV, there is at least one notable resemblance between these two human pathogenic viruses in that their replicative cycle involves an obligate RNA intermediate and a reverse transcription step in the cytoplasm.

In 1985, we found that a broad family of 2′,3′-dideoxynucleosides can suppress replication of HIV-1 in cultured cells through inhibition of the retroviral reverse transcriptase, although each member of this family may exhibit a unique activity and toxicity profile. One such drug, 3′-azido-2′,3′-dideoxythymidine (3′-azido-3′-deoxythymidine, AZT or zidovudine), has now been formally proven to confer prolonged survival and improved qualify of life in patients with advanced HIV infection. Certain clinical benefits also occur in asymptomatic HIV-infected individuals. Two other dideoxynucleosides, 2′,3′-dideoxycytidine (ddC) and 2′,3′-dideoxyinosine (ddI or didanosine) have recently been shown to be active against HIV-1 in patients with AIDS and AIDS-related complex (ARC) in short term Phase I clinical trials. These two drugs are now under Phase II clinical trials in several countries.

One can ask whether experimental therapy for HBV infection, by targeting its reverse transcriptase using 2′,3′-dideoxynucleosides, is worth pursuing [23]. Indeed, several members of the 2′,3′-dideoxynucleoside family have been shown to suppress the replication of duck hepatitis B virus (DHBV) in chronically DHBV-infected Pekin ducks in vivo, although caution in extrapolating results from this animal model to human disease is necessary. In the current study, we asked if dideoxynucleosides could be active against HBV replication in the actively HBV-producing human hepatoblastoma cell line, 2.2.25.

MATERIALS AND METHODS

Cells

A human hepatoblastoma (HepG2)-derived cell line, 2.2.25, transfected with a plasmid containing HBV DNA, was employed in this study. The 2.2.15 cell line carries four 5′-3′ tandem copies of the HBV genome positioned such that two dimers of the genomic DNA are 3′-3′ with respect to each other as chromosomally integrated sequences. These cells episomally produce relaxed circular, covalently closed, and incomplete DNA copies of the HBV genome and express multiple HBV-specific polyadenylated RNAs with lengths of 3.5, 2.5, and 2.1 kilobases. The 2.2.15 (PR) cells and HepG2 cells used in the present study were kindly provided by Philip Roingeard (Harvard

University School of Public Health, Boston, MA), which had been cultured by trypsinizing and seeding them every two weeks for >7 months. The 2.2.15 (GA) cells were kindly provided by George Acs (Mount Sinai School of Medicine, New York, NY). The HLA phenotype analysis and DNA fingerprinting using the hypervariable probe HepG2, confirmed that they were of the same origin carrying the same phenotype, A2, A28, B35, B27.

Nucleoside analogues

2′,3′-dideoxyinosine (ddI) was supplied by Dr. Karl Flora, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, while 2′,3′-dideoxyguanosine (ddG) and 3′-azido-2′,3′-dideoxythymidine (AZT) were purchased from Pharmacia (Piscataway, NJ) and Sigma Chemicals (St. Louis, MO), respectively. 2′,3′-[3H]-dideoxyguanosine (specific activity, 44 Ci/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). The percentage of label in the dideoxyribose moiety averaged 98%, with the remaining tritium being associated with the 8-position of the purine base.

Cell culture and drug treatment

The 2.2.15 cells were trypsinized, resuspended, and seeded in 25 cm^2 tissue culture flasks (Corning; Corning, NY) and cultured in complete media [RPMI 1640 supplemented with 15% undialysed heat-inactivated fetal calf serum (Gibco; Grand Island, NY), 4 mM L-glutamin, 50 units/ml penicillin and 50 ug/ml streptomycin] at 37 C in 5% CO_2 containing air. The culture medium was replaced with fresh complete medium every 2-3 days. Each drug was added to the culture at various time points following cell seeding and concentrations were kept constant throughout the drug treatment period.

Isolation and analysis of DNA

Total DAN was isolated from 2.215 cells as previously described by Sambrook, et al. Briefly, cells were washed in phosphate buffered saline, and lysed in proteinase K-containing solution. Following incubation at 37^0C for 4 h, total DNA was extracted in phenol/chloroform/isoamyl alcohol followed by two extractions with chloroform/isoamyl alcohol. The DNA was precipitated in ethanol overnight, dissolved in TRIS/EDTA buffer, and stored at -20^0C. Undigested samples of DNA were electrophoresed on a 1% agarose gel and transferred to nitrocellulose. Hybridization was performed overnight at 42^0C with a full length HBV genomic DNA probe (AM-12: kindly provided by Dr. John L. Gerin, Georgetown University, Washington, D.C.) radiolabelled by [^{32}P]-nick translation. HBV DNA species were visualized by autoradiography. Relative levels of the densities on the exposed x-ray film representing the episomal HBV DNA were compared by densitometry (X-Rite 301; X-Rite Inc. Grand Rapids, MI).

The percent reduction of HBV DNA content in a sample was determined by the following formula: 100 x(1 - (O.D. for a sample DNA - the background O.D.)/(O.D. for no drug control DNA - the background O.D.)], where O.D. represents the optimal density reading and the background O.D. represents

the lowest density site within each lane. All the samples in each assay were run in one gel to minimize the variability from one experiment to another. The standard deviation in quantitating HBV DNA was typically less than 11% of the mean value when the amount of DNA samples were assessed in quintuplicate in one gel.

Isolation and analysis of HBV-specific RNA

Total RNA was isolated from 2.215 cells by the vanadyl-ribonucleoside complex method. The purified RNA was electrophoreses on a 1% agarose gel containing 1.1 M formaldehyde, transferred overnight to nitrocellulose, and hybridized overnight at 42 C with the radiolabelled AM-12 DNA probe. HBV RNA species were visualized by autoradiography.

Metabolism

Anabolic phosphorylation of ddG was analyzed using a minor modification of previously published procedures using high performance liquid chromatography (HPLC). Briefly, 2.2.15 cells [just before they reached confluency following seeding in 75 cm^2 culture flasks (Costar, Cambridge, MA) in 10 ml of RPMI 1640 complete media] were incubated with [^{3}H]-ddG (5 mCi/ml) in the presence of 5 uM unlabelled ddG for 5 h. Cells were then trypsinized, harvested and extracted with 600 ul of 60% (vol/vol) methanol. The methanol extract was heated at 95 C for 1.5 minutes, clarified by centrifugation, and a 200 ul aliquot was loaded onto a radial compression column of Partisil 10-SAZ equilibrated with 20 mM ammonium phosphate. The nucleotides were eluted isocratically for 5 min, then developed with a highly convex (10 min) gradient followed by a slightly convex (15 min) gradient to 25% and 100% of 700 mM for an additional 10 min (flow rate 2 ml/min). Eluted radioactivity was determined by liquid scintillation spectrometry.

DNA fingerprints analysis

DNA fingerprints were determined for the parental line HepG2 and the two derivative lines 2.2.15 (PR) and 2.2.15 (GA) as previously described [30], with modification as stated elsewhere. Briefly, DNA samples were digested with 50 units of Hae III and subjected to Southern blot analysis with the human hypervariable probe 33.15. Results were verified in duplicate experiments with Hinf I. All bands corresponding to the range of 1.0 to 12 kb were scored; those showing similar molecular weight and intensity were considered to be identical. The composite phenotype of each DNA fragment of an individual comprised the DNA fingerprint.

RESULTS

Reduction of Episomal HBV DNA by Dideoxynucleosides In Vitro

Since the replicative cycle of HBV involves an obligate RNA intermediate and a reverse transcription step in the cytoplasm, we first asked if several members of 2',3'-dideoxynucleosides, potent inhibitors of reverse

transcriptase, could suppress the synthesis of HBV DNA in 2.2.15 (PR) cells. To this end, we compared the amount of episomal viral DNA produced in 2.2.15 (PR) cells cultured in the presence or absence of various concentrations of dG, ddI, or AZT (Table 1-A). It has been shown that 2.2.15 cells begin to produce HBV efficiently at near confluent with little or no detectable episomal HBV DNA during the logarithmic phase of the growth. In the current study, when the cells were cultured for seven days, HBV DNA synthesized in 2.2.15 (PR) cellls was barely detectable; while when cultured for >2 weeks after reaching confluence a significantly increased level of viral DNA was detected in the cells. When these 2.2.15 (PR) cells were cultured with effective drugs, the reduction in the synthesis of episomal HBV DNA was greatest at ≥21 days. For example, when 2.2.15 (PR) cells were harvested and total DNA was extracted 9 days after ddG (50uM) exposure, the amount of HBV DNA was reduced by 77%, however, when the cells were continuously exposed to 50 uM ddG for an additional 12 days in the same experiment, the reduction was more profound at 91%. We therefore tested the effect of drugs in 2.2.15 (PR) cells cultured with or without drugs for ≥21 days in the current study.

First, the cells were cultured without drug until they reached confluence, and each drug was added to the culture (Table 1-A). In an additional 21 days of culture, total DNA was isolated, and undigested DNA samples were analyzed by the Southern blot hybridization technique. Total RNA was extracted from this same set of cultured cells (vide infra). In a Southern blot, without drugs, a dominant band with a mobility of approximately 3.7 kb was readily identified. This band corresponds to the relaxed circular form of episomal HBV DNA. However, when the cells were cultured with 50 uM, 100 uM, and 200 uM dG; 50 uM, 100 uM, and 200 uM ddI; and 10 uM AZT for 3 weeks, we observed a substantial reduction in the amounts of episomal HBV DNA (91%, 89% and 95%; 23%, 59% and 80%; and 64% respectively) (Table 1-A). We then asked whether the decrease in episomal DNA could be dose-related by using relatively lower concentrations of the nucleosides (Table 1-B). In this experiment, the cells were cultured with various concentrations of ddG, ddI and AZT throughout the 3 week period of time in culture. ddG demonstrated a substantial dose-related decrease in viral DNA by 60%, 74% and 92% at 5 uM, 20 uM and 50 uM, respectively (Table 1-B). Similiar, but less intense, dose-related inhibition of DNA synthesis was observed in cultures with either AZT or ddI (Table 1-A, B).

At the drug concentrations used, toxicity of these compounds did not appear to be significant as assessed by microscopic morphology, pellet sizes of the cells after trypsinization and centrifugation, and the yield of total DNA from each cell population (cell counting is inaccurate in 2.2.15 cell cultures, which are epitheloid and do not readily form a single-cell suspension).

Effect of Varying Time of Drug Exposure of HBV Suppression in Vitro

In order to define the time in culture critical for drug treatment, drug exposure was begun at different times after 2.2.15 (PR) cells became confluent. In our assay, 2.2.15 (PR) cells were seeded so that they

reached confluency in 7 days of culture. Drugs were added to the cells on days 0, 6, 8 and 18 in culture (Table 1-C, D, E, F, respectively), and the cells were further cultured for 21 to 24 days keeping the drug concentrations constant. When tested at concentrations that did not affect cellular growth, ddG was the most potent agent against HBV among the three dideoxynucleosides tested in the present study, and produced the highest level of HBV DNA reduction. The reduction was by 95% at 100 uM when ddG was added on day 0 (Table 1-C), by 88% at 50 uM when added on day 6 (Table 1-D); by 47% at 20 uM when added on day 8 (Table 1-E); by 85%, 87% and 95% at 50 uM, 100 uM and 200 uM when added on day 18 (Table 1-F). These findings suggest that (i) preexisting episomal HBV DNA was degraded or secreted in a 21-day period of time in culture, and (ii) the drugs tested here could exert their activity to suppress the de novo HBV DNA snythesis at any time during the culture.

Dideoxynucleosides Do Not Alter the 2.2.15 (PR) HBV RNA Profile

Dideoxynucleosides, following anabolic phosphorylation in the cytoplasm, are thought to block the infectivity and replication of retroviruses by inhibiting reverse transcriptase. If dideoxynucleosides block HBV DHA synthesis and thereby inhibit HBV replication in 2.2.15 (PR) cells at the stage of reverse transcription, the HBV RNA pregenome profile, in theory, may not be appreciably altered as the integrated HBV DNA is transcribed by mRNA by host RNA polymerases which are assumed to be less sensitive to dideoxynucleosides. To address this question, total RNA was extracted from 2.2.15 (PR) cells cultured with 50 uM, 100 uM, and 200 uM ddG, and 50 uM, 100 uM and 200 uM ddI, and analyzed by Northern blot hybridization. In this experiment, ddG and ddI had shown a substantial level of inhibition of HBV DNA synthesis (Table 1-A). Northern blot analysis of the RNA samples revealed two major bands. The larger species (3.5 kb) should represent RNA that serves as the template for HBV DNA synthesis (the pregenome) and also encodes the core protein and DNA polymerase, and the smaller species (2.5 kb) the messenger RNA for the major protein of the envelope. No apparent reduction in the amount of these HBV RNA species was seen as a result of drug exposure.

The Putative Active Metabolite of ddG, ddG-tripohosphate, Is Formed in 2.2.15 Cells

Dideoxynucleoside analogues are successively phosphorylated in the cytoplasm of human cells, ultimately yielding their corresponding dideoxynucleoside-5′-triphosphates, the putative active metabolites of dideoxynucleosides agaisnt reverse transcriptase. In general, the pathways for anabolic phosphorylation are different for the various dideoxynucleosides, and these drugs are not equivalent in either activity or toxicity profiles, in vitro or in vivo. Thus, each dideoxynucleoside must be considered to be a different drug in its own right and must also be considered in the context of biochemcial pharmacology in different cell types. In the case of ddG, anabolic phosphorylation in T cells may depend on 2′-dideoxycytidine kinase (A. Fridland and D.G. Johns, personal communication) and cytosolic 5′-nucleotidase.

We then asked if the 5′-triphosphate of ddG, the most potent agent against HBV in the current study, was formed in 2.2.15 (PR) cells. To evaluate the metabolism of ddG, 212.15 (PR) cells were incubated with [^{3}H]-ddG for 6 h, and the cell lysate was analyzed for the amount of phosphorylated ddG using the reverse phase HPLC. The HPLC profile of the anabolites of ddG obtained was comparable to that seen in CD4+ T-cell lines as published elsewhere [34], and the parent nucleoside, ddG, and its mono-,di-, and triphosphates were clearly detected in the eluate.

Dideoxynucleosides Were Less Active in 2.2.15 (GA) Cells In Vitro

Interestingly, when we used the 2.2.15 (GA) cells as target cells, the activity of dideoxynucleosides to suppress the synthesis of HBV DNA was minimal as compared to that seen in 2.2.15 (PR) cells. For example, in a representative experiment, ddG suppressed the synthesis of extrachromosomal HBV DNA by 2.2.15 (GA) cells by 31, 43, and 37%, when the cells were cultured in the presence of 50, 100 and 200 uM ddG, respectively, throughout the 22-day interval of culture. The morphology of the 2.2.15 (GA) differed from that of the 2.2.15 (PR) cells in that the latter cells had many vacuoles in the cytoplasm. The HPLC analysis using the radiolabelled ddG revealed that the 2.2.15 (GA) cells could also produce ddGTP. Thus, it is possible that the metabolic pathway of normal nucleosides, in particular, 2′-deoxyguanosine, is different between 2.2.15 (PR) cells and 2.2.15 (GA) cells, which is a topic for the future investigation.

DNA fingerprint analysis using Hae III (verified with Hinf I) and the hypervariable probe designated 33.15 also confirmed that the two 2.2.15 cell populations were of the same origin. Of the 25 bands which were scored, one band appeared in 2.2.15 (PR) which was not present in 2.2.15 (GA). This small difference does not alter the statistical basis for confidence that these two lines were of the same source. The probability that these two cell lines would have the same DNA fingerprint but actually be from different lineages can be estimated, from data previously reported by Gilbert et al, at approximate 1×10^{-17}. A unique observation was the presence on ethidium bromide staining after Hae III digestion of numerous (approximately 50) high copy number bands in 2.2.15 (PR) and the parental HepG2 (data not shown). This family of bands was missing the 2.2.15 (GA) DNA. Since the DNA fingerprints were not significantly different between the two cell lines, these bands are most likely the result of amplification of DNA regions or chromosomal segments, as it is quite common for the same cell line, exposed to different conditions in culture, to differ substantially in their karyotypic properties [39]. The one-band difference between 2.2.15 (PR) and 2.2.15 (GA) is most likely a result of this phenomenon.

DISCUSSION

In the present study, we observed that all three dideoxynucleosides tested, ddG, ddI and AZT could suppress HBV replication in the HepG2-derived human hepatoblastoma cell line, 2.2.15 (PR). Among these compounds, ddG was the most potent agent against HBV, and suppressed the episomal HBV DNA

synthesis by up to 95% without affecting cellular growth. ddI exerted a moderate antiviral activity agaisnt HBV as compared to ddG at comparable concentrations. AZT was least effective against HBV replication in 2.2.15 (PR) cells at possible highest concentrations (20 uM) that did not affect cellular growth in the current study. In this regard, Lee and his colleagues have shown a similar rank order in the duck hepatocyte model system as we found in the current 2.2.15 (PR) system (i.e., ddG > ddI > DDC > ddT). The mechanism of this difference remains to be clarified.

THe HBV genome has been shown to contain a sequence highly homologous to the pol gene of retroviruses and the replicative cycle of hepadnaviruses has been shown to involve the reverse transcritpion step. When we studied the metabolism of ddG in 2.2.15 (PR) cells, a relatively low, but distinct level of ddGTP formation was identified. Thus, taking into consideration that (i) ddG as a triphosphate is a recognized potent inhibitor of reverse transcriptase, (ii) anabolic phosphorylation of ddG occurs in the cytoplasm of 2.2.15 (PR) cells, and (iii) the phosphorylation of ddG occurs in the cytoplasm of 2.2.15 (PR) cells, and (iii) the HBV RNA profiles are not apparently affected by the addition of dideoxynucleosides, it is likely that the suppression of HBV replication by dideoxynucleosides takes place at the step of reverse transcription in the replicative cycle of HBV.

In the current study, higher concentrations of dideoxynucleosides (5-20 fold) were required for suppression of HBV replication in 2.2.15 (PR) cells than those to suppress the infectivity and replication of HIV-1 and HIV-2 in vitro. Although the culture medium was replaced with fresh medium containing the same concentration of drugs every 2-3 days, drugs might have been catabolized rather quickly in the 2.2.15 cells, especially for ddG and ddI, both of which may be cleaved by purine nucleoside phosphorylase. This may contribute to the requirement of relatively high concentrations of dideoxypurine nucleosides to suppress HBV DNA synthesis in the present study. It should also be noted that substantial differences in efficiency of the anabolic phosphorylation of dideoxynucleoside analogues exist among various cell populations. The 2.2.15 (PR) cell line might inherently have a low level of kinases essential for phosphorylationof dideoxynucleosides tested here, or might have high levels of corresponding 2′-deoxynucleoside-5′-triphosphates which compete with dideoxynucleoside-5′-triphosphates, thereby limiting their capacity to suppress the reverse transcriptase-mediated viral DNA synthesis.

In our study, ddG-5′-triphosphate was clearly detected in the 2.2.15 cells as in certain CD4+ T-cells. In [^{3}H]-2′,3′-dideoxyguanosine used in the present work, 97% of [^{3}H]-radiolabelling had occureed in the 2′- and 3′-position of the dideoxyribose moiety of ddG and about 3% in the 8-position of the purine base. In this regard, Busso and his colleagues have recently described tha ddGTP could not be detected when H9 cells were incubated for 24 h with based-labelled [8-^{3}]-ddG. However, detection of ddGTP would be difficult with ddG radiolabelled in the base, as used in their experiments, since ddG would be readily subjected to catalysis by purine nucleoside phosphorylase and most of the guanine would enter the salvage pathways for normal purine nucleotide synthesis.

Although the 2.2.15 (GA) cells are in the same lineage as the 2.215 (PR) cells, dideoxynucleosides were much less potent in suppressing HBV DNA synthesis in the former cell population. Since a substantial level of ddGTP was formed in both two populations, one might speculate that differences of metabolic pathway of the corresponding normal nucleoside are involved. For example, a potential drawback of ddG is that 2'-deoxyguanosine can readily reverse the antiretroviral activity of ddG against HIV-1 in vitor perhaps by competing with ddGTP in the form of its 5'-triphosphate and/or interfering with the anabolic phosphorylation of ddG. Thus, the simplest explanation of the lack of sensitivity of 2.2.15 (GA) cells to ddG is that these cells have higher endogenous levels of dGTP, 2'-deoxyadenosine-5'-triphosphate (dATP) etc. Another possibility is that the gene encoding the putative reverse transcriptase of HBV in 2.2.15 (GA) cells underwent mutations in culture so that the aberrant HBV reverse transcriptase lost its sensitivity to ddGTP in vitro, or that 2.2.15 (PR) cells had mutations(s) rendering them to be sensitive to ddG. The pattern of compartmentalization of ddG or its anabolites can also be substantially different in two cell lines. Indeed, the genetic difference in the presence oor absence of the high copy bands we observed may be related to the sensitivity difference between 2.2.15 (PR) and (GA) cells. The mechanism of the different sensitivity to ddG between 2.2.15 (PR) cells and 2.2.15 (GA) cells remains to be studied, and at a minimum these studies underscore the complexicity of in vitro antiviral screening.

The integration of HBV has been linked to the development of hepatocellular carcinoma (HCC) in humans and woodchucks. Integrated viral sequences have nearly always been detected in both human HCC-derived cell lines and human hepatoma tissues. Nevertheless, neither virus replciation nor virus expression is generally seen in the poorly differentiated hepatocytes of advanced tumors. On the other hand, integrated viral sequences are not detected during replication of duck hepatitis B virus (DHBV) except under rare circumstances. Therefore, an integration step of the hepadnarvirus genome is probably unnecessary for hepadvavirus replication and, indeed, the presence of only free viral DNA without integrated sequences can be observed in some chronic carrier stages of HBV infection. Taken together, these data suggest that an effective methodology which suppresses HBV DNA synthesis in actively HBV-producing cells may block de novo infection of hepatocytes and may be a potentially effective therapy.

In the current assay, the inhibition of viral DNA synthesis by dideoxynucleosides was significant whether the cells were started on drugs 0, 6, or 18 days after seeding and then cultured an additional three weeks. If one assumes that de novo HBV infection of hepatocytes is continuously suppressed by drugs and extrachromosomal (unintegrated) viral DNA is unstable, then preexisting unintegrated viral DNA may disappear after a certain period of tieme. Indeed, we have seen that ddC, ddG and ddI produced substantial inhibition of DHBV DNA synthesis in chronically DHBV-infected Pekin ducks in vivo as early as in 5-6 days after administration. It is worth noting that upon administering either ddA or ddI to Pekin ducks for 5 days, 3 out of 6 ducks in both experiments developed severe neurological abnovmalities causing difficulties in walking. In this regard, ddG may be interesting sicne it did not cause any apparent side

effects in Pekin ducks even when it was administered for 8 consecutive weeks. However, one must use extreme caution in extrapolating side effect data from Pekin ducks to humans, since in fact, ddI has been administered to some patients with AIDS for over 2 years without the toxicities seen in ducks.

To date, various strategies have been employed to treat HBV infection; however, it is still a formidable challenge to provide chronic HBV carriers with safe and effective therapy. Interferon has been used in several clinical trials in chronic hepatitis B, and has been found to induce remissions in the disease in 25-50% of patients, although interferon has considerable side effects. Another agent, arabinosyladenine monophosphate (ara-AMP), has also been tested for its activity in patients with chronic hepatitis B. This agent has shown only a transient improvement in the patients. Thus, developing effective drugs for HBV is urgently needed. Several groups have recently, reported that AZT, perhaps the least effective anti-HBV agent in our in vitro study, failed to suppress the replication of HBV in patients with HIV and HBV infection. It should be noted that the improvement of immunodeficiency state in pateints with AIDS by therapy for HIV infection might conceivably potentiate the host's immunity-mediated injury against HBV-harboring hepatocytes, thus aggravating the status of HBV-induced diseases. This may complicate the interpretation of data obtained from patients infected with both HIV and HBV. Thus, in order to assess the possible usefulness of reverse transcriptase inhibitors such as ddG for therapy of HBV infection, a formal clinical trial with patients who are infected with HBV alone would be required.

Recently, several groups have shown that various nucleoside analogues can suppress HBV DNA synthesis in actively HBV-producing hepatoma cell lines in vitro [58, 59], however, the antiviral activity of such compounds against HBV appears to vary to some extent form one cell line to another. These differences might be due to (i) different HBV integration and expression patterns, (ii) different cellular regulation of HBV production, (iii) different metabolic pathways of nucleosides, (iv) mutation(s) yielding drug resistance in virus, and/or (v) different experiemntal procedures, and other unknown factors. Nevertheless, it appears that at least some of the dideoxynucleosides are active against the replication of hepadnaviruses. Our data suggest that dideoxynucleosides, in particualr ddG, inhibit HBV DNA synthesis, and that this inhibition occurs at the step of reverse transcription. Thus, these agents are active against a specific HBV target molecule and may be useful experimental drugs for treatment of HBV infection, although the current data do not address the relative toxicity, efficacy, or therapeutic index of these compounds in vivo.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

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Synthesis and in vitro anti-HIV activity of lipophilic dideoxynucleosides

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Four 2-amino-6-halo-2',3'-dideoxypurine ribofuranosides (ddP), four 6-haol-ddP, two 6-meracapto-ddP, as well as three additional 2',3'-dideoxypurine derivatives were synthesized and evaluated for in vitro activity to suppress the infectivity, replication and cytopathic effect of HIV. 2-Amino-6-fluoro-, 2-amino-6-chloro-, and 6-fluoro-ddPs showed a potent activity against HIV comparable to that of 2',3'-dideoxyinosine (ddI) or 2',3'-dideoxyguanosine (ddG), and completely blocked the infectivity of HIV without affecting the growth of target cells. The comparative order of in vitro anti-HIV activity of the eight 6-haolgen-containing ddPs was 2-amino-5-fluoro, 2-amino-6-chloro, 6-fluoro >2-amino-6-bromo >2-amino-6-iodo, 6-chloro >6-bromo >6-iodo. These compounds also showed a potent activity against HIV-2 and AZT-resistant HIV-1 variants in vitro. Neither the two 6-mercapto-ddPs nor the three 2-.3'-dideoxypurine derivatives were active against HIV-1 in vitro. Several of the 6-halogen-containing ddPs were found to have substantial lipophilic character. The lipophilicity order was: 2-amino-6-iodo > 2-amino-6-bromo >2-amino-6-chloro >2-amino-6-fluoro >ddG >ddI with a log P range from +0.5 to -1.2. All eight 6-haologen-containing ddPs were substrates for adenosine deaminase (ADA). In the presence of an ADA-inhibitor, 2'-deoxycoformycin, all 6-halogen-containing ddPs failed to exert their in vitro antiretroviral effects.

Taken together, these newly synthesized 2-amino-6-halo-ddPs and 6-halo-ddPs compounds may represent a new class of lipophilic prodrugs for ddG and ddI respectively. These
HIV infection and, in particular, HIV-caused neurological disorder.

INTRODUCTION

In the past five years, a number of approaches which may have a potential usefulness for therapy of human immunodeficiency virus (HIV) infection have emerged. One such approach is the use of the broad family of 2′,3′-dideoxynucleosides (ddN) as therapeutic agents against HIV infection. 3′-Azido-2′,3′-dideoxythymidine (AZT or zidovudine), a ddN analogues, was identified in 1985 as a potent antiretroviral agent against HIV. AZT has now been formally proven to reduce the morbidity and mortality of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). Two other members of the ddN family, 2′,3′-dideoxycytidine (ddC) and 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T) have recently been shown to be active in some patients with AIDS and ARC in Phase I clinical trials. A purine ddN member, 2′,3′-dideoxyinosine (ddI), has also recently been shown to be active against HIV-1 in patients with AIDS and advanced ARC in short-term Phase I clinical trials. However, the lipophilicity of purine 2′,3′-dideoxynucleosides, especially ddI, is generally low and perhaps, in part, this limits penetration into the central nervous system (CNS).

The present report describes the synthesis of new dideoxynucleoside analogues and their in vitro activity against HIV. We have now identified several purine 2′,3′-dideoxynucleoside analogues which may represent a new class of lipophilic prodrugs for ddI and ddG. Our observations may also provide additional structure-activity relationships useful in developing other therapeutic purine nucleosides.

MATERIALS AND METHODS

Chemistry

Four 2-amino-6-halo-dideoxypurine ribofuranosides (ddP), four 6-halo-ddP, two 6-mercapto-ddP, and three 2′,3′-dideoxypurine derivatives were synthesized and evaluated for in vitro activity to suppress the infectivity, replication and cytopathic effect of HIV. 2′,2′-dideoxypurine nucleosides (compounds 1-15) were synthesized by using pelleted Escherichia coli JA-300 cells as a source of nucleoside phosphorylase. Our approach for the syntheses of the desired compounds 1-15 is outlined in Schemes I and II.

Antiretroviral drugs with high degree of lipophilicity may theoretically possess an enhanced ability to penetrate the blood-brain-barrier and may efficiently inhibit infectivity and replication of HIV-1 in the CNS. We, therefore, determined n-Octanol/water partition coefficient (P) for each drug by a micro shake-flask procedure.[19] It was found that all eight 6-halogen-containing ddPs had substantially higher octanol partition coefficients than the reference purine dideoxynucleosides, ddA, ddI and ddG (Table I).

Melting points were determined on a Yanaco melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FX60Q instrument. Proton chemical shifts are expressed as values with reference to Me_4Si. ^{13}C chemical shifts are expressed as values with reference to

2, 2-dimethyl-2-silapentane-5-sulfonate. UV spectra were recorded in a HITACHI instrument model 150-20 spectrophotometer. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-AX505H mass spectrometer. Thin-layer chromatography was carried out on E. Merck 60F 254 precoated silica gel plates. 2',3'-dideoxyadenosine (ddA), 2',3'-dideoxyinosine (ddI) and 2',3'-dideoxyguanosine (ddG) were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, while 3'-azido-2',3'-dideoxythymidine (AZT) was purchased from Sigma Chemical Company. 2-amino-6-chloropurine and 2-amino-6-iodopurine were purchased from Sigma Chemical Company, while 2-amino-6-fluoropurine, 2-amino-6-bromopurine and 2',3'-dideoxyuridine were synthesized by published methods.

Antiviral Test Procedures

HIV cytopathic effect inhibition assay was performed as previously described. Briefly, target CD4+ T cells (ATH8) were exposed to a 4.3 x 10^3 $TCID_{50}$ dose of HIV-1 per cell (1,000 viral particles per cell) or a 1.4 x 10^3 $TCID_{50}$ dose of HIV-2 per cell (10 viral particles per cell) for 1 hr, resuspended in 2 ml of fresh complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/ml of penicillin, and 50 ug/ml of streptomycin) containing 15% (vol/vol) interleukin-2 (IL-2, lectin-depleted; Advanced Biotechnologies, Inc., Silver Spring, MD) and 50 U/ml of recombinant IL-2 (Amgen, Thousand Oaks, CA), and the cells were incubated at 37 C in 5% CO_2-containing humidified air. Control cells were treated similarly but were not exposed to the virus. At various time points, viable cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method.

Partition Coefficient Determination

The octanol-water partition coefficiency was determined by micro-flask method, as previously described.[19] Briefly, 20 mM potassium phosphate buffer solution (pH 7.)) containing 10 mg of a test compound (1 ml) was mixed with 1.0 ml 1-octanol. Partitioning was performed using a 2 ml Lidex Mixxor apparatus. The phases were separated, centrifuged and the relative concentration of sample in each phase was determined by high-performance liquid chromatography (HPLC) analysis.

Enzymatic Hydrolysis

Each compound (about 100 mg) was dissolved in 2.0 ml pH 7.5 buffer incubated with 0.3 units of adenosine deaminase (adenosine aminohydrolase) (7.5 ml). A 200 ml aliquot of the enzyme-compound solution was diluted with 0.8 ml cold distilled water and 0.5 ml of the diluted sample ultrafiltered to remove enzyme in a Centrilfree Micropartition unit by centrifugation. The remainder of the sample was maintained on ice for 35 minutes to check reaction quenching at 0 C, after which time it too was ultrafiltered. A 800 ul aliquot of the enzyme-compound solution was placed in a Dubnoff metabolic shaking incubator at 25 C and 100 ul aliquots were analyzed by HPLC. Dideoxynucleosides and decomposition products were

detected at 260 nm with a Gilson 116 variable wavelength detector. For kinetic studies, data were plotted as a function of time and fitted to first order decomposition kinetics using a non-linear least squares curve fitting computer program.

RESULTS AND DISCUSSIONS

Four 2-amino-6-halo-ddPs(compounds 1, 2, 3, and 4 in Scheme I) exerted a potent anti-HIV-1 activity in vitro in the HIV cytopathic effect inhibition assay with respective ED_{50} values of 2.4, 5.5, 6.9 and 7.9 uM. Three 6-halo-ddPs (compounds 5, 6 and 7) also showed significant antiviral activity with respective ED_{50} values of 2.8, 6.9 and 7.7 uM. However, these drugs appeared to be somewhat more suppressive to cell growth as compared to the reference compounds ddI and ddG (compounds 17 and 18). Among ddPs tested, compounds substituted with a fluorine or a chlorine were relatively less toxic for cell growth as compared to those substituted with a bromine or an iodine. Two 6-mercapto-ddPs (compounds 9, 10) and three 2',3'-dideoxypurne derivatives, 2',3'-dideoxyxanthosine (14), 2',3'-dideoxypurine (15), and 2,6-dichloro-ddP (13), were not active against HIV under the conditions used.

In this initial screening assay, compounds 1, 2 and 5 were most potent and least toxic among the newly synthesized ddPs tested. These compounds were further investigated for their antiviral activity against different strains of HIV.

Compounds 1, 2 and 5 were tested for their antiviral activity against HIV-2 $_{LAV2}$ (17) and two recognized AZT-insensitive HIV-1 strains (18) in vitro. As in the assays for activity against HIV01, these three compounds were found to be potent inhibitors of the three different strains of HIV at concentrations of 20 or 50 uM (Table I and Figure 1).

Since 2-amino-6-halo- and 6-halo-purine nucleosides are readily hydrolyzed by ADA, it was likely that the corresponding dideoxynucleosides would also be substrates for this enzyme. For these dideoxynucleoside substrates, ddG was formed at a rate which corresponded to substrate disappearance, while ddI was produced (data not shown) upon enzymatic hydrolysis of the 6-halo-ddPs. In the presence of an excess of ddA, 2-amino-6-fluoro-ddP was as good a substrate as ADA. When the kinetic experiments were repeated in culture media containing 15% fetal calf serum, the 2-amino-6-halo-ddPs were still hydrolyzed to ddG, but at a rate that was approximately 60 times slower than the rate in the presence of excess enzyme. Thus both ddA and 2-amino-6-fluoro-ddP had a approximate 2 hr half-life in RPMI 1640 media. We then asked if 2-amino-6-halo-ddP could have antiviral activity against HIV-1 in ATH8 cells in the presence of 2'-deoxycoformycin (2'-dCF), a potent inhibitor of adenosine deaminase. We found that all these compounds lost their antiviral activity in the presence of 2'-dCF and essentially all the target cells were destroyed by the virus (data not shown).

One of the devastating features of HIV infection is in neurological abnormalities. While the pathogenesis of the HIV-caused neurological abnormalities is as yet incompletely understood, such a disorder may be

directly linked to structural and functional changes due to infection and/or replication of HIV in the CNS, particularly in cells of a M/M lineage. In this regard, HIV-associated neurological disorders in both adults and children with AIDS or ARC have been substantially improved during therapy with AZT. [22,23] Relatively prompt improvements of neurological abnormalities following initiation of therapy with AZT [22,23] may represent the effect of an antiretroviral activity of the drug in the CNS in some patients. Thus, lipophilicity of dideoxynucleosides may constitute an important feature of possible antiretroviral therapies against HIV infection, while the lipophilicity of a given congener may not necessarily improve the therapeutic index of a new drug. It is possible that a drug with a high lipophilicity confers even increased toxicity.

The 6-halo versions of 2′, 3′-dideoxypurine ribofuranosides are of interest in view of activity/structure relationships since the substitution with a halogen atom confers substantial lipophilicity on dideoxypurine ribofuranosides with a retention of antiretroviral activity. 6-halogen-containing ddPs appear to exert antiviral activity only upon conversion to ddG and ddI, respectively. In this regard, it should be noted that there is still no reliable algorithm for predicting which congeners will exert more antiretroviral activity or more toxicity to cells. For example, replacement of an aromatic oxygen of ddI by a hydrogen, generating 2′, 3′-dideoxypurine ribofuranoside, negates the potent antiretroviral activity of ddI (unpublished). The same replacement in ddG, generating 2-amino-2′,3′-dideoxypurine ribofuranoside, also abolishes the antiretroviral activity of ddG (unpublished). Indeed, in the present study, we also found that neither of two 6-mercapto-ddPs (compounds 9, 10 in Table 1) exerted antiretroviral activity in vitro. This may be due to the fact that these 6-mercapto-ddPs are not good substrates for ADA (unpublished data) and may not convert to ddG or ddI.

Taken together, these newly synthesized 6-halo-ddPs may represent a new class of lipophilic prodrugs for ddG or ddI which are active against a wide range of HIV strains. Our current observations may also provide a new strategy to develop lipophilic purine nucleoside derivatives for different clinical applications.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07202-08 BDMS

PERIOD COVERED
October 1, 1990 through September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biostatistics and Data Management Section

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Seth M. Steinberg	Head	BDMS, COP, DCT, NCI
Other:	David J. Venzon	Senior Investigator	BDMS, COP, DCT, NCI
	Inas Elattar	Visiting Scientist	BDMS, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION
Biostatistics and Data Management Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.25	2.25	1.0

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section is the statistical and data management component of the Clinical Oncology Program (COP). The Section provides statistical leadership and data management consultation for major activities of the Program, and is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials of experimental treatments for cancer, and intramural clinical trials for treatment of AIDS. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors, evaluate diagnostic procedures, develop improved staging systems, and assist investigators in the design, execution, and analyses of major in vitro drug testing studies. The Section develops new statistical designs and biometric methods related to the development and evaluation of new cancer and AIDS treatments. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols, and it works closely with interested branches to improve data recording and retrieval. The Section is working to develop specialized clinical data bases for individual branches within the COP.

10. Collaborative Projects Within Clinical Oncology Program

Members of the Biostatistics and Data Management Section provide to the intramural clinical research program both biostatistical and data management expertise. Our efforts in these areas are described in Sections A) and B) below.

A. Members of the Biostatistics and Data Management Section (BDMS) participate in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the Section also serves on the Clinical Research Sub-Panel to review all intramural clinical trials. BDMS staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The Section provides statistical support for the COP as well as advice on the best ways to use available NIH computer systems or microprocessor based professional workstations for clinical and laboratory research. The Section is also presently developing extensive microcomputer based data management systems for several branches in the COP.

A detailed list of COP projects to which members of the Section have provided statistical input follows:

(1) Performed two interim analyses of ALL (leukemia) protocol 77-02, a cooperative study with 181 patients at five institutions.
(2) Performed two interim analyses of two ALL protocols for average and high risk patients; the high risk protocol is a single arm extension of the successful chemotherapy only (no cranial irradiation) arm on the multi-institutional 77-02 protocol, with modifications in Ara-C administration to prevent CNS relapse and to more aggressively treat systemic disease. The average risk protocol is a randomized extension of 77-02, comparing two chemotherapy only arms -- one with high dose methotrexate and one without.
(3) Arranged for randomizations and eligibility checklists for protocols to be conducted through COP Branches.
(4) Served as member on Institutional Review Board.
(5) Performed major update of results on all soft tissue sarcoma protocols, comparing adjuvant chemotherapy to no chemotherapy in patients with extremity tumors, and with head, neck and trunk tumors, comparing limb-sparing surgery to amputation in patients receiving adjuvant chemotherapy, comparing a short course adjuvant chemotherapy regimen (350 mg/m^2 doxorubicin) with standard course (550 mg/m^2); and comparing radiation to no radiation in patients with high grade soft tissue sarcoma of extremities with local surgical resection or with low grade soft tissue sarcoma of head, neck and trunk, or extremities.
(6) Performed analyses for a study assessing differences between two in vitro cell survival curves evaluating 10-EDAM and methotrexate.
(7) Analyzed data on response to ddI in patients with HIV.
(8) Performed analyses of data on immunoglobulin G subtypes and recurrent infections in HIV (+) patients.
(9) Analyzed survival data from animal experiments using an IL-1 receptor antagonist.
(10) Performed analyses of data from experiments on calcium resorption in the presence of suramin.
(11) Analyzed data from the randomized trial of GM-CSF in pediatric patients with cancer.
(12) Performed analyses of data for a study of IL-2 doses administered to patients at original treatment and at retreatment.

(13) Analyzed data on the relationship between GST, progesterone receptors, and estrogen receptor status in patients with breast cancer.
(14) Determined statistical considerations for a randomized trial of patients with fever and neutropenia.
(15) Provided statistical considerations for a Phase II trial evaluating a two-drug treatment for mycosis fungoides.
(16) Performed analyses of prognostic factors in patients with ALL treated on a large randomized clinical trial.
(17) Analyzed data on survival of patients undergoing two lymph node dissections for melanoma.
(18) Provided statistical considerations for a pilot study of interferon in metastatic breast cancer.
(19) Determined an optimal staging algorithm for patients with lung cancer based on costs of individual diagnostic tests.
(20) Performed analyses of data from a protocol using multi-modality therapy for treatment of advanced stage limited non small cell lung carcinoma.
(21) Analyzed data on time to lymphoma diagnosis in patients on three protocols for treatment of AIDS or ARC.
(22) Performed analyses of data on the effects of suramin and PTH on osteoblast counts in experiments with mouse calvariae.
(23) Updated the analyses of survival in patients with small cell lung cancer.
(24) Performed logistic regression analyses of data on the survival of experimental animals treated with tempol.
(25) Performed analyses of factors affecting survival in pediatric patients with neuroblastoma.
(26) Analyzed data from patients on a Phase II trial of suramin for treatment of prostate cancer.
(27) Performed analyses of data from a randomized trial comparing IL-2 alone to IL-2 + LAK for treatment of advanced malignancy.
(28) Provided advice regarding analysis of data on hypermutability of the exon region of the P53 gene.
(29) Performed analyses of data on multiple thoracotomies in patients with soft tissue sarcoma.
(30) Analyzed data from the randomized early stage breast cancer trial.
(31) Performed analyses of data on autopsy findings in patients in patients who died of stage III or IV ovarian cancer.
(32) Analyzed data on prognostic factors in patients treated on a trial of patients with high risk ALL.
(33) Performed analyzes of the relationship of Ras oncogene mutations to survival in patients with NSCLC.
(34) Analyzed data on the development of pancreatitis in patients receiving ddI for treatment of HIV infection.
(35) Performed analyses of data on a series of experiments comparing in vitro growth characteristics of the jun oncogene.
(36) Prepared statistical considerations for a randomized trial in pediatric AIDS using various forms of AZT.
(37) Performed analyses of data from the Phase II trial of 6MP in children with solid tumors.
(38) Analyzed data from a study showing effects of treatment on growth in children who have ALL and were randomized onto a large clinical trial.
(39) Provided statistical considerations for a protocol to treat lymphoblastic lymphoma.
(40) Determined statistical considerations for a protocol relating to the pharmokinetics from PEG L-Asparaginase for treatment of children with ALL.
(41) Provided advice to an administrator regarding interpretation of a Phase III trial of chemotherapy in node-negative breast cancer.
(42) Performed analysis of data from the locally advanced breast cancer trial.
(43) Analyzed data on a study of infections in patients with aplastic anemia.

(44) Performed analysis of data from a Phase I trial of 5-FU, leucovorin, and alpha-interferon for treatment of a gastrointestinal carcinomas.
(45) Analyzed data from a pilot study of chemotherapy and autologous bone marrow transplantation for treatment of Hodgkin's disease.
(46) Performed analyses of the SAP marker in lung cancer.
(47) Performed analyses of data from a study evaluating FLAC + GM-CSF as a treatment for breast cancer.
(48) Analyzed data on clone sizes of cells transfected from several oncogenes.
(49) Tested immunoglobulin levels, IL6, CD4 , and P24 levels at baseline for their association with development of lymphoma in patients receiving AZT for HIV therapy.
(50) Analyzed data from a study examining development of aspergillosis despite use of amphoteracin-B .
(51) Performed analyses of data from a study of adduct formation in patients with colon cancer.
(52) Prepared statistical considerations for a randomized Phase II trial in metastatic prostate cancer.
(53) Provided advice to an investigator regarding the number of patients to test in order to rule out an even distribution of genes.
(54) Performed analysis of T4 data and its relationship to survival in patients with AIDS or ARC.
(55) Prepared statistical considerations for a phase II protocol for treatment of relapsed rhabdomyosarcoma.
(56) Analyzed data on clinical improvement and various measures of improvement in T helper cell function.
(57) Performed analyses of data on alkaline phosphatase levels in media remaining from calcium resorption experiments.
(58) Performed analyses of data on the effects of ara-C treatment in rabbits on in vitro assays of neutrophil function.
(59) Provided advice to an investigator on sample sizes needed to conduct a study of infection rates in cancer patients.
(60) Performed analysis of data comparing groups of patients with chronic granulomatous disease.
(61) Performed analysis of data for a study of dose intensity of chemotherapy in pediatric patients with lymphoma.
(62) Provided statistical review for a protocol designed to use monoclonal antibodies in patients with advanced colorectal or gastric carcinoma.
(63) Analyzed data from a Phase II trial of amiodarone and adriamycin for treatment of breast cancer.
(64) Performed analyses of data on the association between erb-2 and outcome among patients with early stage breast cancer treated on a large randomized trial.
(65) Provided statistical considerations for a protocol which plans to use ras and P53 vaccinations.
(66) Performed analysis of data on the association between HLA data and toxicity in patients treated with IL-2.
(67) Analyzed data from a study of glioblastomas treated with halogenated pyrimidines and radiation.
(68) Provided statistical considerations for a Phase II protocol for treatment of cutaneous T-cell lymphomas with recombinant IL-2 diptheria toxin conjugates.
(69) Provided statistical considerations for a randomized Phase III protocol comparing high and low dose IL-2 for treatment of renal cell cancer.
(70) Provided advice on design of a Phase I trial of three drugs plus G-CSF for treatment of ovarian cancer.

(71) Presented a series of three seminars on statistical methodology in clinical trials to the Pediatric Branch.
(72) Delivered Clinical Oncology Program Grand Rounds on statistical considerations in cancer clinical trials.
(73) Performed analyses of data from an experiment testing for an inhibitor to TNF in the serum of mice inoculated with TNF.
(74) Provided advice regarding analysis of data from a study of the quality of life of patients on a pediatric sarcoma protocol.
(75) Provided statistical considerations for a protocol for a pilot study of chemotherapy and suramin in the treatment of adult T-cell leukemia and lymphoma.
(76) Advised investigators regarding analysis of data on CNS disease in patients with SNC lymphoma.
(77) Provided advice regarding analysis and presentation of data from a trial of CPC chemotherapy +/- WR 2721 for treatment of ovarian cancer.
(78) Performed analyses of effects of several factors on survival in patients with rhandomyosarcoma.
(79) Updated and analyzed data on the in vitro sensitivity of 91 cell lines to five drugs.
(80) Performed analyses on the rates of opportunistic infections in patients on AZT protocols.
(81) Analyzed data on the effects of G-CSF and interferon on superoxide production by human neutrophils.
(82) Performed analysis of data on the effect of MDR expression on time on study in patients with breast cancer.
(83) Analyzed data from a study of the effect of several factors on survival in patients with LN3 type mycosis fungoides.
(84) Provided advice regarding analysis of frequencies of toxicity in patients treated with varying amounts of radiation.
(85) Performed analysis of effects of various factors on survival in patients with breast cancer.
(86) Analyzed data on the effect of G-CSF and interferon on the in vitro response of human neutrophils to various fungal forms.
(87) Provided advice regarding design of a Phase I trial of suramin + somatuline for treatment of prostate cancer.
(88) Performed analysis of EPOCH chemotherapy for treatment of lymphoma.
(89) Prepared statistical considerations for a Phase I study of an immunotoxin for treatment of refractory CD-22 positive B-cell lymphoma.
(90) Prepared statistical considerations for a feasibility and Phase II study of low dose rate chest radiotherapy for treatment of intra-thoracic relapse of small cell lung cancer.
(91) Provided statistical considerations for a phase II study of 5-FU, leucovorin, and interferon-alpha to treat locally advanced pancreatic cancer.
(92) Performed detailed analyses of the quality of life in patients with high-grade soft tissue sarcomas.
(93) Analyzed data on patients with low-grade soft tissue sarcomas treated over many years at NCI.
(94) Performed analyses of laboratory data on the relationship between P24 and other parameters from patients with HIV disease.

B. Data Management Activities

The Section has continued the development and maintenance of several systems which facilitate the monitoring of protocols:

(1) Currently completing the redesign of the Clinical Data Registry (CDR) system. CDR Version II will reflect changes to the data collection forms, table structures, editing procedures, and reports. The Systems Users/Operations Manual is being updated including all additions and enhancements. A Data Managers Manual is also being developed that will set guidelines and standards for forms completion and data transfer.

(2) Continued to update the Cancer Patient Research Information (CAPRI) System until all applicable data was converted to the Clinical Data Registry (CDR) System.

(3) Continued enhancements and modifications to the Protocol Database Management System (PDMS) in 4th Dimension on the MacIntosh for the Medicine Branch. System documentation, including a User's Guide, Programmer's Reference Manual and Program Hierarchy Diagrams, were revised and presented to branch staff.

(4) Completed analyses, design, development, testing and implementation of the Radiation Oncology Branch Data Base Management System (DBMS) using Paradox on an IBM compatible PC. Complete User`s and Programmer's documentation was written and delivered to branch personnel.

(5) Completed a major redesign of the Metabolism Branch Data Management System using FoxPro and Quattro Pro on an IBM compatible PC. Modification, enhancements and additions were completed as requested by branch staff. Programmer's documentation was produced and given to branch staff.

(6) Completed the redesign of the Pathology Tracking System for the Navy Medical Oncology Branch using FoxPro on an IBM compatible PC. The system was converted from dBase III and to FoxPro.

(7) Completed the analysis and redesign of the Navy Patient Listing System, including converting the system from dBase III + to FoxPro on an IBM compatible PC. Added enhancements and modifications as requested, and streamlined the reporting process.

(8) Presently continuing the development of a Master patient database for the Protocol Office of the Medicine Branch. The system which is being written in 4th Dimension on the MacIntosh will be used for tracking all Medicine Branch patients and for assigning clinical associates.

(9) Developed adhoc databases as needed to meet requirements of COP researchers.

A summary list of data management support provided by members of the BDMS for the COP follows:

(1) Provided comprehensive data management support to all branches of the COP through the assignment of Data Managers in each branch. Project Data Managers provided assistance to branch Research Nurses and Investigators in the collection of patient data, completion of data collection forms, systems and database updating and maintenance, retrieval and reporting, and assistance with drug and protocol audits. Data management involved collection and reporting of all types of data, both protocol and disease specific, to meet both the needs of the Investigators and various monitoring agencies.

(2) Developed and revised data collection instruments, as required, for all branches of the COP.

(3) Provided support to insure the patients receiving chemotherapy, especially investigational drugs, have a valid Clinical Center protocol number for pharmacy records.

(4) Monitored daily outpatient clinic appointment lists and maintained a master database of the latest clinic visit by branch for COP patients.

(5) Performed programming, retrievals, analyses and reporting as required by branch and administrative personnel of the COP, including the production of a variety of reports, listings, graphs, and tabulations.

(6) Created, modified and updated mainframe and microcomputer databases for branches of the COP.

(7) Maintained various computer packages and hardware used by the Section.

(8) Provided support of the COP use of personal computers, including assistance and consultation in the selection and installation of hardware, and in the analysis and evaluation of software packages.

(9) Assisted research nurses, principal investigators, and clinical associates in the training associated with the use of personal computers for protocol data management. Training was provided to branch personnel in the use of the branch PC-based systems as well as additional software packages, including desktop publishing and spreadsheet software.

(10) Maintained and modified numerous SAS programs on the IBM 370 mainframe used for producing scheduled and ad hoc protocol, branch and disease specific reports and listings.

(11) Collaborated with Clinical Center staff on abstracting and downloading MIS data for use by branch staff. Set up automated procedures for downloading selected subsets of patients.

(12) Continued support of COP randomization activities, including setting up new branch protocols for intramural and extramural studies. Modified existing randomization materials as requested, and performed randomization of patients.

(13) Continued support of the Medicine Branch in the registration of all patients, including setting up new eligibility checklists for new protocols, modifying existing checklists as changes occurred in the protocols, and performing registrations of all branch patients. Maintained master lists of all registered patients and provided them to branch personnel for compliance monitoring.

(14) Provided registration support for all Navy Medical Oncology Branch patients, including setting up all support materials and registering all eligible patients.

(15) Acted as a coordinating center for three multi-center pediatric leukemia protocols, involving the registration and follow-up of patients, data collection, database maintenance, analysis and reporting.

(16) Served as the coordinating center for two multi-center ovarian cancer protocols, including data collection and maintenance of protocol databases.

(17) Provided extensive data collection, data processing and programming support to the COP Clinical Resource Allocation Program for the Administrative Office.

2. Projects Outside Cop

A. The BDMS also participates in biometric activities outside of the COP. A detailed list of projects outside of COP in which the Section's statisticians have provided statistical input include the following:

1) Performed analyses of data from a Phase I trial of D4T for treatment of AIDS, being conducted at Brown University.

2) Provided advice to a member of the NIH Clinical Center nursing department regarding design of a procedure to assess nausea and vomiting in patients receiving tetraplatin.

3) Provided demonstrations of COP clinical data bases to a visiting physician from the All Union Cancer Research Center in Moscow.

4) Analyzed data on circadian variation in Leu-aminopeptidase activity in five areas of rat brain, for an investigator in Bilbao, Spain.

5) Performed analyses of the prognostic value of flow cytometry and cytophotometry in patients with MFH sarcomas for an investigator at the Armed Forces Institute of Pathology.

6) Provided advice to a physician from NIDDK regarding meta-analyses in medical research.

7) Advised a researcher in the Environmental Epidemiology Branch of NCI regarding design of a case control study for evaluating treatments in males with breast cancer.

8) Performed analyses of data on the relationship between immune status against EBV and HIV exposure for an investigator in Bilbao, Spain.

9) Analyzed data on nasal challenges for an investigator in NIAID.

10) Performed analyses of data from a study comparing MRI to CT with regard to their relative abilities to identify metastatic nodules for an investigator in the NIH Clinical Center Diagnostic Radiology Department.

11) Performed analyses of data to determine factors influencing development of second tumors in patients treated in Brazil over a thirty year period.

12) Served as Chairman of a committee to evaluate proposals for a statistical software system to be used at NIH.

13) Reviewed a manuscript submitted to Cancer Communications for determining cancer promotion or inhibition.

14) Reviewed a manuscript on neuroblastomas submitted to Cancer Research.

15) Provided advice to a researcher in the National Center for Research Resources regarding combination of several p-values from different laboratory experiments.

16) Served as Chairman of an NIAID review committee on clinical data management systems for the intramural AIDS research program of the Institute.

17) Served as statistical and data management advisor to the Scientific Director of the National Center for Nursing Research.

18) Reviewed a manuscript submitted for publication in Biometrics.

19) Provided advice to an investigator from Clinical Center Diagnostic Radiology Department regarding analysis of data comparing diagnostic radiology modalities.

20) Performed analyses of data on factors affecting survival in patients with ATL from Jamaica, at the request of an investigator from NCI-DCE.

B. In addition to data management support for intramural trials, the BDMS provides data management services outside the COP. Project staff have provided operations and/or statistical center support to a number of multi-institutional extramural trials. This support includes performing randomizations, design of data collection instruments, software design and development, production of regular status reports, and production of ad hoc reports and tabulations as directed by the study statistician. The extramural trials supported include:

(1) 7601/7602, Treatment of Early Stage Ovarian Cancer
(2) CCSG-191P, CCSG Protocol for Acute Lymphoblastic Leukemia
(3) CCSG-134P. CCSG Protocol for Poor Prognosis Acute Lymphoblastic Leukemia
(4) CCSG-144P, CCSG Protocol for Average Prognosis Acute Lymphoblastic Leukemia

3. Biometric Research

Current biostatistical research being conducted includes:

(1) A two stage method for selected interactions between variables to be evaluated for prognostic importance.
(2) Development of data management systems which may serve multiple purposes.
(3) Development of models for testing in-vitro synergy of chemotherapeutic agents.
(4) Development of estimation methods for parametric transformations in survival analyses.
(5) Development of appropriate non-parametric techniques for analyzing paired data with missing values.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06523-07 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Metabolism and Mechanism of Action of Etoposide (VP-16, 213)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	B.K. Sinha	Senior Investigator	CPB, COP, DCT, NCI
Others:	H.M. Eliot	Biologist	CPB, COP, DCT, NCI
	H. Yamazaki	Visiting Fellow	CPB, COP, DCT, NCI
	E.G. Mimnaugh	Chemist	CPB, COP, DCT, NCI
	E. Monti	Guest Researcher	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

None.

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biochemical and Molecular Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

VP-1-16 undergoes O-demethylation to generate active intermediates that bind to proteins and DNA. The O-demethylation is P450-dependent. Peroxidases, such as horseradish or prostaglandin synthetase, also activate VP-16 and VM-26 to their O-Quinone derivatives and catalyze the binding of reactive intermediates to DNA. We have shown that both the dihydroxy and O-Quinone derivatives are cytotoxic and induce topo-II-dependent cleavage. The binding sites on the topo-II-DNA complex for these O-demethylated drugs are similar to those of the parent compound. We have also shown that the dihydroxy VP-16 binds metal ions (iron and copper). These metal ion complexes are redox-active and induce DNA strand scission in an oxygen-dependent pathway. Thus, enzymatic activation to reactive intermediates is important in the biological activities of VP-16 and VM-26.

Major Findings:
The semisynthetic podopyllotoxin derivative etoposide (VP-16) has shown activity against a number of human tumors. Although the mechanism of action of this drug is not clear, DNA damage induced by VP-16 has been suggested as a cause of its cytotoxicity. Thus, we have proposed that the cellular damage induced by VP-16 may result from the formation of a reactive intermediate during bioactivation of the drug. We have studied the metabolism of VP-16 by mouse hepatic microsomes. Using HPLC analysis of the chloroform extracts of the microsomal incubation, we showed that VP-16 formed the 3'-4' dihydroxyl derivative (DHVP). The formation of this metabolite (2% of the parent drug) was NADPH-, protein-, VP-16-, and time-dependent, suggesting that the activation was enzymatic. Moreover, DHVP formation was inhibited by SKF-525A and piperonylbutoxide, suggesting that the O-demethylation was also P-450-dependent.

Incubation of [3H] VP-16 with microsomes containing NADPH and DNA resulted in irreversible binding of the drug to DNA and to proteins.

We have found that peroxidase-catalyzed activation of VP-16 forms a number of reactive metabolites. HPLC and mass spectral analysis have shown that VP-16 undergoes aromatization (to AR-VP-16) which is subsequently O-demethylated to O-Quinone (Ar-VP-16-Q). Inhibition studies suggest that the protein binding species result from O-demethylation reactions (VP-16-Q and AR-VP-16-Q), and that DNA binding species are positively charged.

Our studies also indicate that a VP-16 metabolite, dihydroxy VP-16, formed from O-demethylation of VP-16, chelates Fe^{3+} ions and in the presence of H_2O_2 or reduced glutathione forms hydroxyl radicals which induce topo-II-independent DNA cleavage. In addition, Cu^{2+} is also an excellent catalyst for inducing DNA cleavage in an oxygen radical-dependent pathway. We have shown that O-demethylated compounds also bind to topo-II, similar to the way that VP-16 binds to topo-II, and that they induce DNA cleavage as well.

Using alkaline elution studies in both sensitive and resistant MCF-7 cells, we have found that VP-16 induces a significant amount of DNA damage in the sensitive cells. In contrast, very little DNA damage could be detected in the resistant cells. Furthermore, when isolated nuclei were used to assess DNA damage, there was only a two-fold difference in the number of VP-16-induced DNA strand breaks in the sensitive and in the resistant cells.The differences in toxicity (approximately 200-fold) and uptake of VP-16 (2-3 fold) do not completely explain DNA damage induced by VP-16 in these cells and suggest that other factors may also be involved in the mechancs of cell kill by VP-16.

Resistance to VP-16 and to other antitumor drugs results in a decreased drug accumulation; in multi-drug-resistant cell lines, overexpression of P-170 glycoprotein has been implicated in this decreased drug accumulation. In order to characterize VP-16 resistance, we have carried out uptake and efflux of VP-16 in a number of sensitive and resistant human tumor cell lines. Our studies suggest that decreased VP-16 accumulation is not due to overexpression of the P-170 glycoprotein, but rather may be related to energy-dependent modification in drug binding in the resistant cell lines. Furthermore, using photoaffinity labelling of P-170 protein with photoactive vinblastin and verapamil analogs, we have recently shown that VP-16 has a very low binding affinity for the protein, indicating that P-170 is not the major mechanism for VP-16 resistance. In order to further define the mechanisms of VP-16 resistance, we used multidrug-resistant HL60 cells (HL60/ADR). These cells were selected for resistance with adriamycin, and are 200-fold resistant to VP-16; however, they do not overexpress P-170. Using the antibody to topoisomerase II, we have found that the topoisomerase II level was 2-3 fold lower in the drug-resistant cell line compared to that of the WT cells. Moreover, we found that VP-16 induced significant amounts of DNA double strand

breaks (measured by the alkaline unwinding assay) in the parental cell line compared to those of the resistant cell line. Isolated nuclei from the resistant cells were also resistant to VP-16-dependent DNA damage. Interestingly, isolated nuclei from WT cells were relatively more resistant to the drug-dependent DNA breakage than were the intact cells (2.5μM vs. 175 μM VP-16 for 50% of the DNA strand breaks in WT and in HL60/ADR cells, respectively), indicating that some cytosolic factors are necessary for maximal damage. Addition of WT cytosolic proteins significantly increased VP-16-dependent DNA cleavage in isolated WT nuclei. However, addition of cytosolic proteins from HL60/ADR cells had no enhancing effect on DNA cleavage activity in either WT or HL60/ADR nuclei, indicating that this factor was not present in resistant cells. Work is in progress to identify this cytosolic factor. We have recently found that the combination of VP-16 and IL-1 was more cytotoxic to human A375 melanoma cells than was either drug alone. This synergistic interaction appears to result from upregulation of IL-1 receptors by VP-16, thus resulting in an increase in IL-1 binding and the internalization of IL-1.

Publications:

Usui N, Mimnaugh E, Sinha BK. Synergistic antitumor activity of etoposide and human IL-1a against human melanoma cells. J Natl Cancer Inst 1989;81:1904-9.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06524-01 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Myeloperoxidase in Vincristine Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C.E. Myers Chief, Clinical Pharmacology Branch CPB, COP, DCT, NCI

Other: D. Schlaifer Guest Researcher CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

None.

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hemeperoxidases are able to use a wide range of drugs as electron donors. For this reason, this enzyme may play a role in drug detoxification. The purpose of this project is to investigate the role of these enzymes in drug resistance.

Major Findings:
Acute lymphocytic leukemia (ALL) is very responsive to vincristine. On the other hand, vincristine does not have worthwhile activity in acute myelogenous leukemia (AML). One major factor which distinguishes AML from ALL is the expression of myeloperoxidase. Myeloperoxidase is a member of the hemeperoxidase family, which includes such other enzymes as prostaglandin synthetase, lactoperoxidase, and lipoxygenases. These enzymes all mediate one-electron transfer to peroxidases. While they can exhibit considerable specificity as far as the peroxide substrate is concerned, they have a broad and similar specificity for electron donors. As a result, horseradish peroxidase, a very stable hemeperoxidase, is often used to screen potential electron-donating substrates. The vinca alkaloids have been shown to undergo oxidative destruction in the presence of horseradish peroxidase. Thus, the related enzyme, myeloperoxidase (MPO), might also cause oxidative degradation of the vinca alkaloids. We have shown that MPO does accomplish oxidative destruction of vinca alkaloids. In addition, the HL60 promyelocytic leukemia cell line, which expresses MPO, is resistant to vincristine, while a series of MPO-negative cell lines is vincristine-sensitive. None of the lines expresses the P170 glycoprotein.

We have now inserted the MPO gene in an appropriate transfection vector and will see if transfer of this gene will convert the MPO-negative lines to MPO positivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06525-01 CP

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders): Evaluation of Cytotoxicity of Ansamycins to Defined Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.:	L.M. Neckers	Senior Investigator	CPB, COP, DCT, NCI
Other:	L. Whitesell	Biotechnology Fellow	PB, COP, DCT, NCI

COOPERATING UNITS (if any): None.

LAB/BRANCH: Clinical Pharmacology Branch

SECTION: Tumor Cell Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Herbimycin A and geldanamycin (HA and GA, respectively) are benzoquinoid ansamycin antibiotics which have been reported to reverse the transformed phenotype of virus-transformed epithelial cells. While these agents are weak inhibitors of tyrosine kinases in vitro, a result of their addition to cells is delayed reduction in cellular protein phosphotyrosine content and in the activity of certain tyrosine kinases. The focus of this project is to examine these agents' cytotoxicity against cell lines derived from the neuroectoderm and against those cell lines with neurocrine features. Secondarily, our focus is to determine whether any observed cytotoxicity is due to tyrosine kinase inhibition or to another, as yet undefined, mechanism.

We have determined that both drugs are very cytotoxic against both primitive, undifferentiated neuroectoderm-derived tumors and tumors with neurocrine properties. These tumors include medulloblastomas, neuroepitheliomas, colon carcinomas, melanomas, and prostatic carcinomas. More differentiated neuroectoderm-derived cells, such as neuroblastoma, are not affected. Many other cell lines, representative of the hematopoietic system and fibroblasts, are not sensitive at the concentrations employed. We have been able to demonstrate in vivo effectiveness of these drugs as well. Using a subcutaneous athymic mouse-human xenograft model, we observed a significant reduction in tumor mass following subcutaneous drug administration. Tumors studied were prostatic carcinoma (hormone-refractory) and neuroepithelioma. No overt toxicity to the host was seen. We have also demonstrated that these drugs are cytotoxic to a primary explant of a human neuroepithelioma, while not affecting the viability of normal rat brain cortical and cerebellar cultures. The toxicities observed in all cases require an exposure of cells to drug of as little as one hour.

The significance of this project lies in the inherent sensitivity of chemo-refractive malignancies such as prostatic carcinoma and various undifferentiated brain tumors to these agents, as these agents' lipophilicity should permit systemic administration.

Objectives:
The objectives of this project are: (1) to examine the in vitro and in vivo cytotoxicity of ansamycin antibiotics to certain neuroectoderm-derived, and other neurocrine, tumors, correlating differentiation state to sensitivity, and (2) to uncover the mechanism by which cytotoxicity is implemented.

Methods Employed:
Standard cell and molecular biological techniques, such as polyacrylamide gel electrophoresis, Western, Northern, and Southern blotting, protein and nucleic acid isolation, immune complex kinase assay, and flow cytometry will be employed. Cell growth will be assessed by tritiated thymidine incorporation, viable cell number determination, immunocytochemical analysis of bromodeoxyuridine incorporation into cellular DNA, and automated analysis of MTT reduction.

Major Findings:
1. Benzoquinone ansamycins are particularly cytotoxic to undifferentiated neuroectoderm-derived, or neurocrine, cell lines in vitro. These cell lines include prostatic carcinoma, colon carcinoma, melanoma, neuroepithelioma, and medulloblastoma. More differentiated neuroectoderm-derived cell lines, such as neuroblastoma, are much less sensitive.

2. Many other cell lines, including a variety of hematopoietic and fibroblast lines, are quite insensitive to these drugs.

3. A primary explant taken from a patient with neuroepithelioma was as sensitive to the drugs in vitro as were the cell lines, while cerebrocortical and medullary cultures derived from normal rat brain are unaffected by the drugs.

4. Using a subcutaneous athymic mouse/human xenograft model, we have demonstrated that neuroepithelioma and prostatic carcinoma can be effectively treated by in vivo administration of drug with no toxic side effects to the host.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06526-03 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Modulation of Cell Growth by Antisense and Antigene Reagents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	L.M. Neckers	Senior Investigator	CPB, COP, DCT, NCI
Others:	D. Geselowitz	Biotechnology Fellow	CPB, COP, DCT, NCI
	L. Whitesell	Biotechnology Fellow	PB, COP, DCT, NCI
	A. Rosolen	Visiting Fellow	CPB, COP, DCT, NCI
	E. Kyle	Microbiologist	CPB, COP, DCT, NCI
	B. Fahmy	Guest Researcher	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Pediatric Branch, NCI, NIH (I. Magrath); Dermatology Branch, NCI, NIH (S. Katz); Laboratory of Molecular Genetics, NICHHD, NIH (R. Crouch).

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Tumor Cell Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The focus of this project is three-fold: (1) to characterize uptake and intracellular processing of unmodified and modified oligonucleotides; (2) to utilize antisense and antigene technology in several in vitro model systems to identify critical events in cell proliferation/viral replication; and (3) to study the efficacy of antisense and antigene reagents as in vivo modulators of gene expression.

(1) We have characterized the uptake of unmodified oligos as an energy-dependent, endocytic process, mediated by at least one cell surface-binding protein. We have devised a novel technique to study oligo uptake, intracellular localization, and association with protein and nucleic acids. This non-invasive technique will permit subcellular localization over time of an internalized oligo.

(2) We have confirmed that c-myc inhibition is cytostatic for normal and malignant lymphoid cells and that some Burkitt lymphoma cells can be specifically growth-arrested in vitro with a novel c-myc antisense. We have confirmed that N-myc inhibition leads to reduction in growth secondary to alteration in differentiative status of neuroectoderm-derived cell lines.

(3) We have demonstrated that continuous subcutaneous perfusion of an oligo can significantly affect in vivo its targeted gene expression and reproduces other in vitro phenomena observed with either DNA or RNA antisense. This model permits in vivo testing of antisense oligos for efficacy and toxicity. We are developing a continuous perfusion intrathecal model to study the clinical efficacy of antisense in a more relevant pre-clinical in vivo model system.

The significance of this project lies in (a) the identification of critical genes for novel pharmacologic intervention, and (b) testing the in vivo applicability of antisense reagents in well-defined, clinically relevant in vivo model systems.

This project is a combination of the following four terminated projects: Z01 CM 06724-02 M; Z01 CM 06725-02 M; Z01 CM 06726-02 M; and Z01 CM 06733-01 M.

Objectives:
The objectives of this project are (1) to characterize uptake and intracellular processing of unmodified oligonucleotides; (2) to utilize antisense and antigene technology in several in vitro model systems to identify critical events in cell proliferation/viral replication; and (3) to study the in vitro efficacy of antisense reagents as in vivo modulators of gene expression.

Methods Employed:
1. A novel cross-linker/oligo complex has been devised which allows for non-invasive cross-linking of oligo to binding proteins or nucleic acids intracellularly. This technique is used in conjunction with standard protein and nucleic electrophoretic analyses and subcellular fractionation/isolation procedures.
2. Techniques include visualization of specific proteins in cells by immunocytochemistry and Western blotting; growth assays include viable cell counting, MTT assay, and immunochemical quantification of bromo-deoxyuridine incorporation into cellular DNA.
3. Techniques include use of three in vivo models: (1) the subcutaneous athymic mouse/human xenograft model; (2) the intrathecal athymic rat/human xenograft model; and (3) topical application to murine epidermis. Techniques also include stereotaxic implantation of indwelling intrathecal catheters and magnetic resonance imaging of intrathecally tumor-bearing animals.

Major Findings:
1. Invention of a novel method to follow the intracellular trafficking of oligonucleotide and permanently, but non-invasively, cross-link it to associating molecules at any point in time.
2. Identification of a novel set of nuclear oligonucleotide binding proteins.
3. Demonstration that specific inhibition of N-myc expression in neuroectoderm-derived cell lines leads to reduced growth secondary to alteration of differentiative status and that tumorigenicity is not abrogated by N-myc suppression.
4. Demonstration that cytoplasmic over-expression of bacterial RNase H does not improve antisense efficacy.
5. Invention of a luciferase-based luminescent assay for efficacy of triplex oligonucleotides.
6. Demonstration that in vivo administration of oligonucleotides can specifically alter gene expression with no ill side effects.

Publications:
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Rosolen A, Whitesell L, Neckers LM. Antisense oligodeoxynucleotide inhibition of N-myc expression in a neuroectodermal cell line. Advances Neuroblastoma Res 1991;3:29-36.

Schwab G, Siegall CB, Aarden LA, Neckers LM, Nordan RP. Characterization of an interleukin 6-mediated autocrine growth loop in the human multiple myeloma cell line, U266. Blood 1991;77:587-93.

Whitesell L, Rosolen A, Neckers LM. Episome generated N-myc antisense restricts the differentiation potential of neuroectodermal cell lines. Mol Cell Biol 1991;11:1360-71.

Whitesell L, Rosolen A, Neckers LM. N-myc expression is required for neuroectodermal transdifferentiation in vitro. Advances Neuroblastoma Res 1991;3:45-54.

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Neckers LM, Whitesell L, Rosolen A, Geselowitz D. Antisense inhibition of gene expression. In: CRC critical reviews in oncogenesis, in press.

Stein CA, Pal R, Nair BC, Mumbauer S, Hoke G, Neckers LM. Phosphorothioate oligodeoxycytidine inhibits binding of HIV-1 gp120 to CD4. J AIDS, in press.

Ms. Brigid Fahmy was supported by Gilead Sciences via CRADA #CR0088 (Antisense oligonucleotides as anticancer and anti-AIDS agents). Gilead Sciences also supported some of the research effort. (Total CRADA Support: $49,980.00)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06527-01 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Oncologic Aspects of Tyrosine Kinases and Epithelial Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	O. Sartor	Senior Investigator	CPB, COP, DCT, NCI
Others:	C. McLellan (joined 7/17/91)	Biologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
LCDO, NIDR, NIH (K. Robbins, M. Cardinali); CPB, NCI, NIH (J. Trepel).

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Molecular Oncology Group/Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The focus of the project is to study tyrosine kinases, their mechanism of action, substrates that may correlate with transformation, and pharmacological agents which may potentially alter their activity.

(1) We identified highly transforming mutants of the c-fgr proto-oncogene. Surprisingly, the normal c-fgr proto-oncogene was also found to be transforming, and this protein was subsequently observed to be growth-inhibitory in mouse fibroblasts. These findings emphasize that cell growth rate and transfromation are distinct phenotypic properties.

(2) Utilizing mutants of fgr, fyn, and src, we have identified a common 130 kd tyrosine phosphorylated protein (p130) that associates with activated src-family kinases and a 70 kd tyrosine phosphorylated protein that preferentially associates with activated versions of the fyn proto-oncogene.

(3) Efforts to identify the associated p130 have lead to identification of a monoclonal antibody (obtained from Dr. T. Parsons) that reacts with this protein. Furthermore, we have found that p130 is markedly induced after transfection with activated src-family kinases.

(4) Utilizing Dr. Parsons' anti-p85 monoclonal antibody, we identified another protein that associates with the fyn kinase. This tyrosine-phosphorylated protein contains an SH3 domain and appears to localize to regions of membrane adhesion. The p85 molecule is not associated with fgr or src, and current efforts are directed toward mapping the regions of fyn responsible for this association.

(5) Studies in collaboration with K. Robbins and M. Cardinali reveal that suramin can markedly modulate tyrosine phosphorylation and growth in epithelial cancer cell lines. Preliminary observations suggest potentially interesting mechanisms may underlie these alterations.

Objectives:
To determine the mechanisms whereby tyrosine kinases can transform cells; to identify critical substrates for these events; and to identify new targets for pharmacological intervention.

Methods Employed:
We have routinely subcloned full-length cDNAs of normal, mutant, and chimeric proto-oncogenes into eukaryotic expression vectors and have transfected these constructs into NIH 3T3 cells. After transfection and selection of successful transfectants, we have studied the consequence of these transfections on cell growth and morphology as well as on tyrosine phosphorylation. Additionally, we have characterized protein-protein interactions by a combination of Western blotting, immuneprecipitation, kinase assays, and V-8 protease analysis.

Major Findings:
1. Identification of mutations that activate the c-fgr proto-oncogene.

2. Characterization of the growth-inhibitory properties of c-fgr.

3. Identification of proteins that preferentially associate with highly transforming versions of src-family kinases.

4. Identification of proteins that interact preferentially with the fyn proto-oncogene.

5. Marked alterations in tyrosine phosphorylation and growth induced by suramin in epithelial cancer cell lines.

Publications:
Bowers CY, Sartor AO, Reynolds GA, Badger TM. On the actions of the growth hormone-releasing hexapeptide, GHRP. Endocrinol 1991;128:2027-35.

Sartor O, Sameshima JH, Robbins KC. Differential association of cellular proteins with family protein-tyrosine kinases. J Biol Chem 1991;266:6462-66.

Grem JL, McAtee N, Murphy RF, Balis FM, Steinberg SM, Hamilton JM, Sorenson JM, Sartor O, Kramer BS, Goldstein LJ, Gay LM, Caubo KM, Goldspiel B, Allegra CJ. A pilot study of interferon alpha-2a in combination with 5-fluorouracil plus high-dose leucovorin in metastatic gastrointestinal carcinoma. J Clin Oncol (in press).

Sartor O, Moriuchi R, Sameshima J, Severino M, Gutkind JS, Robbins KR. Diverse biologic properties imparted by the c-fgr proto-oncogene. J Cell Biol (submitted).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06528-01 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacterial and Plant-derived Cytotoxins as Novel Antineoplastic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Sausville	Senior Investigator	CPB, COP, DCT, NCI
Other:	G. Kaur	Biologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

NMOB, DCT, NCI, NIH (F. Foss); LP, DCBD&C, NCI, NIH (M.A. Stetler-Stevenson, E. Jaffe); DTP, NCI, NIH (J. Plowman); CPB, NCI, NIH (C. Myers); MB, NCI, NIH (R. Wittes).

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Molecular Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bacterial and plant toxins utilize unique mechanisms of affecting cytotoxicity not currently used in the standard practice of medical oncology. Definition of the mechanisms of action of these agents in vitro will facilitate their rational introduction to the clinic. Prior studies by the principal investigator had demonstrated that cholera toxin could inhibit increases in intracellular calcium in response to [Tyr^4]-bombesin in small cell lung carcinoma (SCLC) cell lines. We have extended these studies by examining the effect of cholera toxin on the growth of lung carcinoma cell lines. Nine of 12 SCLC cell lines and four of 14 non-SCLC cell lines were sensitive to growth inhibition by CT. To define the mechanism by which growth inhibition occurs, we examined the expression of the ganglioside G_{M1}, the receptor for CT. Sensitive SCLC cell lines all had notable expression of G_{M1}, whereas resistant cell lines had reduced or absent G_{M1} expression. In contrast, while non-SCLC cell lines sensitive to CT all had easily detectable G_{M1}, resistant non-SCLC in 70% of cases had detectable G_{M1} expression. Thus, the mechanism for resistance to CT in non-SCLC cannot be related to absence of cell surface receptor. Cholera toxin causes an increase in cyclic AMP (cAMP) after activation of G_s, the stimulatory G protein of adenylate cyclase. No difference in the capacity to generate cAMP in the presence of an inhibitor of cAMP degradation exists in CT-sensitive compared to CT-insensitive non-SCLC. These studies therefore underscore the value of defining the mechanism of CT-mediated growth inhibition in lung carcinoma cells, as the mechanism can be related neither to possessing a receptor for CT nor to the increase in cAMP evoked by the agent.

Clinical trials utilizing a bacterial toxin targeted via IL2 (the diphtheria-toxin-IL2 fusion protein $DAB_{486}IL2$) and a ricin-derived immunotoxin (IgG-RFB4-SMPT-dgA) have been developed to explore the clinical usefulness of macromolecular cytotoxins in cutaneous T-cell lymphoma and relapsed CD22 positive B cell lymphoma, respectively. Pharmacodynamic evaluation of toxin effect in the targeted cell population will be a major focus of these studies, in addition to clinical response.

Objectives:
1. To determine the mechanism by which cholera toxin inhibits the growth of lung carcinoma cell lines.

2. To develop analogs or derivatives of cholera toxin which will lead to novel therapies for $G_{M1}(+)$ neoplasms.

3. To develop novel agents for therapeutic use which utilize cholera toxin A chain expressed intracellularly after delivery with a targeted vector system.

4. To engage in clinical studies with targeted derivatives of bacterial or plant toxins to understand the pharmacokinetic and pharmacodynamic correlates of clinical response to this category of agent.

Methods:
1. Cell culture, metabolic labelling with radioisotope, extraction, chromatography, and radioautographic or scintillation counting of radioactivity.

2. Fluorimetric assay of intracellular calcium concentration.

3. Expression cloning in bacterial vectors.

4. Clinical trial with fluorescent activated cell sorting and immunohistochemical study of tissues/cells.

Major Findings:
1. The effect of cholera toxin (CT) on the growth of 12 small cell lung carcinoma (SCLC) and 14 non-small cell lung carcinoma (NSCLC) cell lines was determined. CT inhibited the growth of nine SCLC cell lines (IC_{50} 27 - 700 ng/ml), all of which had abundant expression of G_{M1} ganglioside, the surface receptor for CT.

2. CT-resistant SCLC had greatly decreased G_{M1} expression. In contrast, CT inhibited four of 14 NSCLC cell lines. Eight of 11 resistant NSCLC had levels of G_{M1} comparable to those of sensitive NSCLC or SCLC, as detected by flow cytometry of fluorescein-labelled CT-B subunit or binding of [^{125}I]-CT.

3. NCI-H661 (NSCLC; $G_{M1}(+)$; CT-resistant) and NCI-H226 (NSCLC; $G_{M1}(+)$; CT-sensitive) had similarly increased levels of intracellular cAMP in the presence of isobutylmethylxanthine (IBMX), but the growth of NCI-H661 remained uninfluenced by CT in the presence of IBMX.

4. We conclude that the expression of surface G_{M1} and the capacity to increase cAMP after CT treatment does not invariably result in NSCLC cell line growth inhibition by CT. In contrast, all SCLC cell lines thus far examined were growth-inhibited if G_{M1} is comparably expressed.

Significance:
1. CT-mediated growth inhibition of lung carcinoma cell lines may suggest novel therapeutic directions in lung carcinomas. Since CT has a B subunit which is devoid of toxicity and an A subunit which mediates ADP-ribosylation of various intracellular targets including G_S, the positive regulator of adenylate cyclase, one can imagine the possibility of attaching the B-subunit to new entities to disrupt the lung tumor cell membrane or introducing the toxic A subunit through more tumor-specific strategies.

2. The clinical development of agents of this class will present a challenge related to understanding whether the targeting function is absolutely required to interact with all cells to achieve antitumor effect. In addition, which particular type of toxicity-causing function (protein synthesis inhibition, membrane damage, etc.) will yield the best therapeutic index is unclear. This project will address these issues in sequence, as agents for different disease categories become available.

Proposed Course:
1. Continue studies on the mechanism of cholera-toxin-mediated inhibition of cell growth.

2. Develop animal models to assess the feasibility of treating patients with either cholera toxin B subunit or holotoxin.

3. Develop a clinical protocol to assess the feasibility of cholera-toxin-based strategies in human lung carcinoma.

4. Engage in clinical trials with targeted toxins, as available from extra or intramural sources. Particular attention will be given to the biologic interaction in patients of the targeted toxin with some feature of tumor cell biology, in addition to examining pharmacokinetic measures and clinical indicators of response.

Publications:
Viallet J, Sharoni Y, Frucht H, Jensen RJ, Minna JD, Sausville EA. Cholera toxin inhibits signal transduction by several mitogens and the in vitro growth of human small cell lung cancer. J Clin Invest 1990;86:1904-12.

Sausville E, principal investigator. A pilot study to examine the safety and pharmacodynamics of an IL-2 diphtheria toxin fragment fusion protein (DAB486IL2) in patients with cutaneous T-cell lymphoma. NCI Clinical Protocol 91-C-170 (CPB 277).

Sausville E, principal investigator. A phase I study of continuous infuxion immunotoxin IgG-RFB4-SMPT-dgA in refractory CD22-positive B-cell lymphoma. NCI Clinical Protocol 91-C-176 (CPB 276).

Patent Application:
Sausville E, Viallet J, Minna J. US Patent Application No. 7/438,643: Inhibition of Malignant Cells Having G_{M1} Ganglioside Sites by Administration of Cholera Toxin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06529-01 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Preclinical Pharmacology of Protein Kinase Antagonists

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Sausville	Senior Investigator	CPB, COP, DCT, NCI
Others:	G. Kaur	Biologist	CPB, COP, DCT, NCI
	P. Worland	Visiting Associate	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
DTP, DCT, NCI, NIH (E. Acton); LP, DCBD&C, NCI, NIH (M. Stetler-Stevenson).

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Molecular Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Protein kinase activity is of clear importance to the action of a variety of growth factors and cellular oncogenes. Therefore, interruption of protein kinase activity could lead to novel therapeutic agents. Three classes of compounds have been studied, all of which have the proven capacity to inhibit either serine/threonine or tyrosine kinases.

AG17 and AG592 are tyrphostin analogs of erbstatin. These compounds both inhibit the growth of a number of breast carcinoma cell lines with IC_{50}s of approximately 0.5-5 uM. The inhibition of cell growth does not correlate with the expression of known tyrosine kinase activities in this panel of cell lines.

UCN-01 and UCN-02, derivatives of staurosporin, inhibit the growth of lung and prostate carcinoma cell lines, with IC_{50}s between 0.1 and 1 uM. These compounds were found to have broad but non-selective cytotoxicity in the NCI-Developmental Therapeutics Program Screen for active compounds. The mechanism of their growth-inhibitory effect is not clear.

L86-8275 is a flavone with known capacity to inhibit both EGF-receptor kinase and cAMP-dependent kinase. It inhibited growth of breast carcinoma cell lines with IC_{50}s of approximately 0.1 uM. Mechanistic studies with this compound reveal that it inhibits macromolecular synthesis of DNA, RNA, and protein within eight hours of addition; it causes a block in the G_2 phase of the cell cycle; its growth-inhibitory effect is reversible. Thus, its activity and potency distinguishes it from other previously described flavones.

Subsequent studies will focus on whether the above growth-inhibitory effects are relatable to the inhibition of kinase activity in each of these cases, and whether there is augmentation of activity of conventional cytotoxic agents by these compounds.

U.S. GOVERNMENT PRINTING OFFICE 1991 O- 864 939

Objectives:
1. To determine if drugs which inhibit protein kinase activity may lead to novel anti-neoplastic treatments. The specific kinase activities and drugs to be targeted here are:
(a) TYROSINE PHOSPHORYLATION. Compounds to be studied include the flavone L868275 and the tyrphostins AG-17 and AG-592.
(b) PROTEIN KINASE C. Staurosporin analogs UCN-01 and UCN-02, which have been proposed to be selective for protein kinase C-stimulated signals, will be studied.
The listed compounds will be examined in breast, prostate, and lung cell lines. The specific question to be addressed will be the mechanism by which growth inhibition is effected.

2. To determine the concentration x time relationship for cell growth inhibition by each of the listed classes of compounds. This data, combined with animal toxicology data, will allow projection of concentrations to be targeted in clinical trial.

3. To determine the interaction of the listed drug classes with conventional categories of antineoplastic agents in drug-sensitive and -resistant cell lines.

Methods Employed:
1. Tissue culture, with assessment of the effect of drug on cell growth by metabolic labelling and colorimetric assays of viability including the MTT assay.

2. Enzymatic assay of kinase activity employing transfer of phosphate from ATPγ $^{32}PO_4$ to model substrates.

3. Flow-cytometric assay of cell cycle distribution.

Major Findings:
1. Of a total of 52 analogs of erbstatin ("tyrphostins") screened, only two, AG17 and AG592, demonstrated growth-inhibitory activity at <10 μM in a panel of nine breast carcinoma cell lines. No obvious correlation with the pattern of growth inhibition by these compounds and the expression of particular classes of tyrosine kinase activities in these cell lines has emerged.

2. UCNO1 and UCNO2 are growth-inhibitory for the non-small cell lung carcinoma cell lines NCI-H460 and NCI-H522 with IC_{50} of ~1 and ~5 μM, respectively. These compounds are also growth-inhibitory for the small cell lung carcinoma cell line NCI-N592 and the prostate carcinoma cell line PC3 at ~0.1 and ~1μM, respectively.

3. The flavone L868275 inhibits all breast carcinoma cell lines tested thus far with an IC_{50} of ~0.1 μM. It is reversible after treatment for 24 hours and is without overt toxicity for non-growing cells. Although this compound can inhibit tyrosine kinase activity of isolated breast carcinoma cell line membranes with respect to synthetic substrates, the estimated IC_{50} for this reaction is at least two orders of magnitude higher than the concentration at which activity in cells is observed. The form of the inhibition is competitive with ATP. L868275 inhibits globally the incorporation of thymidine, leucine, and uridine into macromolecules, and the onset of this effect is within 3-6 hours of addition at the IC_{50}. This compound causes a block in G_2; it possibly causes a block at the G_1/S border.

Proposed Course:
1. Determine relationship of tyrphostin inhibition of cell growth to tyrosine kinase inhibition.

2. Determine the relationship of UCNO1 and UCNO2 inhibition of cell growth to protein kinase-C inhibition.

3. Examine the capacity of L868275 to inhibit specific kinase reactions in living cells.

4. Determine the minimal necessary contact time of all three classes of compounds to effect growth inhibition.

5. Establish whether additive or synergistic growth-inhibitory effects occur with other classes of cytotoxic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06530-01 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
G-proteins and their Effectors as Targets for Cancer Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.:	E. Sausville	Senior Investigator	CPB, COP, DCT, NCI
Others:	P. Worland	Visiting Associate	CPB, COP, DCT, NCI
	D. Dobrzanski	Clinical Associate	MB, COP, DCT, NCI
	C. Myers	Senior Investigator	CPB, COP, DCT, NCI
	J. Trepel	Senior Investigator	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Clinical Pharmacology Branch, NCI (C. Myers, J. Trepel); NINDS, NIH (J. Battey).

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Molecular Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.1	2.1	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Guanine-nucleotide binding proteins (G-proteins) transduce signals from cell surface receptors coupled to adenylate cyclase, phospholipases, and ion channels. This laboratory has sought to develop drugs which alter the action of G-proteins or their effectors in human lung and prostate carcinoma cell lines. These studies will lead to clinical trials to extend these studies to human subjects.

Bombesin agonist receptors, coupled to phospholipase-C through a pertussis toxin-insensitive G-protein, have been studied in lung tumor cell lines. We have found that bombesin-peptide receptors are of two types: a form with higher affinity for [Tyr^4] bombesin than for neuromedin B, and a second type with higher affinity for neuromedin B. These receptors are expressed in approximately 80% of human small cell lung carcinoma (SCLC) cell lines, and in approximately 20% of non-SCLC cell lines. The structure of human bombesin receptors led to a consideration of features of the molecules important for its action. Mastoparans are wasp venom toxins with structural similarity to the third intracytoplasmic loop of the bombesin receptor(s). Mastoparans inhibit small cell lung carcinoma cell growth, perhaps by altering G-protein-receptor coupling. A transfected neuromedin B receptor-bearing cell line has been developed that will allow the further definition of the mechanism of growth modulation by bombesin-related peptides.

The stimulatory protein of adenylate cyclase, G_s, causes increased cyclic AMP. Increased cAMP inhibits prostate carcinoma cell growth in vitro. A clinical protocol in relapsed prostate carcinoma has been developed to examine whether increases in cyclic AMP effected by the phosphodiesterase inhibitor pentoxifylline leads to stabilization or improvement of disease.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864 939

Objectives:
1. Definition of phospholipase-coupled G-proteins in carcinoma cell lines.

2. Pharmacologic manipulation of receptor coupling to G-proteins.

3. Modification of G-protein effector functions.

Methods:
1. cDNA cloning.

2. Protein expression in procaryotic and eucaryotic vectors.

3. Peptide synthesis.

4. Fluourescence assay of intracellular calcium concentration.

5. Metabolic labelling of tissue culture cells.

6. Clinical trial using phosphodiesterase inhibitor.

Major Findings:
1. A human small cell lung carcinoma (SCLC) cell line responds with increased intracellular calcium to both [Tyr^4]-bombesin and neuromedin B, but shows differential sensitivity to the inhibitor [D-Phe^{6}]BN(6-13)ethylester, which inhibits the bombesin but not neuromedin B responses. Thus, two different bombesin receptor subtypes could be expressed in the same cell line. This point was proven by the derivation of distinct human clones for a [Tyr^4] bombesin-preferring (GRP-R) and a neuromedin-B-preferring (NMB-R) receptor from this cell line. The GRP-R is expressed in 4/7 and the NMB-R is expressed in 3/7 SCLC cell lines.

2. The murine NMB-R gene was introduced into a receptor-negative cell line. The transfected receptor mediates increases in intracellular CA^{2+}, phosphatidylinositol turnover, and thymidine incorporation when the cells are confluent. When cells are exponentially growing in the presence of the ligand, there is a slower growth rate compared to control. Thus, neuromedin B, when acting through its receptor, can be growth-stimulatory or -inhibitory depending on the context of its presentation to cells.

3. Consideration of the primary sequence of the GRP-R and NMB-R described above in (1) suggests a similarity between the third intracytoplasmic loop of the receptor and the structure of mastoparans. Mastoparans are wasp venom toxins known to activate guanine nucleotide binding proteins by non-receptor-mediated interaction. A series of mastoparan derivatives synthesized in our laboratory inhibits the growth of cells both expressing and not expressing the GRP-R and NMB-R with IC_{50}'s between 1 and 10 μM.

Significance:
1. These studies clearly demonstrate that efforts to develop a *specific* bombesin receptor antagonist or to interrupt the action (e.g., by antibodies) of a *single* class of bombesin agonists will not be useful in a significant proportion of lung carcinomas, because these cells express *multiple* similar growth factor receptors.

2. Efforts should focus on the development of approaches that would antagonize pathways common to the action of *multiple* growth factors.

3. The neuromedin B receptor is demonstrated to be growth-stimulatory or growth-inhibitory, depending on the context in which it is stimulated.

Proposed Course:
1. Continue to define intracellular substrates of growth factors with G-protein coupled transduction.

2. Define in detail the mechanism of action of mastoparan-induced cell growth inhibition.

3. Define in detail the important mediators of neuropeptide-mediated growth modulation.

4. Extend to other tumor systems, notably prostate carcinoma, efforts to define the effect of modulating G-protein function on growth. This will be attempted for effectors coupled to adenylate cyclase as well as for effectors coupled to phospholipase activation.

Publications:
Lebacq-Verheyden AM, Trepel J, Sausville EA, Battey JF. Bombesin and gastrin releasing peptide: neuropeptides, secretogogues, and growth factors. In: Sporn M, Roberts A, eds. Handbook of experimental pharmacology; vol. 95 II. Berlin: Springer-Verlag, 1990,71-124.

Sausville E, principal investigator. Chemotherapy in metastatic prostate carcinoma refractory to hormonal manipulation and suramin: a randomized phase II study with crossover of weekly doxorubicin compared to pentoxifylline/thioTEPA. NCI Clinical Protocol 91-C-120 (CPB-270).

Corjay MH, Dobrzanski DJ, Way JM, Viallet J, Shapira H, Worland P, Sausville EA, Battey JF. Two distinct bombesin receptor subtypes are expressed and functional in human lung carcinoma cells. J Biol Chem (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06719-03 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Signal Transduction Events and the Regulation of Cell Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Trepel	Senior Investigator	CPB, COP, DCT, NCI
Others:	Y.-J. Bang	Visiting Fellow	CPB, COP, DCT, NCI
	W.-K. Kang	Visiting Fellow	CPB, COP, DCT, NCI
	W.G. Fang	Visiting Fellow	CPB, COP, DCT, NCI
	F. Pirnia	Microbiologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Laboratory of Chemoprevention, NCI, NIH (S.-J. Kim, M.B. Sporn); Department of Urology, Stanford University Medical Center (D. Peehl); Department of Medical Neurosciences, Walter Reed Army Institute of Research (M. Koenig); Laboratory of Pathology, NCI, NIH (M. Tsokos); Digestive Diseases Branch, NIDDK, NIH (R.T. Jensen)

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Cell Signalling and Oncogenesis Group/Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project is designed to increase our understanding of the biology of prostate cancer and to develop a new approach to the treatment of advanced prostatic cancer through the study of the signal transduction events regulating the growth of human prostate carcinoma cell lines. This work is currently focused on (1) effects of cAMP on growth and differentiation, and (2) cytotoxicity through activation of P_2-purinergic receptors. We found that elevation of intracellular cAMP is highly growth-inhibitory to all prostate carcinoma cell lines tested, and induces neuronal morphology in two of four cell lines. All of the cell lines expressed one or more markers of neuroendocrine differentiation, including dense core granules, NSE, S100, and neurofilament proteins. In collaboration with the Molecular Oncology Group, CPB, we tested for the expression and activity of the neuroendocrine marker $pp60^{c-src}$ and found high levels of src kinase activity that were increased after cAMP treatment. In collaboration with the Tumor Cell Biology Section, CPB, we found that two ansamycin antibiotics reported to inhibit src activity were highly potent cytotoxic agents in all prostatic carcinoma cell lines. Studies of the mechanism of cAMP-induced growth inhibition demonstrated that cAMP induces the secretion of bioactive TGF-Beta2, an increase in TGF-Beta2 transcription, and activation of cAMP response elements in the TGF-Beta2 promoter. P_2-purinergic receptor studies demonstrated that androgen-independent prostate carcinoma cell lines express P_2-purinergic receptors that are coupled to phospholipase C activation, acute Ca^{2+} mobilization, the induction of prolonged Ca^{2+} oscillations, growth arrest, and cell death. In the androgen-sensitive cell line LNCaP, however, P_2 agonists did not stimulate phospholipase C activity, did not induce Ca^{2+} release, and did not inhibit growth, while both cell types expressed specific P_2 receptors, with comparable K_d and B_{max}. These data strongly implicate phospholipase C activation and prolonged Ca^{2+} mobilization in the growth-inhibitory and cytotoxic effect of P_2-purinergic receptor agonists.

Objectives:
The objective of this project is to increase our understanding of the biology of prostate cancer and to develop a new approach to the treatment of advanced prostatic cancer by studying the signal transduction events regulating the growth of human prostate carcinoma cell lines. This work is currently focused on two aspects of growth regulation in human prostate carcinoma cells: (1) neuroendocrine differentiation and growth inhibition through elevation of intracellular cAMP, and (2) cytotoxicity through activation of phospholipase C-linked P_2-purinergic receptors.

Methods Employed:
1. Signal transduction studies are performed using spectrofluorometric analysis of intracellular Ca^{2+} levels, high-pressure liquid chromatographic analysis of phosphatidylinositol turnover, radioligand binding assays, and ACAS (Adherent Cell Analysis and Sorting) image analysis of single-cell Ca^{2+} transients and intracellular Ca^{2+} oscillations.

2. Studies of neuroendocrine markers are performed using immunocytochemistry and Western blot analysis, light microscopy, and transmission electron microscopy. Expression and activity of $pp60^{c\text{-}src}$ are studied using Western blot and immune complex kinase assay. Studies of TGF-β are performed using enzyme-linked immunosorbent assay, Western blot, Northern blot, CCL-64 binding assay, transient transfections, and CAT assays.

Major Findings:
1. P_2-purinergic receptors are specifically coupled to phospholipase C activation, acute Ca^{2+} mobilization, prolonged intracellular Ca^{2+} oscillations, and cell death, in androgen-independent prostate carcinoma cells.
In the androgen-sensitive cell line LNCaP, P_2-purinergic receptors are expressed, but completely uncoupled from phospholipase C, Ca^{2+} mobilization, and growth inhibition.

2. Cyclic AMP analogs and phosphodiesterase inhibitors, including the clinically available drug pentoxiphylline, are markedly cytotoxic to all prostate carcinoma cell lines tested. This finding is being incorporated into a new phase II trial using suramin + pentoxiphylline/thiotepa versus suramin + doxorubicin in hormone/suramin-refractory prostate cancer.

3. Human prostate carcinoma cell lines express certain neuroendocrine markers *de novo*, and expression of neuroendocrine markers can be markedly upregulated by elevation of intracellular cAMP. This biphenotypic potential in prostate cancer (epithelial and neuroendocrine) is similar to the emerging concept in lung cancer that all lung cancers, including small cell lung cancer, are derived from a single multipotential cell.

4. Human prostate carcinoma cell lines express high levels of the protooncogene product $pp60^{c\text{-}src}$. Herbimycin and geldanamycin, two ansamycin antibiotics reported to inhibit *src* activity, are highly potent cytotoxic agents in all prostatic carcinoma cell lines.

5. In collaboration with the Laboratory of Chemoprevention, we have demonstrated a new molecular mechanism for the growth-inhibitory effect of cyclic AMP: in the human prostate carcinoma cell line PC-3, cyclic AMP induces the secretion of bioactive TGF-β2, an increase in TGF-β2 transcription, and activation of cyclic AMP response elements in the TGF-β2 promoter.

Publications:

Lebacq-Verheyden A-M, Trepel JB, Sausville EA, Battey JF. Bombesin and gastrin releasing peptide: neuropeptides, secretogogues, and growth factors. In Roberts AB, Sporn MB, eds. Peptide growth factors and their receptors. Handbook of experimental pharmacology; vol. 95 II. Berlin: Springer-Verlag, 1990;71-124.

Myers C, Trepel J, Neckers L, Linehan M. Potential roles of growth factors, their agonists and antagonists in adjuvant therapy. In: Sixth international conference on the adjuvant therapy of cancer. Philadelphia: WB Saunders, 1990,14-20.

Park J-G, Frucht H, LaRocca RV, Bliss DP, Jr, Kurita Y, Chen T-R, Henslee JG, Trepel JB, Jensen RT, Johnson BE, Bang Y-J, Kim J-P, Gazdar AF. Characteristics of cell lines established from human gastric carcinoma. Cancer Res 1990;50:2773-80.

Sausville EA, Trepel JB, Moyer JD. Inhibitors of bombesin-stimulated intracellular signals: interruption of an autocrine pathway as a therapeutic strategy. Prog Clin Biol Res 1990;354A:193-207.

Sharoni Y, Viallet J, Trepel JB, Sausville EA. Effect of guanine and adenine nucleotides on bombesin-stimulated phospholipase C activity in membranes from Swiss 3T3 and small cell lung carcinoma cells. Cancer Res 1990;50:5257-62.

Patent Application:

Trepel JB, Fang W-G, Pirnia F, Myers CE. US Serial No. 509,183: Use of Purinergic Receptor Agonists as Antineoplastic Agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06721-03 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Analysis of Drug Resistance by Flow Microfluorometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: J.B. Trepel	Senior Investigator	CPB, COP, DCT, NCI
Other: F. Pirnia	Microbiologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Medicine Branch, NCI, NIH (K. Cowan, K. Dixon, C. Allegra, P. Johnson, T. Fojo, C. Herzog, S. Bates, P. Elwood, K.-N. Chung, J. Grem); Georgetown University Medical Center (R. Glazer, S. Ahmad).

LAB/BRANCH

Clinical Pharmacology Branch

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NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We have established assays to examine expression of the drug resistance phenotype using flow cytometric analysis of intact single cells. There are significant advantages to this approach, including the ability to derive rapid, semiquantitative data on tens of thousands of cells and the ability to study the heterogeneity of drug resistance within clinical specimens. The techniques developed include analysis of P-glycoprotein expression using the monoclonal antibody MRK-16, analysis of the expression of thymidylate synthetase using a monoclonal antibody developed by Dr. Patrick Johnson, and analysis of the expression of the folate binding protein and properties of the reduced folate transporter, using fluoresceinated methotrexate. In the past year, we have used these techniques in a wide variety of applications in collaborative studies. These applications include examination of the reduced folate transporter in breast cancer cell lines; expression of P-glycoprotein in breast cancer cell lines transfected with the mdr 1 gene and the gene for protein kinase C alpha; comparison of flow cytometry with Western blot, Northern blot, and RNA in situ for the analysis of P-glycoprotein in colon cancer cell lines expressing clinically relevant levels of P-glycoprotein and drug resistance; examination of the expression of the folate binding protein in CHO, MCF-7, and L cells transiently transfected with a folate binding protein expression vector; analysis of thymidylate synthetase expression in colon cancer cell lines; and analysis of the cell cycle phase distribution after treatment of human colon cancer cells with the novel antimetabolite MRPP.

Objectives:
The objective of this project is to utilize the unique capabilities of flow cytometry for analysis of the expression of markers of the drug resistance phenotype. While most of these studies are currently focused on in vitro regulation of drug resistance, an important goal of this project is the application of these techniques to the study of drug resistance and resistance reversal in clinical specimens.

Methods Employed:
Immunocytochemistry, flow cytometric analysis.

Major Findings:
1. In a collaboration with Dr. Cowan and Dr. Dixon of the Medicine Branch, we demonstrated that it is possible, in monolayer cells, to distinguish cells that do express reduced folate transporter from cells that do not. These studies demonstrate the feasibility of flow cytometric analysis of the expression of the reduced folate transporter in solid tumors.

2. In a collaboration with Dr. Herzog, Dr. Fojo, and Dr. Bates of the Medicine Branch, we demonstrated that flow cytometric analysis is a rapid, sensitive, semiquantitative technique for analyzing P-glycoprotein expression; this technique can readily detect P-glycoprotein in cells expressing clinically relevant levels of resistance.

3. In a collaboration with Dr. Glazer and Dr. Shakeel of Georgetown University Medical Center and Dr. Cowan of the Medicine Branch, we found that transfection of P-glycoprotein-expressing MCF-7 breast cancer cells with protein kinase Cα resulted in an increase in drug resistance with little modulation of P-glycoprotein expression as detected by flow analysis.

Publication:
Yu G, Ahmad S, Aquino A, Fairchild CR, Trepel JB, Ohno S, Suzuki K, Tsuruo T, Cowan KH, Glazer RI. Transfection with protein kinase Cα confers increased multidrug resistance to MCF-7 cells expressing P-glycoprotein. Cancer Commun 1991;3:181-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06722-03 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of IL-6-Mediated Myeloma Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.P. Nordan	Senior Investigator	CPB, COP, DCT, NCI
Others:	F. D'Alessandro	Visiting Associate	CPB, COP, DCT, NCI
	G. Schwab (through 4/91)	Visiting Fellow	CPB, COP, DCT, NCI
	M. Loeloff	Biologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Genetics, DCBD, NCI, NIH (B. Mock); Laboratory of Cellular and Developmental Biology, NIDDK, NIH (A. Greenberg, C. Landos, A. Kimmel); Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam (L. Aarden).

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

A primary goal of this laboratory is to increase our understanding of IL-6-mediated myeloma growth with the ultimate goal of identifying areas which may provide targets for therapeutic intervention. We have performed studies aimed at (1) structural characterization of the IL-6 receptor and (2) analysis of the mechanisms by which these cells escape the requirement for this growth factor.

Recent studies in our lab have also revealed that, in addition to autocrine growth, the progression to IL-6-dependence can be mediated by a nonautocrine mechanism. We have gained insight into this mechanism by using somatic cell hybrid experiments in which IL-6-dependence is restored by the introduction of normal (wild type) DNA via cell fusion. The introduction of wild type DNA via cell fusion presumably reconstitutes a negative growth/tumor suppressor gene which is normally regulated by IL-6. Presumably, such a gene would be expected to play a role in IL-6 signal transduction. We are now in the process of identifying this gene. We have created a panel of IL-6-dependent rat-mouse somatic cell hybrids. This panel of cells will allow us to identify the chromosome on which this gene resides and, eventually, to clone the gene responsible for transition to autonomous growth.

We have continued the structural characterization of the IL-6 receptor. Although published reports have identified only one IL-6-binding protein, gp80, in the receptor complex, affinity crosslinking studies in our laboratory have identified a 150 kDa crosslinked complex that contains a 130 kDa IL-6-binding membrane glycoprotein. Two other complexes with masses of 100 and 120 kDa are composed of gp80 crosslinked to one and two molecules of IL-6, respectively. Our results indicate that (1) the 130 kDa molecule associates directly with IL-6 and (2) the functional receptor complex may consist of dimers of gp80 plus two molecules of IL-6 and at least one gp130 molecule. We are also pursuing the identity of a protein found crosslinked to IL-6 in a 70 kDa complex.

We have established that, in IL-6-dependent murine myeloma cells, the mechanism of death after IL-6 withdrawal is apoptosis. Chronic exposure to phorbol esters (1) delays apoptosis and (2) enhances IL-6-mediated proliferation, thus implicating protein kinase C as a mediator of an anti-growth pathway.

Objectives:
A primary goal of this laboratory is to increase our understanding of IL-6-mediated myeloma growth with the ultimate goal of identifying areas which may provide targets for therapeutic intervention. Previous studies by the principal investigator identified and characterized a cytokine, now called interleukin 6 (IL-6), that supported the in vitro growth of early murine plasma cell tumors (myelomas). It has subsequently been shown that human myelomas also proliferate in vitro in response to IL-6. The early mouse myelomas require an inflammatory peritoneal oil granuloma for in vivo growth and fail to proliferate in vivo in the absence of this microenvironment. The eventual progression of these tumors to a fully malignant phenotype in vivo is associated with a concomitant transition to IL-6-independent growth in vitro. Our working hypothesis is that the early tumor cells require IL-6 for in vivo growth (supplied by the local microenvironment), with the subsequent loss of IL-6-dependence representing a key step in the progression to a fully malignant tumor. Since the establishment of this laboratory within the Tumor Biology Section, we have performed studies aimed at (1) elucidating the mechanisms of IL-6-mediated growth of human and murine myelomas (the characterization of the IL-6 receptor, in particular), and (2) analyzing the mechanisms utilized by these cells to escape the requirement for this growth factor.

Methods Employed:
To determine if IL-6-independent tumor cells have become autonomous by an autocrine or non-autocrine mechanism, supernatants are tested for IL-6 using a sensitive bioassay and cells are directly tested for IL-6 production via coculture with highly sensitive IL-6-dependent cells. Cells are also evaluated for expression of IL-6 mRNA by Northern hybridization and by reverse transcription and PCR amplification. Characterization of the IL-6 receptor complex has employed the use of covalent or metabolic radiolabeling of cells, cytokines, and antibodies. Monoclonal antibodies directed against members of the IL-6 receptor complex are developed, produced, and purified in this laboratory. Affinity crosslinking procedures and immunoprecipitations are used to identify the complexes which are present within the IL-6 receptor complex. Our experiments also employ construction and expression of recombinant genes in eukaryotic cells, including members of the IL-6 receptor.

Major Findings:
1. The transition of myelomas from IL-6-dependent growth to autonomous (IL-6-independent) growth can involve either autocrine or non-autocrine mechanisms. Non-autocrine autonomous rat and murine myelomas can be restored to IL-6-dependence by the introduction of normal (wild type) DNA. The ability of normal DNA to restore IL-6-dependence suggests that the transition to non-autocrine autonomous growth may involve the functional loss of a putative growth regulatory (tumor suppressor) gene.

2. We have shown that, in addition to the known 80 kDa IL-6-binding receptor molecule, gp80, a 130 kDa member of the IL-6 receptor complex also directly associates with IL-6. In addition, two molecules of IL-6 are found crosslinked to gp80, suggesting that the functional complex consists of two gp80 molecules, two IL-6 molecules, and at least one 130 kDa chain.

3. We have established that, in IL-6-dependent murine myeloma cells, the mechanism of death after IL-6 withdrawal is apoptosis. Chronic exposure to phorbol esters (1) delays apoptosis and (2) enhances proliferation in the presence of IL-6, thus implicating protein kinase C as a possible mediator of a putative anti-growth pathway.

4. In collaboration with the members of the Laboratory of Cellular and Developmental Biology, we have found that IL-6 appears to be involved in the regulation of lipoprotein lipase activity in adipocytes and, thus, may play a role in cachexia.

Publications:

Colamonici OR, D'Alessandro F, Diaz MO, Gregory SA, Neckers LM, Nordan R. Characterization of three monoclonal antibodies that recognize the interferon alpha 2 receptor. Proc Natl Acad Sci USA 1990;87:7230-4.

Siegall C, Schwab G, Nordan RP, FitzGerald DJ, Pastan I. Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells. Cancer Res 1990;50:7786-8.

Riekmann P, D'Alessandro F, Nordan RP, Fauci AS, Kehrl JH. IL-6 and tumor necrosis factor-alpha. Autocrine and paracrine cytokines involved in B cell function. J Immunol 1991;146:3462-8.

Schwab G, Siegall CB, Aarden LA, Neckers LM, Nordan RP. Characterization of an interleukin-6-mediated autocrine growth loop in the human multiple myeloma cell line, U266. Blood 1991;77:587-93.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06723-03 CP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Subunits of the Interleukin 2 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	O.R. Colamonici	Visiting Associate	CPB, COP, DCT, NCI
Others:	L.M. Neckers	Senior Investigator	CPB, COP, DCT, NCI
	A. Rosolen	Visiting Fellow	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
NCI-Navy Oncology Branch, COP, DCT, NCI, NIH; Pediatric Branch, COP, DCT, NCI, NIH.

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Tumor Cell Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.5	.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three forms of the IL-2 receptor have been reported: low, intermediate, and high affinity. Each form corresponds to the expression of two different receptor subunits: p55-alpha chain and p75-beta chain. Sole expression of p55 yields low affinity receptors, sole expression of p75 yields intermediate affinity receptors, and co-expression of both subunits yields high affinity receptors. Resonance energy transfer studies have suggested the presence of another peptide subunit of the IL-2 receptor with an approximate molecular mass of 95,000 Daltons. The requirement of another protein to form a functional IL-2 receptor is supported by experiments in which fibroblasts transfected with p75 cDNA express the protein on the surface but do not bind IL-2, whereas transfected T-cells express p75 and bind IL-2.

We have discovered and characterized the presence of a putative new IL-2 receptor subunit with a molecular mass of 95,000-110,000 Daltons. This subunit is present in low, intermediate, and perhaps, in high affinity receptor complexes. Its presence is required to form intermediate affinity receptors with p75, but it is not necessary to obtain IL-2 binding to p55. The p95 protein, termed the gamma subunit of the IL-2 receptor, is not ICAM1, as determined by monoclonal antibody competitor studies.

This project was terminated in November 1990 when Dr. O.R. Colamonici left the NIH to go to the University of Chicago.

Major Findings:

1. We have described a novel third subunit of the IL-2 receptor, designated the γ subunit.

2. The γ subunit of the IL-2 receptor is required for binding of IL-2 to intermediate affinity, but not to low affinity, IL-2 receptors.

Publications:

Colamonici OR, Neckers LM, Rosolen A. Putative γ subunit of the IL2 receptor is detected in low, intermediate and high affinity IL2 receptor-bearing cells. J Immunol 1990:145:155-60.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06728-03 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Tyrosine Protein Kinases in Hematopoietic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	I.D. Horak	Senior Investigator	CPB, COP, DCT, NCI
Others:	A.L. Burkhardt	Microbiologist	CPB, COP, DCT, NCI
	Z.-H. Li	Visiting Fellow	CPB, COP, DCT, NCI
	B. Matoskova	Visiting Fellow	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Tumor Virus Biology, DCE, NCI, NIH (E.M. Horak, J.B. Bolen); Metabolism Branch, DCBDC, NCI, NIH (A. Grant, T.A. Waldmann); Experimental Immunology, DCBDC, NCI, NIH (P.J. Lucas, R.E. Gress).

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Tyrosine Protein Kinases Group/Biophysical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our project has three foci: (1) to characterize the regulation and function of the src family of tyrosine protein kinases (TPK's) in normal hematopoietic cells; (2) to characterize the regulation and function of the src family of TPK's in retrovirus-associated diseases such as ATLL; and (3) to utilize TPK inhibitors to block T-cell activation and proliferation signals. We hope to apply the results of these three foci to the treatment of T-cell neoplastic diseases.

(1) We have prepared numerous unique biochemical reagents for our studies and have analyzed in detail the expression of c-src, c-yes, fyn, lck, hck, lyn, and c-fgr in hematopoietic cells. The results of these studies have defined the expression of the src family of TPK's in fresh human hematopoietic cells. Protein kinases of the src family, such as lck, have been shown to be important for the generation of IL-2-dependent proliferation signals. Using IL-2-dependent T-cell lines, we further characterized signal transduction pathway(s) initiated by the binding of IL-2 to the high affinity IL-2 receptor. We showed that IL-2-induced signal transduction in T-cells is mediated by $p56^{lck}$.

(2) Recently, we have found that the normal pattern of TPK expression is disrupted in HTLV-I-associated adult T-cell leukemia and lymphoma during the transition from IL-2-dependence to IL-2-independence. The level of $p56^{lck}$ protein is decreased 10-100-fold, with proportional coordinate changes in the expression of lck RNA in IL-2-dependent ATLL cell lines, as well as in normal T lymphocytes acutely infected with HTLV-I *in vitro*. The increased expression of $p56^{lyn}$ protein observed in these ATLL cell lines correlates with the increased abundance of lyn steady-state RNA levels. Preliminary results from several ATLL patients suggest a correlation between the ratio of $p56^{lck}/p56^{lyn}$ expression and the response to anti-TAC monoclonal antibody therapy. In normal T-cells, surface protein CD4 is an integral membrane glycoprotein noncovalently associated with the TPK $p56^{lck}$. We have shown that both HIV-1 and the virus glycoprotein gp 120 fail to elicit detectable $p56^{lck}$-dependent TPK activation.

(3) Deregulation of TPK's has been associated with a number of human cancers, and specific inhibitors of these enzymes are attractive synthetic targets. A number of small molecules have been shown to inhibit the binding of tyrosine-containing substrates to the enzymes. Specific inhibitors of TPK's not only provide useful tools for studying the function of these enzymes but also offer new therapeutic approaches for treatment of certain cancers.

Objectives:
The objectives of this project are (1) to characterize the regulation and function of the *src* family of tyrosine protein kinases (TPK's) in normal hematopoietic cells; (2) to characterize the regulation and function of the *src* family of TPK's in retrovirus-associated diseases such as ATLL; and (3) to utilize tyrosine protein kinase inhibitors to block T-cell activation and proliferation signals and apply these results to the treatment of T-cell neoplastic diseases.

Methods Employed:
1. A novel technique using TrpE fusion protein to generate polyclonal antibodies.

2. Techniques including visualization of specific proteins by Western blotting and kinase assays.

3. Techniques including the use of in vitro studies and DNA cell cycle analysis.

Major Findings:
1. The generation of new molecular and biochemical reagents allowed a detailed analysis of the expression of the *src* family of tyrosine protein kinases in fresh hematopoietic cells and established their role in the activation and proliferation of T and B lymphocytes.

2. Deregulation of the *src* family members in certain tumors (e.g., ATLL, colon cancer) may enable the bypassing of proliferation control points, leading to uncontrolled cell growth.

3. Specific inhibitors of TPK's are very effective in controlling T-cell proliferation in vitro. This observation will provide a useful tool for studying the structure and function of these enzymes and may also offer new therapeutic approaches for the treatment of certain cancers (e.g., ATLL, HIV-associated NHL, colon cancer).

Publications:
Horak ID, Corcoran ML, Thompson PA, Wahl LM, Bolen JB. Expression of $p60^{fyn}$ in human platelets. Oncogene 1990; 5:597-602.

Horak ID, Popovic M, Horak EM, Lucas PJ, Gress PE, June CH, Bolen JB. No T-Cell tyrosine protein kinase signalling or calcium mobilization after CD4 association with HIV-1 or HIV gp 120. Nature 1990;348:557-60.

Bolen JB, Thompson PA, Eisman E, Horak ID. Expression and interactions of the *src* family of tyrosine protein kinases in T lymphocytes. Advances in Cancer Res 1991;57:103-49.

Burke TR, Jr., Li Z-H, Bolen JB, Chapekar M, Gang Y, Glazer RI, Rice KC, Marquez VE. Examination of the possible mediation of antineoplastic effects of opiates through the inhibition of tyrosine-specific protein kinases. Biochem Pharm 1991;41:R17-20.

Horak ID, Gress RE, Lucas PJ, Horak EM, Waldmann TA, Bolen JB. T-lymphocyte interleukin 2-dependent tyrosine protein kinase signal transduction involves the activation of $p56^{lck}$. PNAS USA 1991;88:1996-2000.

Burkhardt AL, Brunswick M, Bolen JB, Mond JJ. Anti-immunoglobulin stimulation of B lymphocytes activates *src*-related tyrosine protein kinases. PNAS USA (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06730-03 CP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polyanions Used as Anti-Neoplastic and Anti-HIV Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C.E. Myers	Chief, Clinical Pharmacology Branch	CPB, COP, DCT, NCI
Others:	M. Cooper	Senior Investigator	CPB, COP, DCT, NCI
	M. Ranson	Special Volunteer	CPB, COP, DCT, NCI
	T. Toko	Guest Researcher	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

None.

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Biophysical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The use of polyanions as anti-neoplastic and anti-HIV agents was investigated. Examples of these types of compounds are phosphorothioate oligodeoxynucleotides and the bis-naphthalene sulfonic acids (e.g., suramin). Suramin administration has been shown to cause elaboration of a heparan sulfate which is excreted in the urine. This heparan sulfate was isolated and its biologic effects characterized.

Major Findings:
1. The heparan sulfate has been purified to homogeneity. It has been shown to slow or arrest the growth of a wide variety of human tumor cell lines in tissue culture. The 24-hour excretion of this material has been shown to be tightly correlated ($r > 0.9$) with the duration of suramin administration rather than with the amount of drug given or blood level attained. This suggests that the process leading to the synthesis of this compound is very sensitive to relatively low levels of drug. It may well be that accumulation of this heparan sulfate may plan an important role in the anti-tumor activity of this drug.

2. Suramin exhibits broad activity against a panel of carcinoma of the stomach cell lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06119 22 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytogenetic Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacqueline Whang-Peng	Head, Cytogenetic Oncology Section	MB, COP, DCT, NCI
Others:	Turid Knutsen	Medical Technologist	MB, COP, DCT, NCI
	Wei-Peng Zhao	General Fellow	MB, COP, DCT, NCI
	Susheela Mungamum	Guest Researcher	MB, COP, DCT, NCI
	Gulsan Yavnz	Guest Researcher	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Environmental Epidemiology Branch, NCI; NCI/Navy Medical Oncology Branch, NCI; Surgery Branch, NCI; Pediatric Oncology Branch, NCI; Laboratory of Tumor Virus Biology, NCI

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Cytogenetic Oncology Section has been examining specimens and tissue culture lines established from patients with hematologic malignancies and solid tumors in order to identify specific chromosomal changes associated with or diagnostic of these diseases. The breakpoints of these tumors indicate areas to look for new dominant oncogenes activated by translocations while the areas of deletions and loss of material by non-reciprocal translocations highlight areas to search for recessive oncogenes. These cytogenetic studies provide additional evidence that multiple genetic lesions are associated with the development of malignant tumors. We are presently conducting chromosomal in situ hybridization studies using either ^{3}H labeled probes or biotinylated probes to localize viral integration sites, and to localize other genes that may be important to the development of malignant diseases. We are also using chromosome painting to study the relationship of various chromosomes involved in translocations (such as the 9 and 22 in CML) in interphase cells. We plan to study the potential role of DNA topoisomerases in mediating illegitimate recombination in mammalian cells using the human c-abl protooncogene as a model system.

PROJECTS IN PROGRESS:

1. Cytogenetic studies of human neoplastic hematologic, and congenital diseases, with special emphasis on AIDS patients who develop leukemia or lymphoma. Specific disease studies include prostatic cancer, non-small cell carcinoma of lung, renal cell carcinoma, small cell tumors in childhood, acute lymphocytic leukemia, preleukemia, secondary leukemia, etc.

2. Localization of genes in normal chromosomes, using in-situ hybridization (^{3}H-labeled and biotinylated probes).

3. Localization of chromosome abnormalities in resting cells, using the chromosome painting technique; this will be applied to tumors which have few or no dividing cells.

4. Cytogenetic studies in HIV-I infected cell lines. Results have shown additional chromosomes abnormalities in four infected cell lines. Chromosome 17 is the most frequently involved chromosome in three of the four lines, followed by chromosomes 3 and 21. No significant abnormalities were seen in the normal lymphocytes from six individuals whose cells were infected in vitro with HIV-I. Cytogenetic studies are in progress in HTLV-II lines to further understand the significance of chromosome 17 involvement.

5. Use of chromosomes painting in interface cells to measure the distance between chromosomes 9 and 22 in normal individuals and in patients with CML. These studies will be done to evaluate the relationship between these two chromosomes and the formation of the 9;22 translocation.

6. Study of DNA topoisomerase-mediated genome instability. DNA rearrangements have been shown to be tightly linked to tumorigenesis. While a number of illegitimate recombination processes may be responsible for DNA rearrangements, little is known about the mechanism of any of these processes. DNA topoisomerases have been suggested to be responsible for some forms of DNA rearrangements because of their breakage-reunion activity. We plan to study the potential role of DNA topoisomerases in mediating illegitimate recombination in mammalian cells using the human c-abl protooncogene as a model system (in collaboration with Dr. Leroy Liu, Johns Hopkin's University, Baltimore).

PROJECTS COMPLETED:

1. Cytogenetic studies of esophageal carcinoma (Whang-Peng, et al, 1990). A total of 14 short- and long-term cell lines derived from esophageal cancer were studied. The presence in the primary explant of extensive cancer were studied. The presence in the primary explant of extensive numerical and structural abnormalities involving every chromosome including the sex chromosomes indicate that these abnormalities occur early in the malignant cells. The chromosomes most frequently involved in the structural abnormalities were 1, 9, and 11, each occurring in 13 of the 14 lines. The most frequent breakpoints for these abnormalities occurred at 3p14, 11q11q12, and 9q11q12, as well as the centromeric regions of all the acrocentric chromosomes. An HSR at 11q12 was found in three of the cell lines.

2. Using antiserum, DNA topoisomerase II levels in PHA-stimulated human lymphocytes were measured by immunoblotting. An increase in intracellular topo II level paralleled the entry of cells into proliferation. An increase in the topo II level resulted from an increase in the amount of topo II mRNA. The appearance of topo II mRNA was seen at 45 hours after stimulation. The

same RNA blot was rehybridized with a thymidine kinase probe. The maximal level of thymidine kinase mRNA was observed at 39 hours after PHA stimulation. It was found that the

expression of the topo II gene was later than the onset of DNA replication. Thus, this study suggests that topo I, which is constantly expressed throughout the cell cycle, might participate in the initiation of DNA replication, while topo II is involved in solving the DNA topological problems accompanying DNA strand separation during DNA replication (Hwong et al., 1990).

3. Chromosome abnormalities have been observed in about 50% of patients with acute leukemia. There have been several published reports which have emphasized the chromosomal changes in relation to the age of the patient and the morphologic type of acute leukemia. All observations suggest that there are both age related similarities and differences. The karyotype is an important independent prognostic factor in acute leukemia; however, age alone (especially about age 70) is the single most important factor for a poor prognosis (Whang-Peng, 1991).

4. Cytogenetic studies were performed on 27 cell lines and 4 fresh malignant pleural and pericardial effusions from 30 patients with non-small cell cancer. Many clonal structural (deletions and nonreciprocal translocations) and numerical abnormalities were found in each specimen. Statistical analysis revealed that these changes were nonrandomly distributed among the chromosomes. A statistically significant number of chromosomal breakpoints were seen in regions 1q1, 1q3, 3p1, 3p2, 7q1, 13p11, 14p1, 15p1, and 17q1 when the regions were compared to the total haploid complement. In addition, when a given region was compared to other regions within the same chromosome, statistically significant numbers of breakpoints were noted for regions 1q3, 5q1, 7q1, 13p1, 14p1, 15p1, 16q2, 17q1, and 21p1. Specific chromosome bands showing the most frequent involvement in structural abnormalities were (in descending order) 3p14.2, 3q21, 19q13, 11p15, 1q11, 7q11, 1q21, 3p23, and 3p21. The breakpoints indicate areas to look for new dominant oncogenes activated by translocations, while the areas of deletions and loss of material and nonreciprocal translocations highlight areas to search for recessive oncogenes. These cytogenetic studies represent strong evidence that multiple genetic lesions are associated with the development of metastatic lung cancer, and provide a roadmap to search for new genes involved in the pathogenesis of lung cancer (Whang-Peng et al, 1991).

5. Cytogenetic studies were performed on 12 cases of rhabdomyosarcoma (RMS) and an additional 40 cases from the literature were reviewed. The patients were divided into three categories: alveolar (A), embryonal (E), and primitive (P). The chromosome marker t(2;13) (q37;q14), thought to be characteristic for RMS-A, was found in 70% of cases with RMS-A; other cases of RMS-A showed chromosomal variants involving these breakpoints, namely t(2;11) (q37;q37), t(1;13) (p21;q14), and t(1;13) (p36;q14). In the RMS-E had variant cases, only one instance of the typical t(2;13) marker was noted, but six cases of RMS-E had variant or complex translocations involving the same breakpoints on chromosome 2 and 13; abnormalities of chromosome 11 were also observed. RMS-P represents a heterogeneous group of patients. One of our three cases had both an ins t(5;13) marker involving a breakpoint on 13q14, and the t(11;22) marker seen in Ewing's sarcoma. It is difficult to say whether these cases more closely resemble RMS or Ewing's or whether the tumors are derived from more than one cell type (Whang-Peng et al, 1991).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06513 15 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology of Antimetabolite Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carmen Allegra	Senior Investigator	MB, COP, DCT, NCI
Others:	Bruce A. Chabner	Director, DCT	OD, DCT, NCI
	Donna Boarman	Chemist	MB, COP, DCT, NCI
	John Wright	Senior Staff Fellow	MB, COP, DCT, NCI
	James C. Drake	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Cell Biology and Metabolism Branch, NICHHD, NIH; NCI-Navy Oncology Branch, COP, DCT, NCI; Critical Care Medicine Department, Clinical Center, NIH; NCI Pediatric Oncology Branch, Clinical Center, NIH

LAB/BRANCH

Medicine Branch

SECTION

Gastrointestinal Tumor Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
11.0	8.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section for the study of gastrointestinal tumors is divided into two broad areas that include the development of strategies for the treatment of solid tumors, in particular, those arising from the gastrointestinal tract, and the development of therapies for the treatment of opportunistic infections in patients with AIDS. The antineoplastic investigations revolve around the development of a complete understanding of the mechanisms of action and the mechanisms of resistance to the antimetabolite class of antineoplastic agents, specifically, 5-fluorouracil and methotrexate. The central focus of this area is to improve the therapy of solid tumors by 1) modulating the activity of the available antimetabolite agents in an effort to improve activity and circumvent resistance mechanisms defined from both preclinical and clinical investigations 2) enhancing the dose intensity of antimetabolite agents through use of biologic agents such as interferon and colony stimulating factors and 3) investigating the activity and mechanisms of action and resistance of novel agents for the treatment of gastrointestinal malignancies. In addition, we are investigating the growth factor requirements for colorectal carcinoma and adenoma cells in an attempt to characterize and interfere with these growth requirements. By investigating the progression from adenoma to carcinoma we hope to understand how various growth factors may be involved in malignant transformation with the ultimate goal of preventing such transformation.

The investigations of therapies for opportunistic infections is focused on the interactions of antifolate agents on the metabolic pathways in toxoplasma gondii, pneumocystis carinii and cryptosporidium. In addition to the use of basic biochemical technologies, we are using the tools of molecular biology to clone, sequence and express clinically relevant target enzymes for characterization and as an aide in the search for new therapeutic agents.

Project Description

Additional Personnel Associated with Project:

Sydell Zinn	Biologist	MB, COP, DCT, NCI
Edward Chu	Senior Staff Fellow	MB, COP, DCT, NCI
Jean Grem	Medical Staff Fellow	MB, COP, DCT, NCI
Patrick Johnston	Senior Staff Fellow	MB, COP, DCT, NCI
Pamela Daychild	Biologist	MB, COP, DCT, NCI
Lorrin Yee	Clinical Associate	MB, COP, DCT, NCI
Pedro Politi	Clinical Associate	MB, COP, DCT, NCI
Chris Takimoto	Clinical Associate	MB, COP, DCT, NCI
Peter Brandon	Stay-in-School	MB, COP, DCT, NCI

1. Biochemical Modulation of 5-Fluorouracil

5-FU is the single most active agent thus far identified for the treatment of gastrointestinal cancers. A multiplicity of trials using this agent in combination with other antineoplastics has not resulted in a significant increase in response rate or duration of response over that achieved with 5-FU alone. Acquired and de novo drug resistance appear to be the major impediments to the use of the presently available chemotherapeutic agents. Laboratory investigations have demonstrated that the formation of the ternary complex of thymidylate synthase-fluorodeoxyuradine monophosphate-5-10 methylene tetrahydrofolate as being a critical step for the cytotoxic effects of the fluoropyrimidines. The stability of the ternary complex has been clearly shown to be dependent on the concentration of the folate substrate. In vivo and in vitro preclinical studies demonstrate that the potency of the fluoropyrimidines may be enhanced (3-6- fold) by the addition of high concentrations of folate in the form of leucovorin. These preclinical studies spawned a series of phase 2 studies in colorectal and breast cancer using the combination of 5-FU with leucovorin. These studies showed an improved response rate in untreated colorectal cancer and two have shown a significant survival benefit.

Trails at the clinical center in heavily treated patients with metastatic breast cancer demonstrated that 5-FU plus leucovorin was an active regimen in this population despite the fact that 90% of the patients received and failed prior therapy with 5-FU containing regimens. This study also demonstrated that the addition of leucovorin to 5-FU was responsible for a marked enhancement in the stability of the critical ternary complex in serial tumor samples harvested from patients undergoing therapy. This study taken with the data from the treatment of patients with advanced colorectal carcinoma suggests that thymidylate synthase is a clinically relevant chemotherapeutic target. A additional critical piece of information that came from these studies was the observation that the target enzyme, thymidylate synthase, was acutely inducible by exposure to 5-FU. Subsequent preclinical studies have shown that this induction is responsible for drug resistance in certain colon cell lines. In addition, we have found that gamma-interferon is capable of interdicting the acute induction of thymidylate synthase and results in enhanced sensitivity to the fluoropyrimidines. The molecular mechanisms responsible for regulating this induction and alternate strategies to circumvent enzyme induction have been under active investigation. Using colon and rectal cell lines, we have found that enzyme control occurs at the level of protein translation as no changes in the mRNA levels are apparent in the face of marked enzyme changes following exposure to 5FU. Subsequent studies using an in vitro translational system have revealed that control of the thymidylate synthase translation occurs via a specific autoregulatory interaction of the protein with its own mRNA. Furthermore the binding site occupancy of the enzyme is a determinant of its ability to interact with the message. The identification of the protein-mRNA autoregulatory loop is unprecedented in mammalian systems and may provide a

paradigm for the regulation of other critical chemotherapeutic targets.

In addition to calcium leucovorin and interferon, we have identified cis-Platin as an agent capable of positive interaction with 5-FU. This interaction has been characterized in several cell lines and we are presently in the process of defining the mechanisms of this potentially useful interaction. Preliminary studies suggest that the locus of the interaction is at the level of DNA repair rather than at the level of protein interactions or metabolic alterations. Over the past six months we have collaborated with Drs. Chrambach and Rampino (NICHHD) to develop a sensitive system for quantitating the degree of DNA fragmentation without the need for radiolabelling. Presumably, this new technique will be applicable to patient material as well as in vitro systems.

Thymidylate Synthase Quantitation

Because of the importance of thymidylate synthase as a therapeutic target, we have sought to develop new and more sensitive methods for its quantitation. Over the past year we have developed a series of monoclonal antibodies against the recombinant human enzyme and have used these antibodies for quantitating the expression of thymidylate synthase. We have used these antibodies to quantitate the level of thymidylate synthase in peripheral blood lymphocytes and malignant cells in culture using both ELISA and Western immunoblot analysis. Immunohistochemical techniques have been developed for enzyme quantitation in fixed and paraffin embedded tissue. We are presently investigating the prognostic significance of thymidylate synthase expression in patients with colon, rectum and breast cancers.

New Therapeutic Agents for Solid Tumors

Efforts are continuing to identify new agents for the treatment of gastrointestinal malignancies and to understand their mechanisms of action and resistance. Cyclopentenyl cytosine (CPEC) is one such agent and we are presently in the process of clarifying its mechanism of action so that it may be applied clinically in a scientifically sound fashion both alone and with other agents. CPEC has been shown to be a potent inhibitor of cytidylate synthetase. In addition, have found that CPEC is incorporated into RNA . While the significance of this incorporation is unclear, we are currently in the process of characterizing the species of RNA into which the CPEC is incorporated and the quantity incorporated. A potential second mechanism of action would make CPEC unique from other cytidine nucleotide inhibitors which appear to produce toxicity via pure enzymatic inhibition. CPEC is expected to be available for clinical trials in the Medicine Branch within this fiscal year and a phase 1 study has been approved by the NCI ICRS and is awaiting final approval from the FDA.

A new folate analog, D1694, is capable of potent inhibition of thymidylate synthase with inhibition constants in the nanomolar range for the polyglutamated metabolites. We are presently investigating the interaction of this new analog with human thymidylate synthase and its effect on the interaction of anti-thymidylate synthase antibodies with the enzyme. A phase 1 clinical trial with D1694 has been provisionally approved by the NCI ICRS and is awaiting final approval from the FDA .

2. Antifolate Projects

We have continued to investigate the mechanism by which dihydrofolate reductase inhibitors produce metabolic inhibition. The inhibition of thymidylate synthase following antifolate exposure appears to be a multifactorial process including partial substrate depletion and direct inhibition by the polyglutamated metabolites of methotrexate and dihydrofolate. The extent to which substrate depletion accounts for enzyme inhibition appears to be exquisitely cell line dependent. Substrate depletion appears to be responsible for the majority of enzyme inhibition in the rat hepatoma line

H35, while it appears only partially responsible in the murine leukemia cell line L1210 and in human breast (MCF-7) and colon (H630) cell lines. We are presently investigating the basis for these apparent differences by applying a computerized model of the folate pathways developed by Dr. Morrison. The metabolic variables responsible for the differences in folate pools will then be tested in appropriately modified cell lines as verification of the results from the computer simulations.

Given the utility of the leucovorin/5FU combination for the treatment of patients with solid malignancies, we have been investigating the determinants of leucovorin efficacy. Two of the critical determinants occur at the cellular level and include metabolism to the various one-carbon substituted forms and polyglutamates. We have found that the conversion of leucovorin to 5-10 methylene tetrahydrofolate is time and dose dependent in human breast and colon cell lines. We were unable to identify saturation of this metabolism at doses up to 50 μM. By contrast, metabolism to the polyglutamate forms was principally dependent on the time of exposure rather than dose. This metabolism appeared to be saturable with highest levels achieved by approximately 24 hours. The polyglutamate metabolites had a prolonged intracellular half-life (~20 hours for the pentaglutamate) and were approximately 50-fold more able to form ternary complex with thymidylate synthase and FdUMP when compared to the monoglutamate form. These studies suggest that time of exposure is the critical factor in the optimal use of leucovorin with 5-FU.

In addition to the mechanism of action of the antifolates, we have investigated the mechanisms of resistance to these agents. Of interest, we found that a human breast carcinoma cell line resistant to adrimycin was crossresistant to methotrexate. This line expressed high levels of MDR 1 and presumably was insensitive to the anthracycline via this mechanism. Investigation of the traditional mechanisms of methotrexate resistance revealed that the resistant cells contains a mutated dihydrofolate reductase. The characteristics of this enzyme and the precise mutation are the topic of ongoing investigations.

The apparent insensitivity of human colon carcinoma cells to the antifolates has never been fully explained. In an attempt to understand the potential determinants of this insensitivity we studied the mechanisms of resistance to the lipophilic antifolate, trimetrexate, in human colon carcinoma cell lines. A through examination of the classical mechanisms of resistance to trimetrexate suggested that a novel mechanism may be responsible for drug insensitivity. We found that the basal levels of dihydrofolate reductase were similar in three colon cell lines whose sensitivity to trimetrexate differed by approximately 100-fold. Interestingly, exposure to the antifol resulted in an acute induction of dihydrofolate reductase in proportion to the degree of drug sensitivity. Further studies to elucidate the potential causal role in resistance of this dynamic phenomenon are in process.

3. Clinical Trials

The current group of intramural clinical trials for the treatment of patients with advanced adenocarcinoma of the gastrointestinal tract have been formulated from completed clinical trials and preclinical observations made in our laboratories. Each of these trails is based on the combination of 5-FU with leucovorin plus an additional manipulation designed to further enhance the synergy of the basic combination.

MB 239/259 Jean Grem - Principal Investigator

Interferon is a relatively poorly understood agent with a host of cellular effects. We have combined this agent with 5-FU /leucovorin for a twofold purpose: 1) several laboratories using murine models have shown that the toxic/therapeutic ratio of 5-FU may be increased by the addition of interferon. In recognition of the steep dose-response curve for 5-FU, the addition of

interferon may allow a more dose-intense regimen to be safely administered. 2) We have found that interferon and 5-FU can interact in a positive fashion. Interferon appears to inhibit the induction of the target enzyme, thymidylate synthase, with 5-FU exposure. These two effects may have important clinical implications. Indeed, a study by Wadler etal. has found that the use of interferon with 5-FU results in high response rates in patients with previously untreated colorectal cancer (60%). These results have been confirmed by several other groups who have reported response rates in the 25-40% range. Based on these data, a pilot trial combining 5-FU with leucovorin given on a daily for five days regimen days 2-5 and alfa-interferon given daily on days 1-7 was initiated at the NCI. This trial was closed in early 1991. We found that these agents could be administered with acceptable toxicity. Of particular importance was the lack of neurologic toxicities which had been reported in the trials using weekly 5-FU with three times weekly interferon. While no responses were noted in patients who had previously failed 5-FU, a response rate of 45% was found in previously untreated patients. The pharmacokinetics of 5-FU were studied in patients with and without the addition of interferon. The simultaneous administration of alfa-interferon resulted in a 1.3-fold increase in total drug exposure (AUC) at the 5 million units per meter squared dose. This dose was chosen for further investigation because it was well tolerated and resulted in an apparent increase in drug exposure. A phase 2 trial testing this combination in a homogeneous population of untreated patients with advanced and measurable colorectal carcinoma is underway.

MB 245 Jean Grem - Principal Investigator

In a separate attempt to increase the dose intensity of 5-FU, we have added colony stimulating factor to the combination of 5-FU/leucovorin. CSF's have been shown to ameliorate the myelotoxicity associated with cytotoxics and may have a mitigating effect on the associated mucositis.

MB 249 Jean Grem - Principal Investigator

Several clinical trails have indicated that the use of PALA with 5-FU may be synergistic. In contrast to previous trials using this combination, the present trials have used a low-dose of PALA that was capable of producing the desired biochemical effect but not the MTD of the drug. Use of high-doses of PALA in previous trials required decreases in 5-FU doses and thus loss of potential benefit. We have designed a trial using PALA with 5-FU and leucovorin with the knowledge that PALA may further enhance the formation and stability of the critical ternary complex. As is true with all current colorectal trials in the Medicine Branch, this trial incorporates both clinical and biochemical/molecular endpoints. The thrust of this work will be to directly measure inhibition of the target enzyme in patient tumor samples and peripheral blood lymphocytes.

Monoclonal Antibody Studies Carmen Allegra - Principal Investigator

The monoclonal antibody COL 1 is available for clinical investigation. A phase 1 study has been provisionally approved by the NCI ICRS and we are awaiting final FDA approval. The focus of this trial will be to determine the MTD of the 131I conjugate and to examine the tissue-to-tumor ratio of the radiolabel through imaging studies and timed tissue biopsies. This reagent will be used with therapeutic intent as well as for imaging primarily in patients with gastrointestinal malignancies.

Cyclopentenyl Cytosine Jean Grem - Principal Investigator

This antimetabolite will enter phase 1 testing pending final approval by the FDA. THe phase 1 protocol has been approved in final form by the NCI ICRS. In addition to the usual endpoints of MTD and toxicity, this trial will incorporate pharmacokinetic analysis and examination of target

enzyme inhibition in tumor tissue and peripheral blood lymphocytes.

Carmen Allegra - Principal Investigator

D1694 is a potent folate-analog inhibitor of thymidylate synthase. A phase 1 trial has been provisionally approved by the NCI ICRS and is awaiting final FDA approval. As in the other Gastrointestinal Tumor Section protocols, this study will also include biochemical endpoints as well as the clinical endpoints of MTD, toxicity and pharmacokinetics. Our intention is to study the kinetics of thymidylate synthase inhibition by D1694 using our monoclonal antibody technology and to make correlations with clinical parameters.

Interbranch Studies

With the development of more active regimens, we have initiated a program of neoadjuvant therapy in patients with local and locally advanced gastric and pancreas carcinomas. The purpose of these protocols is to evaluate the activity and value of modulated fluorouracil regimens when used in the neoadjuvant setting and the procure tumor tissue for investigations regarding the mechanisms of antimetabolite resistance. In addition, we have initiated the development of a multimodality program for the treatment of patients with esophageal carcinoma. This study will employ neoadjuvant chemotherapy, chemo-radiation and surgery.

4. Opportunistic Infections Project

The dihydropteroate synthetase (DHPS) enzyme has been extensively investigated in T. gondii organisms. The use of sequential dye affinity chromatographic techniques have been developed to purify the enzyme over 100,000- fold. Over twenty sulfonamide and over 40 sulfone analogs have been screened for inhibitory activity against this enzyme. The sulfone compounds were unexpectedly the most potent class of analogs with typical inhibitory constants $< 1\ \mu M$. Since DHPS is an important target for drug development, we have been interested in investigating the characteristics of the enzyme isolated from pneumocystis organisms. This enzyme is expressed in low levels and is even less stable than the enzyme from T. gondii. Ideally, an investigation would include physical characteristics, characterization of the kinetic interactions of the enzyme with its substrates and interaction of the enzyme with various inhibitors. The latter characterization would be the most critical given the unacceptable toxicity associated with the presently available inhibitors. We have adapted two strategies to address these issues. One is to use unpurified enzyme harvested from the lungs of steroid treated rats. This method is slow and tedious due to the unreliable nature of organism availability. The second is to attempt to clone the cDNA encoding for the enzyme so that it could be expressed at high levels in bacteria. We are applying the tools of molecular biology in collaboration with Drs. Kovacs and Masur (CC). Collaborations with Dr. Jeffery Edman have resulted in the cloning and expression of the pneumocystis dihydrofolate reductase and thymidylate synthase from our present pneumocystis cDNA library. Attempts to clone the pneumocystis DHPS have not yet met with success, but we are continuing our exploration using the latest available technologies.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06516 09 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Drug Resistance in Human Breast Cancer Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Kenneth H. Cowan	Head, Medical Breast Cancer Section	MB, COP, DCT, NCI
Others:	Charles Morrow	Biotechnician	MB, COP, DCT, NCI
	Jeffrey Moscow	Biotechnician	MB, COP, DCT, NCI
	Masayuki Nakagawa	Visiting Fellow	MB, COP, DCT, NCI
	Kathy Dixon	Visiting Fellow	MB, COP, DCT, NCI
	Julie Horton	Senior Staff Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

NIH Pathology, Surgery Branch, Radiation Oncology Branch

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10	8	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory has been investigating the genetic and biochemical changes associated with drug resistance in human breast cancer. We have identified a number of changes associated with the development of multi-drug resistance in human breast cancer cells including over-expression of the mdr1/P-glycoprotein drug efflux, as well as increased expression of the π class isozyme of glutathione transferase in the selenium dependent glutathione peroxidase gene. In order to study the role of these genes in development of resistance, we have transfected expression vectors for the human mdr1 gene and for the alpha, μ, and π class GST genes into drug sensitive breast cancer cells. We have also examined the possible interaction of GST and protein kinase C with P-glycoprotein through cotransfection experiments. In order to study the regulation of the genes overexpressed in drug resistant cells we have cloned the genomic promotor and flanking sequences for the human mdr1 gene, the glutathione transferese II gene, the selenium dependent glutathione peroxidase gene. The functional activity of the promotors were analyzed by fusing these elements to the CAT gene.

Other studies from our lab have identified changes in drug accumulation in association with mitoxantrone resistance. Resistance in the latter appears to be be due to markedly diminished methotrexate transport. In contrast resistance to mitoxantrone is associated with enhanced drug efflux in the absence of P-glycoprotein expression with enhanced drug efflux that is not mediated by mdr1/P-glycoprotein expression. However, membrane protein has been identified in the resistant cells which cross reacts with antibodies directed against the human P-glycoprotein. Another cell line selected for methotrexate resistance has developed a marked decrease in methotrexate accumulation. This defect is apparently due to a marked decrease in the reduced folate transport system in the resistant cells. Both of these cell lines provide useful models to study the molecular biology changes associated with the development of drug resistance in human breast cancer cells.

Project Description

Additional Personnel Assigned on Project:

Jean Gudas	Senior Staff Fellow	MB, COP, DCT, NCI
Lucy Gilbert	Visiting Fellow	MB, COP, DCT, NCI
Merrill Goldsmith	Microbiologist	MB, COP, DCT, NCI
Mary Jane Madden	Chemist	MB, COP, DCT, NCI

A. *mdr*1 Gene Regulation

In order to study the regulation of the expression of the multidrug resistant gene *mdr*1/P-glycoprotein, we have cloned and sequenced the human *mdr*1 promotor and 4.7 kb of upstream DNA. In order to study the sequences involved in the regulation of this drug resistance gene, a series of promotor-CAT fusion genes were constructed and studied following transfection into sensitive and multidrug resistant cell lines. In these studies, sequences involved in the transcriptional regulation of expression and proper initiation of the *mdr*1 gene have been identified. Current studies are directed towards identification of the nuclear factors involved in the initiation and mdr transcript formation and in the regulation of mdr1 gene transcription in multidrug resistance cells.

B. Protein Kinase C and *mdr*1/P-glycoprotein Function

Multidrug resistance in human breast cancer cell lines has been shown to be associated with increased expression of the phosphoglycoprotein P-glycoprotein and as well as increased expression of protein kinase C (PKC). The role of PKC in drug resistance was studied by transfecting an expression vector containing the cDNA for PKC into a breast cancer cell line previously transfected with the human *mdr*1 gene. These studies have demonstrated that increased expression of PKC enhances the level of multidrug resistance in mdr1 transfected cells. This increase in multidrug resistance is associated with enhanced phosphorylation of the P-glycoprotein in the transfected cells as well as increased drug efflux. These studies have shown that protein PKC can enhance the magnitude of multidrug resistance, an effect that may be mediated either directly or indirectly through phosphorylation of P-glycoprotein. Studies are now directed towards identifying other protein kinases that may be involved in modulation of P-glycoprotein function and enhanced multidrug resistance.

C. Role of Glutathione Transferases in Drug Resistance

Previous studies from our laboratory have demonstrated that multidrug resistance in human breast cancer cells is associated with over expression of P-glycoprotein, as well as increased expression of a number of drug metabolizing and scavenging enzymes including glutathione transferase and glutathione peroxidase. The increase in glutathione transferase activity in the multidrug resistant cell line was shown to be due to increased expression of the pi class transferase isozyme. In order to study the role of glutathione transferases in drug resistance, eukaryotic expression vectors containing cDNA's for the three classes of cytosolic GSTs (alpha, μ, and π) were constructed and transfected into human MCF-7 breast cancer cells. Since this cell line contains very low levels of

endogenous GST activity, it represents a good model system in which to study the effect of increased GST expression on drug resistance. We have transfected the human GSTπ, two distinct cDNA's for the human GST alpha (GST A1, A2), and human GSTμ into MCF-7 cells. Clones containing increased GST expression were selected using the neomycin resistance gene as selective as a selective marker. In each case, increased GST expression resulted in resistance to ethacrynic acid, a known substrate for each of the GST classes. Similarly, GSTa and GSTπ transfected cells develop cross resistance to benzo(a)pyrene and benzo(a)pyrene-diol-epoxide. However, GSTa, GSTμ, or GSTπ overexpressing cells were found not to be resistant to any of a diverse variety of anticancer drugs including adriamycin, cisplatin, chlorambucil, and BCNU. Thus, increased GST expression are the levels achieved in these studies is sufficient by itself to cause resistance to the anticancer drug agents tested.

We have also explored the possibility whether GST could play an important function as an intracellular drug binding protein that could interact with P-glycoprotein by delivering protein bound drug to the P-glycoprotein pump. In these studies cells were first transfected with the mdr1 gene and multidrug resistant clones were selected. Three distinct clones of mdr1 expressing MCF-7 cells were then transfected with a GSTπ expression vector and clones expressing increased levels of GSTπ were then examined for multidrug resistance phenotype. These studies demonstrated that increased *mdr*1 expression was sufficient to cause the multidrug resistance phenotype. Furthermore, increased expression of GSTπ in *mdr*1 positive cells did not enhance the level of resistance or alter the pattern of multidrug resistance. Thus GSTπ does not apparently interact with the P-glycoprotein drug efflux pump.

D. Regulation of Expression of the Human GSTπ Gene

Previous studies from our laboratory have shown that the expression of GSTπ is inversely correlated with the expression of estrogen receptor in human breast cancer cell lines. In order to further characterize the expression of GSTπ in human tumors, we have developed an immunohistochemical assay for GSTπ as well as estrogen and progesterone receptors using formalin fixed human breast cancers.

These studies have shown an excellent inverse correlation between the expression of GSTπ and estrogen receptors in primary human breast cancer ($P_2<0.001$). Furthermore, preliminary data in patients with node negative breast cancer, the expression of GSTπ appears to be correlated with a poorer prognosis.

In order to understand the regulation of expression of GSTπ in human breast cancer, we have cloned and sequenced the human GSTπ genomic gene. The GSTπ promotor was analyzed by fusing the promotor and flanking sequences to the bacterial CAT gene. A series of GSTπ-CAT fusion gene construct was constructed to analyze the influence of the 5' flanking sequences on the regulation of expression of GSTπ.

GSTπ-CAT transfection studies and nuclear run-on analysis of ER+ and ER- breast cancer cell lines have demonstrated that the increased expression of GSTπ in ER- breast cancers is regulated through post-transcriptional control mechanisms. In order to study the post-transcriptional regulation of GSTπ expression, we have transfected both ER+ and ER- cell lines with a eukyatic expression vector containing the GSTπ cDNA. Individual clones of cells were isolated and the stability of the RNA was analyzed. These studies demonstrated that the stability of the GSTπ

transcript from the transfected expression vector was essentially identical to that of the endogenous gene in ER- cell lines. Furthermore, the stability of GSTπ in ER+ cell lines transfected with GSTπ expression vector was the same that in ER- cell lines. Thus, the post-transcriptional mechanisms involved in the increased expression of GSTπ in ER- cell lines, does not involve either the 5' or 3' untranslated regions.

E. Glutathione Peroxidase Gene Regulation

As noted above, multidrug resistance in human breast cancer cells is also associated with increased expression of the selenium-dependent glutathione peroxidase (hGPx) gene. Other studies have implicated the expression of this enzyme in an adriamycin resistance. Studies from our laboratory have demonstrated that the expression of the human glutathione peroxidase gene (like GSTπ) is also increased in ER- negative breast cancer cells relative to that ER+ breast cancer cells.

In order to study the regulation of expression of this gene we have cloned the human glutathione peroxidase genomic gene. Nucleotide sequence analyses of this gene and its flanking sequences have demonstrated that the 3' end of the human *rhoH*12 oncogene lies within two kb upstream of the glutathione peroxidase gene. Furthermore, flanking the r*hoH*12 and peroxidase genes are tandem *alu* repeat elements. In addition, another transcribed open reading frame was found between the 3' end of the *roH*12 gene and the 5' end of the peroxidase gene. This sequence has no homology with any previously identified protein or DNA sequences.

A series of LGPX-CAT fusion genes were used to study the 5' regulatory sequences of the peroxidase gene. In addition, nuclear run-on analysis were performed in ER+ and ER- cells and indicate that the peroxidase gene, like the GSTπ gene, is regulated in breast cancer cells through post transcriptional control mechanism.

F. Mitoxantrone Resistance in Human Breast Cancer Cells

We have isolated a mitoxantrone resistant human breast cancer cell line that is >3000 fold resistant to mitoxantrone. These cells developed low levels of cross-resistance to adriamycin and VP16 but not to alkylating agents or cisplatinum. Resistance in the cell line is associated with decreased mitoxantrone accumulation and enhanced drug elimination. Northern blot and Western blot analyses failed to identify any *mdr*1 RNA or protein (P-glycoprotein) in the mitoxantrone resistant cells. However, using a polyclonal antibody directed against a conserved region of the P-glycoprotein/*mdr*1 gene product, increased levels of immunoreactive proteins were found in the membrane of the mitoxantrone resistant cells. The role of these membrane proteins in the development of atypical multidrug resistance is currently under investigation.

G. Methotrexate Resistant Human Breast Cancer Cells

We have previously isolated methotrexate resistant human breast cancer cell lines that displayed decreased methotrexate transport and decreased polyglutamation of methotrexate. Current studies have indicated that the transport of methotrexate into the drug sensitive breast cancer cell line occurs by the reduced folate transport system (low affinity high capacity transport system). No expression of the folate binding protein (folate receptor) is detected in the sensitive or resistant cell lines. Resistance in this human breast cancer cell line is associated with a marked decrease in methotrexate uptake into the resistant cell line. Studies have been initiated to examine in more detail the defects in the resistant cell line and to develop ways to overcome this resistance.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM 06716 04 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Platinum Drug Resistance in Human Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Eddie Reed	Senior Investigator	MB, COP, DCT, NCI
Other:	Ricardo Parker	Biotechnology Fellow	MB, COP, DCT, NCI
	Meenakshi Dabholkar	Visiting Fellow	MB, COP, DCT, NCI
	Freida Bostick-Bruton	Biologist	MB, COP, DCT, NCI
	Terri Cornelison	Senior Staff Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Pharmacology, DTP/DCT/NCI; U.S.C. Cancer Center, Los Angeles, California; Laboratory of Cellular Carcinogenesis and Tumor Promotion, DCE/NCI

LAB/BRANCH

Medicine Branch

SECTION

Section of Genitourinary Malignancy

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This unit continues to conduct work to investigate the clinical and molecular parameters of resistance to platinum compounds. Work is being performed using fresh human materials, and established cell lines of malignant and non-malignant origin.

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Project Description

Major findings include:

1) We have shown that in human ovarian cancer cell lines, DNA repair appears to be the primary mechanism of resistance to cisplatin. Alterations of transmembrane transport appears to be important as well, but cytosolic inactivation of drug appears not to play a role in determining the level of resistance to platinum compounds.

2) We have developed a shuttle vector assay by which one can assess functional platinum-DNA adduct repair capacity in human T lymphocytes, or in human malignant cells. Studies are in progress to determine whether such an assay can be used clinically.

3) In collaboration with Vilhelm Bohr of LMP/DIP/NCI, we have begun to examine the role of gene-specific repair of platinum-DNA adducts in platinum drug resistance. This does occur in Chinese hamster ovary cells and in human malignant cells. The precise relationship between such repair and clinical resistance to platinum compounds is being explored.

4) We have preliminary evidence to suggest that the human excision nuclease ERCC1 is one of the key genes which effects enhanced DNA repair of platinum-DNA lesions. This preliminary evidence shows a relationship between gene expression and resistance to platinum agents. Work is in progress to modulate the level of gene expression and determine how this will effect the level of platinum resistance.

5) In patients who receive cisplatin/carboplatin as their only therapy, the level of platinum-DNA damage measured in leukocytes is directly related to clinical response to therapy. This observation was made in a cohort of patients with 15 different tumor types, suggesting that the molecular mechanisms which modulate platinum drug resistance are not unique to tumor tissue and probably reflect the pharmacogenetic makeup of the individual.

Publications:

Dabholkar M, Eastman A, Reed E. Host cell reactivation of cisplatin damaged pRSVcat in a human lymphoid cell line. Carcinogenesis, II 1990:1761-64.

Reed E, Gupta-Burt S, Litterst CL, Poirier MC. Characterization of the DNA damage recognized by an antiserum elicited against cis-diamminedichloroplatinum (II)-modified DNA. Carcinogenesis, II:1990;2117-21.

Reed E. Cisplatin. In: Pinedo HM, Chabner BA, Longo DL, eds. Cancer Chemotherapy and Biological Response Modifiers Annual - Volume II. Amsterdam: Elsevier Science Publishers B.V., 1990:522-26.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06718 03 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Folate Binding/Transport Proteins (FBPs)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Patrick C. Elwood	Senior Investigator	MB, COP, DCT, NCI
Others:	Koong-Nah Chung	Senior Staff Fellow	MB, COP, DCT, NCI
	Stephanie Page	Biologist	MB, COP, DCT, NCI
	Yutaka Saikawa	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human folate binding proteins (FBP), or folate receptor, bind and transport physioloic reduced folates and methotrexate, a chemotherapeutically-active folate analogue. Others have reported that defects in drug influx account for acquired methotrexate drug resistance in 40-60% of mutant cell lines studied. The Molecular Cell Biology Section is investigating the following:

1. Structure, function, and molecular biology of human FBPs;
2. Role of the FBPs in transport of folates and in the development of acquired methotrexate resistance; and
3. Expression of the FBPs.

U.S. GOVERNMENT PRINTING OFFICE: 1991 O—864 939

Major Findings:

1. Structure, Function, and Molecular Biology of Human FBPs.

A. Molecular Biology: We have previously isolated and characterized human folate binding proteins from human placenta, human milk, and human tissue culture cells. Based on in vitro ligand inhibition studies and inhibition of transport by anti-human FBP antisera, the membrane form of the FBPs mediates folate and antifolate transport in these systems. By means of oligonucleotides derived from partial amino acid sequence of purified KB cell FBP, we have previously reported the cloning, sequencing and characterization of a KB cell FBP cDNA contained in human "KB cell" and human placental cDNA libraries.

In the past year, we have completed the sequencing and characterization of a second distinct human "placental" FBP cDNA. The "placental" cDNA is the most abundant (16/18 clones) FBP cDNA contained in the human placental cDNA library but is not expressed in KB cells; contains 125 bp and 166 bp 5' and 3' untranslated regions (UTRs); and contains a 753 bp open reading frame (ORF). Compared to the KB cell FBP cDNA, the nucleotide sequence of the UTRs of the placental FBP cDNA are heterogeneous whereas the ORF are homologous (70% overall) indicating that the FBP cDNAs are encoded by separate genes and that the encoded FBP proteins constitute a gene family with conserved high affinity folate binding sites which most likely arose from a common ancestral gene. The "placental" FBP cDNA is similar to that previously reported by others except that the ORF contains 3 bp differences which result in one amino acid change in the deduced amino acid sequence, and the 5' UTR is shorter and contains a different 5'-most nucleotide sequence. The 5 prime extent of the "placental" gene is heterogeneous in length based on sequence analysis of 3 cloned placental cDNAs and 11 PCR-cloned cDNAs, primer extension assays of placental RNA, and RNAase protection assays (see below).

To determine the chromosomal organization of the FBPs and to obtain the genomic regulatory sequences for expression analysis, we have isolated 5 distinct genomic clones (arbitrarily named C1, C2, C3, C36, C66) containing DNA homologous to the KB cell cDNA from a genomic human leukocyte EMBL3 library (Clontech). The genomic clones have been characterized by restriction mapping, hybridization analysis with "KB cell" and "placental" cDNA-specific 5' and 3' probes, and partial sequence analysis. Based on comparison to the "placental" FBP cDNA nucleotide sequence, clones C1 and C2 contain the placental FBP gene which spans approximately 6 Kb and is comprised of 5 exons (127-472 bp) and 4 introns (117-2136) flanked by consensus donor and acceptor splice sequences. The major transcriptional start sites (TSS) of the placental gene are between 146 and 152 bp upstream from the ATG translation initiation site (thus exon 1 is between 100 to 106 bp in length) and is heterogeneous. We hae determined the nucleotide sequence aproximately 700 bp upstream to the TSS which contains "TATAA boxes" at -367 and -555. The function of these potential eukaryotic promoters and other regulatory sequences is unknown. We plan to determine the upstream DNA sequence and to study the regulatory elements contained in the gene by standard CAT analysis.

Based on nucletoide sequence, the KB cell cDNA was not encoded by the above genomic clones. However, we subsequently isolated 3 hybridization positive genomic clones by screening a second human lymphocyte genomic (lambda dash) library (Stratagene) with a 5' "KB cell" specific cDNA probe. The restriction and hybridization map of 2 unique clones (probably allelic forms or duplicated genes) has been determined, and we have determined the DNA sequence of hybridization positive restriction fragments encompassing the gene including 2500 and 4000 bp upstream from the TSS. Based on comparison of the genomic clones to nucleotide sequences of the 2 published "KB cell" cDNAs and an unpublished "KB

cell" cDNA sequence (recently isolated from my laboratory) characterized by a distinct 5' UTR relative to length and sequence, the organization of the KB cell gene is extremely similar to that of the placental gene supporting a close evolutionary relationship. The KB cell gene is characterized by 5 exons (136-404 bp) and 4 introns (149-2800 bp) flanked by consensus splice sequences. Exon 1 contains the majority (e.g. 203 of 210 bp) of the 5' UTR and the nucleotide sequence of exon 1 differentiates the 3 "KB cell" cDNAs. The domains containing the 3 potential exon I sequences homologous to the 5' UTRs of the 3KB cell cDNAs are arranged in tandem order in the KB cell FBP gene approximately 1000-2000 bp upstream to the translation initiation site which is contained in exon II. We are currently mapping the 5 prime boundaries of this gene by primer extension and RNAase protection assays to delimit the gene boundary(ies) and to determine the abundance and importance relative to tissue specific expression, to differentiation specific expression, and to translational efficiency of each potential exon 1 prior to analysis of potential upstream regulatory elements by standard CAT assays.

B. Cell Biology: In humans, folate binding proteins exist in two forms (membrane-associated or soluble extracellular forms) which exhibit unique biochemical properties includng cellular localization but are related in a precursor-product manner. To further study this relationship, we determined the conditions under which the membrane form is converted in vitro to the soluble form and demonstrated that a membrane-bound metalloprotease is involved. Furthermore, comparison of the amino acid sequence deduced from the human KB cell cDNA, the amino acid sequence determined from the milk soluble FBP, the amino termini of the KB cell membrane-associated and soluble FBPs, and the changes in specific activity of endogenously labeled FBP during conversion, conversion to the soluble form (S-FBP) involves cleavage of an extremely hydrophobic -COOH termini from the membrane-associated FBP (M-FBP) which is involved in membrane anchoring and detergent binding characteristic of M-FBP. This data suggests that M-FBP is anchored to the membrane by the hydrophobic carboxyl termini whereas data published by others implicate a GPI tail or anchor attaching M-FBP to the membrane. To further elucidate the nature of the membrane anchor, we are transfecting the KB cell cDNA into mouse L cells, a cell line which is deficient in the enzyme(s) required for attaching a covalent GPI tail. Preliminary data suggests that these cells express low levels of functional folate receptor after transient transfection with full length KB cell cDNA.

To identify functional regions and their importance we have initiated transfection experiments to be used in site directed mutagenesis experiments. We have identified an expression vector containing a CMV promoter which will express easily quantifiable levels of a functional folate receptor when transiently transfected into human MCF7 mammary carcinoma cells, L cells (see above) and CHO cells. By means of PCR and oligonucleotides containing specific single mutations or deletions, we are currently constructing cDNA mutants to identify the ligand binding site, to determine the minimum COOH termini resulting in membrane anchoring, and to investigate the functional importance/role of serine phosphorylation sites (PKC consensus sequence at Ser^{79}).

2. Role of the FBPs in Transport of Folates and in the Development of Acquired Methotrexate Resistance.

We have selected and isolated 9 methotrexate (mtx) resistant mutant KB cell clones cultured in physiologic concentrations of extracellular folates (1-10 nM). Potential mechanisms of resistance (DHFR activity; mtx polyglutamation; drug efflux; and transport defects with specific emphasis on the expression and function of the folate receptor) were analyzed in each

resistant cell line. Transport defects (drug influx) associated with decreased expression (5% to 70% of wild type (wt) KB cells) of the folate receptor were present in all (9/9) clones and were associated with increased DHFR activity in 44% (4/9) ranging from 1.5 to 9 fold higher than wt KB cells. Drug efflux and polyglutamation in the mutants were similar to wt KB cells. Thus, transport defects (associated with decreased folate receptor expression) is the most common mechanism of methotrexate observed under these conditions. We have cloned the folate receptor from each of these cell lines for determination of their cDNA sequence.

The phenotype of decreased folate receptor expression is stable after 6-9 months in drug free media in approximately 50% of the mutants suggesting a mutational change which results in decreased transcription. We are currently cloning by PCR the 5' domains of KB cell gene from these stable mutants to investigate this observation.

By SDS-PAGE and western blot analysis of membrane preparations of folate deplete methotrexate resistant mutants, we have identified a 50 Kd, membrane-associated, non-glycosylated protein (pI=7.0) which is overexpressed in 4-5 mutant cell lines. In 3 cell lines, this phenotype is stable for > than 30 passes into drug free media. Folate depletion of wt KB cells also increases the expression of this protein; whereas folate repletion is associated with a decrease in expression. These observations suggest a role for this protein in folate homeostasis. Interestingly, a rabbit anti-human folate receptor antiserum hybridizes with this protein on western blot analysis suggesting that the 50 Kd protein shares epitopes with the folate receptor, or associates with and co-purifies with the folate receptor. We have immunized a rabbit with SDS-PAGE gel purified protein and are initiating experiments to purify and clone this protein for further characterization and functional analysis.

3. Expression of the FBPs (Translational Control).

The transcriptional studies of cis and trans elements pertinent to this section are alluded to above in the genomic cloning projects. We have isolated and fully characterized 2 KB cell cDNAs which differ only in the length and sequence of the 5' UTR (presumably as a result of alternative splicing of exon 1, see above). Preliminary evidence suggests that although in vitro transcription of each cDNA is similar, however, the translation efficiency (S^{35}methionine incorporation in protein/ug cRNA added) of the respective cRNAs are 10-15 fold different. To determine the nature of these differences (e.g. cis elements such as stem-loop structures or trans elements such as nucleic acid binding proteins), we are studying the transcriptional efficiency of each construct in detail and are designing deletion constructs of each KB cell cDNA for in vitro expression analysis.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06727 03 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Oncogenes Activation in Human Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Maria Zajac-Kaye	Senior Staff Fellow	MB, COP, DCT, NCI
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	Noa Ben-Baruch	Clinical Associate	MB, COP, DCT, NCI
	Michele Exum	General Fellow	MB, COP, DCT, NCI
	Melissa Blake	Technician (Stay-in-School)	MB, COP, DCT, NCI
	Nadia Alexandrova	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.6	2.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We have investigated the mechanisms of oncogenes regulation in human malignancies. Deregulation of the *c-myc* gene accompanies all cases of human Burkitt's lymphoma (BL) and many other types of human tumors, therefore the mechanism underlying the transcriptional regulation of the *c-myc* gene and the role of its gene product have been investigated. The three major projects are the following:

A. Transcriptional regulation of the *c-myc* oncogene in normal and neoplastic cells.

B. Effect of differentiating agents on the function of transcription factors and their role in the regulation of the *c-myc* gene.

C. Search for the function of the *c-myc* proteins.

A. Transcriptional regulation of the *c-myc* oncogene in normal and neoplastic cells.

Our objectives are to define DNA cis elements and regulatory binding proteins which play a role in the control of c-myc expression and use this information to develop reagents that may modulate *c-myc* activity in normal and neoplastic cells. Our effort has been concentrated on downstream regions of the *c-myc* gene because acquired somatic mutations have been shown to cluster in the intron I sequence of *c-myc* DNA in Burkitt's lymphoma (BL) cell lines. Our published work identified a 138 Kd phosphoprotein (designated MIF for Myc Intron Factor) whose binding was abolished due to mutations present in the 20 bp MIF recognition sequence in the intron I of the *c-myc* gene from BL DNA. Phosphorylation of this protein on serine residue was shown to be required for binding to its recognition sequence. Functional analysis of MIF led to identification of additional cis elements located adjacent to MIF binding site. We showed that this frequently mutated region in the intron I of the *c-myc* gene contains a cluster of four cis elements which bind nuclear proteins, which we have designated MIF-1 through MIF-4. We found that MIF-3 by itself is a strong negative regulator of the major promoter in the *c-myc* gene and that MIF-3 is also frequently mutated in BL *c-myc* DNA. Comparison of the DNA binding sequences for all four factors showed homology between the MIF-1, -2 and -3 sites and also between the MIF-3 and -4 sites. Thus, the interaction among these four cis elements and their binding factors may be important in the control of *c-myc* expression and it may be perturbed in BL due to mutations frequently observed in all four binding sites. PCR sequencing will allow us to determine whether intron I region in the *c-myc* gene is also a mutational hot spot in other tumor types which show overexpression of the *c-myc* gene. Purification, characterization and cloning of the genes encoding these proteins will allow us to better understand their role in the regulation of the *c-myc* gene and in oncogenesis. Our results suggest that the intron I cis elements, the binding factors MIF-1, 2, 3 and 4 and the kinases which phosphorylates MIF-1 may comprise an important physiological circuit for the regulation of *c-myc* expression. These studies may yield valuable information on the role of transcription factors:DNA interaction in normal and in cancer cells and may potentially provide a novel approach to impede *c-myc* activity by developing reagents such as synthetic peptides, which could specifically bind to the mutated DNA consensus sequence to modulate expression of the *c-myc* gene. Such reagents might have future therapeutic significance.

B. Effect of differentiating agents on the function of transcription factors and their role in the regulation of the *c-myc* gene.

In response to differentiating agents such as retinoic acid, TPA and DMSO, *c-myc* expression rapidly decreases, while conversely, overexpression of *c-myc* blocks cellular differentiation. The objective of this project is to identify cis elements in the *c-myc* gene which are involved in the proliferation and differentiation of the human promyelocytic cell line (HL60) and the human histiocytic leukemia cell line (U937) as a model for oncogene manipulation and therapy. Both cell lines provide a system where *c-myc* expression (linked with cell growth and differentiation) could be physiologically modulated and easily examined. We have observed dramatic and reproducible differences in myc DNA:nuclear protein complexes in response to retinoic acid, TPA, DMSO and suramin treatment, indicating that nuclear regulatory proteins that bind to the *c-myc* gene are affected by the agents used. Our goal is to determine, whether these agents can effect the activity of these nuclear proteins, thus altering their specific binding to DNA (for example: changing phosphorylation state by inhibiting phosphatase or activating kinases) or whether induction of differentiation was linked to the induction of specific binding factors. We have partially purified the protein which binds to the MIF-1 recognition sequence from undifferenciated HL60 cells and our results suggests that undifferentiated HL60 cells synthesize a protein which has specificity for the MIF-1 sequence but is distinct from the MIF-1 protein,

while the 138 kd MIF-1 is induced only during the differentiation process. The functional role of these two distinct proteins which showed specificity for the same DNA binding sequence as well as the possible role of MIF-2 to MIF-4 in the control of the differentiation and proliferation of these cells is under investigation. Understanding the molecular mechanism by which nuclear regulatory proteins can by modulated may allow us to develop reagents to turn off uncontrolled expression of the *c-myc* gene.

C. Search for the function of the c-myc proteins.

Although there is a large body of evidence on the role of *c-myc* protein in cell proliferation, mitogenesis and differentiation, we still lack direct evidence on the molecular function of the *c-myc* proteins. There are four potential open reading frames (ORF) in the *c-myc* gene, however only three proteins have been identified in vivo. These are p67, p64 and p58. The p67 and p64 are derived from alternative initiation codons but share the same reading frame in exon 2 and 3. These proteins are conserved between species. The p58 protein is believed to be encoded by the exon 1 ORF which is only found in human *c-myc* gene. The objective of this project is to determine whether the p58 protein which is disrupted in almost every BL cell line has any biological significance. Our first set of experiments was to determine whether the p58 protein can interact with cellular proteins. We have prepared a construct in which the exon I ORF of the *c-myc* gene is fused to the gluthatione-S-transferase gene and the expressed fusion protein can than be purified from bacterial proteins by gluthatione beads. Preliminary experiments suggests that the myc fusion protein can interact with several proteins from HeLa and HL60 cells. Deletional analysis would allow us to establish the sequence in the *c-myc* protein required for this interaction and would provide us with the tool to use this specific myc fusion protein to screen an expression library to pull out the gene of the protein that the p58 myc may be interacting with. The ability of p58 *c-myc* to regulate expression of other genes either alone or with its cellular partner could be investigated. This technology would also be extended to the analysis of the p64 and p67 *c-myc* proteins. The possible interaction of proteins encoded by cellular antioncogenes (such as the retinoblastoma gene product) with the proteins encoded by the oncogenes (such as the *c-myc* gene product) to neutralize its oncogenic function is under investigation. These studies may help to understand the molecular function of the *c-myc* product and may lead to development of trans-dominant mutants (both oncoproteins as well as suppressor proteins) which may impede specific function of the *c-myc* gene.

PUBLICATIONS

Zajac-Kaye M, Yu B, Ben-Baruch N. Downstream regulatory elements in the *c-myc* gene. In: Potter M, Melchers F, eds. Current topics in microbiology and immunology; mechanism in B cell neoplasia. Berlin, Heidelberg: Springer-Verlag, vol. 166, 1990;279-284.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06731 03 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression and Regulation of the mdr1 Gene and Transforming Growth Factor Alpha

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Susan Bates	Senior Investigator	MB, COP, DCT, NCI
Others:	Liz Murphy	Biologist	MB, COP, DCT, NCI
	Stefania Scala	General Fellow	MB, COP, DCT, NCI
	Tung Ba Le	General Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pathology, DCBD, NCI (Maria Tsokos)

LAB/BRANCH

Medicine Branch

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of the laboratory work this year has been on multidrug resistance with special emphasis on breast cancer, and the regulation of both expression and function of the P-glycoprotein gene. We have evaluated differentiating agents in breast cancer, colon cancer, and neuroblastoma. In each system differentiating agents have differing effects, ranging from downregulation of the gene to increased expression with decreased function. Simultaneously, we have studied levels of expression in patient samples, from patients on drug resistance reversal trials for refractory lymphoma and breast cancer.

Studies of Expression, Regulation, and Reversal of Multidrug Resistance

Studies of P-glycoprotein Mediated Drug Resistance

1. Previous work in our laboratory had demonstrated that the polymerase chain reaction methodology could be used quantitatively to determine the level of expression of mdr-1 in small samples of tumor tissue. This methodology was applied to samples from patients entering onto the EPOCH protocol for refractory lymphoma in order to determine relationships between response, prior therapy, and mdr-1 expression. Samples were obtained as often as possible from patients before and after treatment with EPOCH. These studies demonstrated that mdr-1 expression was measurable in most patients, and present in half at levels known to be effectively antagonized in vitro in cytotoxicity assays. Immunohistochemical analysis of P-glycoprotein, and of B and T cell markers was performed on concurrent tissue samples to validate the mRNA results.

Studies in lymphoma in our laboratory also demonstrated expression of mdr-1 mRNA in normal T cells. Studies now are underway to determine whether this expression is increased by exposure to chemotherapy and whether the P-glycoprotein is associated with drug efflux in the T cells. Since the level of expression in the lymphoma cells and in the normal T cells is often comparable, these cells are being used to develop a sensitive and specific methodology for assessment of doxorubicin accumulation and effect of P-glycoprotein antagonists on accumulation in patient samples.

2. Modulation of P-glycoprotein expression by differentiating agents was studied in vitro in human breast cancer cell lines. A number of agents were evaluated, with the findings that one, 8-Cl-cAMP, was able to effectively down regulate expression of P-glycoprotein and mdr-1 mRNA. This agent was a weak differentiating agent, but rapidly inhibited mdr-1 mRNA expression, P-glycoprotein synthesis as measured by S-35 methinine labeling, and increased drug accumulation in the cells. We postulated that this effect was related to differentiation in that a number of features which had developed in the acquisition of drug resistance were reverted to the parental phenotype in the 8-Cl-cAMP treated cells. These included decreased expression of keratin and increased expression of EGF receptor.

3. In collaboration with Tito Fojo, we have performed studies on the effect of the differentiating agent sodium butyrate in a human colon cancer cell line, SW620. This cell line and its resistant derivatives demonstrate loss of P-glycoprotein function which occurs over a period of 48 hours, while levels of P-glycoprotein expression are increasing. This effect is linked to a progressive decline in the total phosphorylation of the protein, which is reversible within 12 hours of removal of the sodium butyrate.

4. The clinical trial in refractory breast cancer combining continuous infusion adriamycin and amiodarone as a P-glycoprotein antagonist has been closed to new accrual as a new trial design is under development. The current trial demonstrated increased expression of P-glycoprotein in half of the patients; and this expression predicted for shortened time to treatment failure.

5. Previous studies in neuroblastoma had demonstrated that the differentiating agent retinoic acid was able to increase expression of P-glycoprotein. These studies were then extended to patient samples in collaboration with Maria Tsokos of the Pathology Department to demonstrate that increased P-glycoprotein expression was found in more well-differentiated neuroblastomas. This work is being published, and we have now turned to the in vitro cell culture models to try to understand why the increase in P-glycoprotein expression is often not associated with active drug

efflux. These studies are being performed in collaboration with Marian Meyers and June Biedler of Memorial Sloan-Kettering; and demonstrate thus far that phosphorylation is not altered in the treated cells.

6. In order to study drug resistance in breast cancer, several cell lines have been selected for drug resistance in vitro during the past year. These models should provide the opportunity to further our understanding of the link in breast cancer between drug resistance, loss of estrogen receptor expression, and the increase in EGF receptor which was observed initially in two MCF-7 cell lines studied. Two of the cell lines demonstrate this phenotype, regardless of the mechanism of drug resistance obtained.

7. One adriamycin-resistant MCF-7 cell line which demonstrates increased EGF receptor has been studied with agents designed to interfere with the ligand/receptor pathway. This subline demonstrates enhanced sensitivity to growth inhibition by these agents compared to parental cells. Studies are underway to determine whether the drug resistance can be reversed by addition of the agents in combination with chemotherapy.

PUBLICATIONS

Deutsch, LA, Valverius EM, Mickley LA, Rosen N, Bates S. Modulation of EGF receptor expression by differentiating agents in human colon carcinoma cell lines. Cancer Communications 1990; 2 (no 10) 345-55.

Deutsch LA, Rudick JR, Fojo AT, Bates SE. Use of the polymerase chain reaction in the quantitation of mdr-1 gene expression. Biochemistry 1990;29:10351-56.

Bates SE, Shieh CY, Mickley LA, Dietich H, Lauriaux L, Fojo AT. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/Pgp) found in adrenocortical carcinoma. J Clin Endocrinol Metab, in press.

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Lai GM, Chen YN, Mickley LA, Fojo AT, Bates SE. P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma cell lines. Int J Cancer, in press.

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Bates SE, Shieh CY, Tsokos M. Expression of mdr-1/Pgp in neuroblastoma. Am J Path, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06732 03 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Modulation of the Expression of a Multidrug Resistance Gene (mdr-1)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Antonio T. Fojo	Senior Investigator	MB, COP, DCT, NCI
Others:	Lyn A. Mickley	Biologist	MB, COP, DCT, NCI
	Cynthia Herzog	Clinical Associate	MB, COP, DCT, NCI
	Manuel Alvarez	Guest Researcher	MB, COP, DCT, NCI
	Zhirong Zhan	General Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The focus of the laboratory continues to center around the problems of drug resistance. As in the past, three major areas continue to be actively investigated. They are: multidrug resistance mediated by P-glycoprotein, adriamycin resistance associated with overexpression of a 95 kilodalton membrane protein and mechanisms of cisplatin resistance.

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Multidrug Resistance Mediated by P-glycoprotein

This field has been of interest to the principal investigator for eight years. Work in this field is continuing. The emphasis continues on understanding several aspects of this field with a final goal of applying the findings to clinical trials. Several projects have been ongoing in the laboratory.

1. Work was completed on the primary sequence of over 100 P-glycoprotein from a wide variety of sources. The results unequivocally demonstrate the infrequent occurrence of acquired mutations during the selection of cells for multidrug resistance. In over 30 drug resistant sublines studied, acquired mutations were found in only two, without evidence in one that the mutation affected the resistance pattern. In addition, a high degree of sequence conservation was found in P-glycoproteins from these sources. Although highly conserved, genetic polymorphism was demonstrated in several colon cancer cell lines selected in the laboratory for drug resistance in a step wise manner, and this finding allowed at the molecular level the ability to precisely follow the acquisition of drug resistance. Overexpression of one or both alleles can occur during the course of drug selection, with gene amplification following or coinciding with overexpression of an individual allele. The evidence indicates that during the course of selection, a single clone emerges as a dominant cell, advancing its mechanism of resistance. The latter observation, is a finding of clinical interest and will be tested in patient samples in the next phase of this study. Plans to study this as well as the evolution of both mechanisms of resistance, especially the topoisomerases will be the direction this work assumes in the clinical arena. It is hoped with this approach to try to begin to understand the evolution of drug resistance in clinical samples.

2. The utilization of differentiating agents for reversal of drug resistance has also continued utilizing primarily colon cancer models, with plans to extend these observations to other models in the future. These studies, done in collaboration with Dr. Susan Bates have clearly demonstrated that differentiating agents can reverse multidrug resistance and that this reversal is additive and possibly synergistic with the effect of verapamil. The mechanism underlying this observation appears to be alteration in the phosphorylation of P-glycoprotein.

3. Continued emphasis on the study of models with low levels of P-glycoprotein has continued with research in unselected cell lines, expressing clinical levels of P-glycoprotein. These studies have begun to shift with a greater emphasis on models relevant to malignant lymphomas, and the development in the laboratory of several models of drug resistance in lymphoma. Plans are to examine these in detail, with special attention to drug scheduling and combinations of reversal agents.

Mechanisms of Cisplatin Resistance

This field is evolving into what will clearly become a major focus of the laboratory. To this extent, 15 cell lines have been isolated in the laboratory, including platinum resistant cell lines, as well as drug sensitive revertants. Studies to date have demonstrated a tight correlation between the level of a 55 kd protein and cisplatin resistance. In addition, a tight correlation has been demonstrated between constitutive metallothionein expression and platinum resistance. In collaboration with Dr. Eddie Reed, a correlation has also been shown between platinum accumulation and drug resistance. Current directions in this effort are proceeding in several directions simultaneously. Antibodies are being raised to the 55 kilodalton protein from two different sources, with testing of sera now in process. In addition, the protein has been purified and attempts at obtaining amino acid sequence are in progress. Demonstration of cytogenetic evidence indicating the possibility of gene amplification, as evidenced by the

presence of an abnormally banding region, will be followed in the near future by attempts to identify the existence of amplified sequence amenable to molecular cloning. The availability of excellent models, and the unequivocal findings to date promise that this field will become increasingly amenable to more in depth understanding of what it is hoped will be a unique mechanism of cisplatin resistance not previously identified.

Adriamycin Resistance Associated with Overexpression of a 95 Kilodalton Membrane Protein

Attempts continue to further investigate this mechanism. Collaborative efforts have demonstrated expression in human leukemia samples and constitutive expression in a highly resistant lung cancer cell line.

PUBLICATIONS

Chen YN, Mickley LA, Hwang JL, Acton E, Fojo AT. Characterization of adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. J Biol Chem 1990;265:10073-80.

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Bates SE, Shieh CY, Mickley LA, Dichek H, Loriaux DL, Fojo AT. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein (Pgp) found in adrenocortical carcinoma. J Clin Endocrinol Metab, in press.

Schilder KJ, Hall L, Monks A, Handel LM, Fornace AJ, Young RC, Ozols RF, Fojo AT, Hamilton TC. Metallothionein gene expression and resistance to cisplatin in human ovarian cancer. Int J Cancer, in press.

Lai GM, Moscow JA, Alvarez MG, Fojo AT, Bates SE. Contribution of glutathione and glutathione-dependent enzymes in the reversal of adriamycin resistance in colon carcinoma cell lines. Int J Cancer, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06734 01 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

The Role of Signal Transduction in the Regulation of Nuclear Oncogenes and Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Maria Zajac-Kaye	Senior Staff Fellow	MB, COP, DCT, NCI
Others:	Noa Ben-Baruch	Clinical Associate	MB, COP, DCT, NCI
	Bennett Yu	Clinical Associate	MB, COP, DCT, NCI
	Melissa Blake	Technician (Stay-in-School)	MB, COP, DCT, NCI
	Michele Exum	General Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We are interested in signal transduction pathways between the external stimuli generated at the plasma membrane and the nuclear oncogenes or other cellular and viral genes that can regulate gene expression and cell division. Such links may be provided by oncogenes located in the cytoplasm with protein kinase activity. Thus, we have investigated the possible role *raf* kinase may play in transmitting a signal to the nucleus to turn on or off expression of specific genes. The two major projects are the following:

A. Regulation of nuclear oncogenes expression by activated *raf* kinase.

B. Regulation of viral gene expression by activated *raf* kinase.

A. Regulation of nuclear oncogenes expression by activated *raf* kinase.

Recent experiments from our laboratory indicates that the *v-raf* oncogene, which constitutively expresses a kinase activity, may be transmitting a signal to the nucleus by phosphorylating nuclear substrates capable of regulating *c-myc* transcription. A *v-raf* gene, in which point mutations abolishes kinase activity, showed no effect. To study the mechanism of this regulation we have constructed a series of vectors in which regulatory elements of the *c-myc* promoter region are deleted. Cotransfection experiments of *raf* with these *c-myc* mutants fused to the CAT indicator gene will allow us to determine which regions of the *c-myc* gene are required for the response to the *raf* oncogene. The role of other protein kinase oncogenes which are capable of transmitting signal to the nucleus to regulate the *c-myc* gene will be evaluated as well. Understanding the mechanism by which oncogene products, disrupt signal transduction pathways thus affecting control of cell growth, will allow to design reagents to compensate for these effects and revert tumorigenicity.

B. Regulation of viral gene expression by activated *raf* kinase.

We have found that the *raf* oncogene can regulate transcription of the cytomegalovirus (CMV) promoter region. It has been shown that the CMV promoter contains several copies of NF-1 and NF-κB binding sequences. NF-1 is a cellular factor which plays a role both in DNA replication and in RNA transcription and NF-κB was first described as a protein which binds to the immunoglobulin κ enhancer. The NF-κB factor is present in an active form in the nucleus of a restricted set of cell types (mature B cells, differentiated monocytes, and some T cells). In most other cell types NF-κB is bound to an inhibitor and is present in an inactive form in the cytoplasm. Phosphorylation of the inhibitor molecule dissociates the complex and NF-κB relocates to the nucleus in its active form. Thus, the *raf* kinase may phosphorylate the inhibitor molecule and activate the NF-κB factor and we are currently testing this hypothesis. Moreover,we have shown, that activated *raf* kinase can activate NF-κB in a cotransfection experiments. In addition, this finding will also have implication in the regulation of the HIV-1 which is also under strong influence of NF-κB enhancer. These studies will investigate the role of oncogenes in the induction of viral genes and will also help to identify other cellular genes or oncogenes which are regulated by the cascade events of the signal transduction pathway.

PUBLICATION

Zajac-Kaye M, Yu B, Ben-Baruch N. Downstream regulatory elements in the *c-myc* gene. In: Potter M, Melchers F, eds. Current topics in microbiology and immunology; mechanism in B cell neoplasia. Berlin, Heidelberg: Springer-Verlag, vol. 166, 1990;279-284.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06735 01 M

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Treatment Trials in Breast Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Joyce A. O'Shaughnessy	Senior Investigator	MB, COP, DCT, NCI
Others:	Kenneth H. Cowan	Senior Investigator	MB, COP, DCT, NCI
	Andrea Denicoff	Research Nurse	MB, COP, DCT, NCI
	DianeSavarese	Staff Fellow	MB, COP, DCT, NCI
	Noa Ben-Baruch	Visiting Associate	MB, COP, DCT, NCI
	Jason Fisherman	Senior Investigator	CTEP, DCT, NCI

COOPERATING UNITS *(if any)*

NIH Pathology, Surgery Branch, Radiation Oncology Branch, Laboratory of Tumor Immunology

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Medical Breast Cancer Section has been investigating new therapeutic strategies for the treatment of breast cancer. These clinical trials have focused mainly on locally advanced breast cancer and metastatic disease; however, the therapeutic strategies developed in these advanced stages have recently been taken forward into early stage disease. The three major areas of clinical investigation over the past year have included: 1) overcoming drug resistance with dose intensive therapies; 2) the study of hematopoietic growth factors with intensive chemotherapy; and 3) new agents for breast cancer. Laboratory investigations of the breast cancer tissue obtained from patients are conducted in conjunction with the clinical trials as described in the following sub-projects. Future plans include the further development of highly dose intensive chemotherapy regimens in conjunction with peripheral blood stem cells autografts. Another approach will involve the continuation of the study of hemopoietic growth factors including interleukin-3 (IL-3) in combination with GM-CSF. A new strategy that will be developed and initiated in the coming year involves Phase I trials of radiolabelled monoclonal antibodies against breast cancer antigens. We also plan to continue our work with taxol and adriamycin, using this regimen as induction therapy for patients with metastatic disease. We will also develop a new treatment regimen combining tamoxifen, 4-HPR and alpha interferon. These three agents have demonstrated synergistic activity up against breast cancer through their induction of growth suppressing factors. Lastly, the Medical Breast Cancer Section will move over the next year to conduct gene therapy trials in which the mdr-1 gene is inserted into peripheral blood stem cells. Reconstitution of patients' bone marrows with these genetically altered stem cells will thereby allow us to study the administration of high dose mdr-1-associated chemotherapy agents.

Project Description

Additional Personnel Assigned on Project:

Mary McCabe	Clinical Trial Specialist	CTEP, DCT, NCI
Maria Merino	Pathologist	DBCB, NCI
David Danforth	Senior Investigator	SB, COP, DCT, NCI
Lori Pierce	Senior Investigator	ROB, COP, DCT, NCI
Jeffrey Schlom	Chief	NCI

Clinical Treatment Trials in Breast Cancer

Increasing the dose intensity of a cytotoxic drug or regimen can theoretically overcome several possible mechanisms of drug resistance including: 1) inadequate drug delivery to tumor, rapid drug clearance or inactivation; 2) inadequate drug uptake into cells; 3) increased concentration of a target enzyme; and 4) rapid inactivation or efflux of the drug such as that observed with the expression of P-glycoprotein in multi-drug resistant cells. In humans, the efficacy and toxicity of most cytotoxic drugs increase with higher doses of drug delivered. Interesting and provocative correlations between dose intensity and effectiveness have been drawn from several uncontrolled clinical studies. At the present time, however, the hypothesis that increasing the dose delivered per unit time to breast cancer patients will improve their outcome is still investigational.

A. Phase II Study of FLAC/GM-CSF in Patients with Locally Advanced and Metastatic Breast Cancer

The development of novel and dose-intensive regimens for the treatment of breast cancer has been a major priority of the Medical Breast Cancer Section. The availability of the hematopoietic growth factors has provided new opportunities to escalate the doses of drugs whose dose-limiting toxicity is myelosuppression. Over the past 2 and one half years, we have conducted Phase I and II trials of dose-intensive FLAC (5-Fluorouracil, Leucovorin, Adriamycin, Cytoxan) with GM-CSF (granulocyte - macrophage colony-stimulating factor) in patients with locally advanced and metastatic breast cancer. The average delivered doses of drugs in these trials are 30 to 50% higher than doses conventially administered; response rates have also been higher - 100% for locally advanced and 89% for metastatic disease. To date, over 80 patients with stage III and IV breast cancer have been treated with FLAC GM-CSF. 100% of patients have experienced grade IV hematologic toxicity. In 25% of the over 300 cycles of chemotherapy administered, patients have required hospitalization for fever and neutropenia. In locally advanced patients, the neoadjuvant FLAC/GM-CSF has resulted in 33% of patients achieving pathologic complete responses. In these patients, radiation therapy alone is administered after chemotherapy instead of the standard mastectomy plus radiation therapy. In this way we are studying whether long-term local control is achievable without mastectomy in the setting of pathologically - confirmed eradication of tumor.

In conjunction with this Phase II study, selected patients are being studied for their hematologic stem cells response to GM-CSF. In collaboration with the Transfusion Medicine Department, the peripheral blood stem cells of patients are quantitated during the period of recovery following the chemotherapy-induced nadir. Our preliminary findings to date indicate a vigorous stem cell response to GM-CSF following the nadir in cycle 1. Interestingly, the stem cell response for the subsequent cycles 2 and 3 are significantly less vigorous compared to cycle 1. These studies have major implications for our future studies which will attempt to harvest large numbers of peripheral blood stem cells from patients for future autografts. In addition, these studies may shed insight into the effects of the growth factors on the stem cell population with repeated cycles of

chemotherapy. Our FLAC/GM-CSF regimen has recently been taken into the neoadjuvant and adjuvant treatment of Stage II breast cancer in a randomized trial at NCI. It is hoped that the increased dose intensity of this regimen will improve the cure rate of these early stage patients with micrometastatic disease.

The Phase II study of FLAC/GM-CSF in Stage IV breast cancer has been completed recently. We anticipate that accrual to the Stage III study will be completed in the coming year.

B. Phase I Study of FLAC plus IL-3 plus GM-CSF

Our second generation study of FLAC plus hematopoietic growth factors will begin in the next several months. Interleukin - 3 (IL-3) is a multi-lineage growth factor that stimulates the proliferation of early myeloid precursors including megakaryocytes. Animal studies have demonstrated marked synergy between IL-3 and GM-CSF when given sequentially, especially regarding circulating CFU-GM's, where a 63-fold rise in these progenitors was found. We will conduct a Phase I study of FLAC + IL-3 + GM-CSF to determine the optimal dose of IL-3 for enhancing granulocyte and platelet recovery from chemotherapy. The dose intensity of FLAC chemotherapy will also be a major endpoint of this trial as the ultimate goal of combination growth factor therapy is not only toxicity reduction, but also improved response rates.

This study will include a detailed analysis of the recovery patterns of peripheral blood stem cells and progenitors following FLAC + IL-3 + GM-CSF. These data will be critical in our future efforts to collect peripheral blood stem cells (PBSC) for autografts and gene transfer studies.

C. Phase II Study of Fazarabine in Patients with Metastatic Breast Cancer

Fazarabine is a new anti-metabolite which combines the structural features of two effective anti-neoplastic agents, cytosine arabinoside (Ara-C) and 5-azacytidine. Fazarabine (arabinosyl-5-azacytosine) inhibits DNA synthesis but has little effect on either RNA or protein synthesis. Fazarabine has also been shown to induce differentiation in HL60 cells, a property which presumably reflects its ability to inhibit DNA methylation. Pre-clinical in vitro and in vivo studies suggest a broad spectrum of activity of fazarabine. In contrast with both parent compounds which have little activity against solid tumors, fazarabine displays a wide spectrum of activity in the NCI panel of human tumor xenografts. Pre-clinical anti-tumor studies also indicate that the schedule of fazarabine administration is important with more frequent administration of fazarabine being associated with enhanced cytotoxic effects.

The Medical Breast Cancer Section conducted a Phase I study of fazarabine in 27 patients with advanced malignancies that were refractory to standard treatments. All but one patient had been previously treated with a mean of 2.5 different chemotherapy regimens. Eighty-three cycles were administered with doses ranging from 0.2 to 5.94 mm/m^2/hr for 72 hours. The dose limiting toxicity for fazarabine was bone marrow suppression and it occurred mainly in the form of leucopenia and thrombocytopenia. Out of 83 cycles of therapy, only 4 episodes of fever and neutropenia occurred requiring hospitalization for intravenous antibiotics. No major bleeding occurred as a result of the thrombocytopenia. In this Phase I study, three minor clinical responses were seen in patients with metastatic adenocarcinoma of unknown origin, metastatic colon cancer and in multiply relapsed embryonal testicular cancer. This study suggested that fazarabine may have activity in solid tumors, particularly in testicular cancer and adenocarcinoma. The Phase II dose for previously treated patients was determined to be 2.0 mg/m^2/hr as a 72 hour continuous intravenous infusion. For previously untreated patients; the recommended dose is 2.5mg/m^2/hr for 72 hours. This Phase I study has recently been published. A Phase II study of colon cancer was

conducted in our section; zero out of 14 responses were seen. We concluded therefore, that fazarabine does not have significant activity against metastatic colon cancer at the doses studied. The manuscript for this study is being prepared.

Over the past year, we have conducted a Phase II study of fazarabine in patients with metastatic breast cancer. Eligibility criteria include measurable disease and previous treatment with no more than one prior chemotherapy regimen. To date, 23 (21 evaluable for response; 2 too early) patients have been accrued to this Phase II study and three partial responses have been noted. Toxicity has been limited to grade 3 and 4 mylosuppression with no serious infections documented. Two episodes of grade 4 hepatic toxicity have been noted. The treatment with fazarabine has been very well tolerated overall. The accrual goal for this study is 28 patients and we anticipate that this will be accomplished within the next several months. Because it appears that fazarabine has activity against metastatic breast cancer, we will consider a future study combining fazarabine with G-CSF in order to increase the dose of fazarabine delivered.

D. Phase I Study of Taxol and Adriamycin with G-CSF in Previously Untreated Metastatic Breast Cancer Patients

Taxol is an anti-mitotic cytotoxic agent derived from the bark of the western yew tree, Taxus brevifolia. In 1977, Taxol was chosen for clinical evaluation based on good activity against B16 melanoma and human mammary tumor xenografts. In addition, taxol has a unique mechanism of action. Unlike other anti-mitotic agents such as colchicine and podophyllotoxin which inhibit microtubule assembly, taxol promotes microtubule assembly and stabilizes tubulin polymer formation. Taxol has been studied in several Phase I trials; the cumulative data suggest that the dose limiting toxicity is reversible mylosuppression. In addition, substantive peripheral neuropathy has been observed at doses of 275 mg/m^2 and higher. Phase I and II studies have suggested that taxol may have significant activity in human ovarian cancer, malignant melanoma, and non-small cell lung cancer.

In a Phase II study of metastatic breast cancer patients at M.D. Anderson, 25 evaluable patients were treated with taxol. Fourteen patients had received prior adjuvant therapy and 11 patients had received prior therapy for metastatic disease. Twenty-three patients had been treated with prior adriamycin; 6 had progressed while on adriamycin. Taxol was administered as a 24 hour continuous infusion on day one of a 21 day cycle. Eighteen patients started at a dose of 250mg/m^2. The starting dose was subsequently reduced 25% to 200 mg/m^2 and, depending on toxicities, subsequent doses were reduced by 10% decrements. The major toxicity was myelosuppression in this study. In this Phase II study, there were three complete responses with an overall response rate of 48%. This 48% response rate in previously treated patients with metastatic disease suggests that taxol is a significantly active new agent for metastatic breast cancer.

Our Phase I study, started in March, 1991, combines taxol and adriamycin together with G-CSF. Adriamycin is the most active single agent for the treatment of breast cancer and results in remissions in 40 to 55% of patients. In our study, taxol and adriamycin are administered as 72 hour continuous intravenous infusions. It has been shown that prolonged infusion of adriamycin reduces peak plasma levels and may diminish both cardiac toxicity and nausea and vomiting. In addition, prolonged exposure of tumor cells to chemotherapeutic agents may also mitigate the effects of multi-drug resistance proteins. It has been shown for vincristine, another microtubule-active agent, that the drug's net effect on cycling cells can be increased with prolonged exposure,

and evidence exists that some adriamycin resistant cell lines develop cross resistance to taxol via an mdr mechanism.

In our Phase I study, patients with previously untreated metastatic breast cancer are treated with a 72 hour continuous infusion of taxol and adriamycin. G-CSF is then administered subcutaneously daily from days 4 through 18. Cycles are repeated every 21 days. The starting doses of taxol and adriamycin are 160 mg/m^2 and 45 mg/m^2, respectively. For subsequent dose levels the adriamycin is escalated by 15 mg/m^2 the taxol subsequently escalated in increments of 20mg/m^2. To date, the major toxicity observed has been mylosuppression which has been reversible in all cases. No significant peripheral neuropathy or cardiac toxicity has been observed. The Medical Breast Cancer Section is collaborating with the National Heart, Lung and Blood Institute to closely monitor the potential cardiac toxicity of this new regimen. Accrual to this study and dose escalation are continuing.

Upon completion of this Phase I study, we will consider altering the schedule of administration to study whether taxol and adriamycin in combination with G-CSF can be administered every 14 days. Preliminary data from our current study suggest that the hematopoietic nadir from this regimen occurs early (day 7) and that the hematologic recovery is quite rapid with G-CSF. It may be that the dose intensity of this regimen can be further increased with a 14 day cycle of administration. Because our Phase I study is limited to patients with measurable metastatic disease, we will have some preliminary data on the activity of this new regimen in previously untreated metastatic breast cancer patients.

E. A Phase I Study of High-Dose Piroxantrone Alone and in Combination With Granulocyte Colony Stimulating Factor (G-CSF)

Piroxantrone (oxantrazole) is one of a series of anthracycline derivatives initially designed and synthesized to decrease cardiac toxicity while maintaining significant tumor activity. Reactive oxygen species such as semiquinone free radicals have been postulated to be important in the development of anthryacycline induced cardiac toxicity via peroxidative injury to membrane lipids in cardiac tissue. Piroxantrone has a decreased potential for superoxide free radical formation through chromophore modifications of the anthracenedeione nucleus. In rat liver microsomal preparations, superoxide dismutase-sensitive oxygen consumption (a measure of free radical formation) is 5 to 10 times lower with piroxantrone as compared with adriamycin. In addition, piroxantrone appears less cardiac toxic than doxorubicin in the cultured fetal mouse heart model.

Although the mechanism of cytotoxicity and anti-tumor activity is not completely understood, piroxantrone has been shown to intercalate into DNA, induce single and double strand breaks, and inhibit DNA synthesis. The single strand breaks are protein associated and slowly repaired, raising the question of whether piroxantrone may function in conjunction with topoisomerase II. RNA and protein synthesis are also inhibited to a lesser degree than DNA synthesis, in contrast to adriamycin and mitoxantrone where the effects on RNA and DNA synthesis are equivalent.

In the initial anti-tumor screens, piroxantrone showed a broad spectrum of activity both in vivo and in vitro, demonstrating improved survival and anti-tumor activity in almost all murine tumor models tested. Two human Phase I studies have been conducted with piroxantrone. The first study was conducted at the Mayo Clinic and the dose limiting toxicity was leukopenia. The recommended Phase II starting dose was 160 mg/m^2. In this study, responses were seen in two

patients: one breast cancer patient and another with metastatic melanoma. In the second Phase I study at Johns Hopkins, the dose limiting toxicity again was leukopenia. The recommended Phase II starting dose in this study was 150 mg/m^2 . No patients achieved a partial or complete response during this study.

In our Phase I study of piroxantrone with G-CSF, the primary objective is to establish a new maximally tolerated dose for this combination. We will explore the ability of G-CSF to increase either the absolute dose of piroxantrone administered or the overall dose intensity delivered. In addition, we will examine the pharmacokinetics of metabolism and elimination of the piroxantrone when administered both alone and in combination with G-CSF. Another major endpoint of this study is a careful examination of the cardiac toxicity of piroxantrone.

This study was activated in March, 1991 and accrual has been rapid. Two dose levels of piroxantrone, 160 mg/m^2 and 185 mg/m^2, have been evaluated and no significant dose limiting toxicity in combination with G-CSF has been observed. The dose of piroxantrone will be escalated by 25% for subsequent levels. Our preliminary data suggest that the MTD of piroxantrone will be significantly higher in combination with G-CSF than when given singly.

F. A Pilot Study of the Ability of Alpha Interferon to Upregulate the Expression of Tumor Associated Antigens

Certain tumor cell surface proteins are capable of eliciting an immune response in vivo; these proteins have been termed tumor-associated antigens (TAA's). Interferons are proteins with anti-viral, anti-proliferative, immunomodulating and differentiating effects. It has been shown by several investigators that interferons are capable of enhancing the expression of class I and II histocompatibility antigens on the surface of human tumor cells. In vitro, breast cancer cell lines that constitutively express low levels of tumor-associated antigens are highly responsive to induction of expression by interferons. Greiner et al. have demonstrated a dose dependent increase in the expression of the antigen TAG-72 on MCF-7 cells with alpha interferon per ml. Similar results have been shown for CEA and the antigen recognized by the monoclonal antibody B6.2.

Interferons have also been demonstrated to increase expression of estrogen and progesterone receptors. In a preliminary study from Memorial Sloan Kettering, three million units of interferon alpha given daily for 14 days was used to modulate the expression of the estrogen and progesterone receptors in women with metastatic breast cancer. One of five patients initially ER/PR negative converted to positive, and two of four patients initially ER/PR positive showed increases in receptor levels. One of these latter patients who had been refractory to hormone therapy achieved a partial a response with the combination of tamoxifen and interferon.
The goal of our present study is to examine the ability of systemically administered interferon-alpha to upregulate the expression of TAG-72, estrogen and progesterone receptors, and other tumor-associated antigens. The upregulation of these antigens by alpha interferon will be correlated with the serum levels of interferon achieved with subcutaneous administration. We will also examine the ability of alpha interferon to induce shedding of tumor cell surface antigens by measuring circulating TAA serum levels pre- and post-interferon administration. Patients will be treated with 5 MU/m^2 of alpha interferon subcutaneously daily for 3 days. Biopsies of tumor will be obtained pre- and post-interferon administration. This tissue will be evaluated by immunohistochemistry for tumor-associated antigen levels and estrogen and progesterone receptors. These laboratory

examinations will be conducted in the laboratory of Drs. Jack Greiner and Jeffrey Schlom at NCI. In addition, serum will be obtained for circulating tumor-associated antigens pre- and post-interferon administration.

This study has accrued 4 of a planned 20 patients to date. The demonstration that systemic administration of alpha interferon leads to upregulation of the TAG-72 antigen will be helpful in designing future therapy trials with radiolabelled monoclonal antibodies against this antigen.

G. Future Plans

1. Retroviral Transfer of Drug Resistant Genes

The transgenic experiments by Pastan and Gottesman have demonstrated that transfer of the mdr gene into bone marrow stem cells is feasible and rational. Dr. Nienhuis et al. has infected mouse bone marrow stem cells with a retroviral vector containing the mdr-1 cDNA and has demonstrated expression of the mdr protein in vivo. The Medical Breast Cancer Section will collaborate with Dr. Nienhuis to develop an mdr-1 gene therapy project that will be taken into treatment trials in breast cancer patients. The proposed protocol would harvest patients' peripheral blood stem cells co-cultivate them with host stromal cells and the mdr-1 retroviral vector. Cells would then be selected for the CD34 and Kit markers which enrich for progenitors and stem cells. Patients would be treated with high dose alkylator therapy and reconstituted with the mdr-1 infected stem cells. Post reconstitution, the patients would be treated with a high dose chemotherapy regimen involving natural products, perhaps taxol and adriamycin. The expression of the infected mdr-1 gene in the host bone marrow and peripheral blood cells will be examined using PCR techniques and the toxicity induced by the adriamycin/taxol regimen compared to patients who are reconstituted with cells not containing the mdr-1 retroviral vector. In this way we hope to establish a link between the expression of the mdr-1 gene in the bone marrow cells and protection against hematologic toxicity.

2. High Dose Alkylators and Peripheral Blood Stem Cell Autograft as Intensification Therapy

Investigators around the country are currently utilizing one or two cycles of very high dose chemotherapy plus stem cell autografts as intensification therapy in patients with advanced breast cancer. We are interested in developing a multiple cycle, very high dose combination chemotherapy regimen with peripheral blood stem cell support. It is not yet known whether more prolonged therapy with very high dose agents will achieve greater tumor eradication compared with "the big bang" of one or two cycles. Theoretically, tumor cells that are able to escape the lethal effects of a single cycle of high dose drugs through enhanced DNA repair, for example, might succumb to repeated cycles of the same. In addition, it may be that such a low growth fraction tumor would be more effectively treated with more prolonged intensification therapy.

We plan to conduct a Phase I study of combination high dose alkylator therapy together with hematopoietic growth factors every 28 days for a total of 3 or 4 cycles. Once the dose has been reached where hematopoietic recovery has not occurred by 28 days with growth factors alone, peripheral blood stem cell support will be added to attempt further escalation of the high dose alkylators. The goals of this study will be to explore the feasibility of administering 3 or 4 cycles of very high dose therapy, and to determine the maximally tolerated doses of the agents with hematopoietic growth factors alone and with peripheral blood stem cell support. This study will be an important preliminary to our future gene therapy trials with the mdr-1 gene.

3. Radiolabelled Monoclonal Antibodies

The NCI intramural program has and will have access to several novel murine, chimeric and single chain antigen monoclonal antibodies against adenocarcinomas in collaboration with Dr. Jeffrey Schlom. These monoclonal antibodies are being radiolabelled with novel isotopes such as the beta emitter Lutecium-177 and represent a new treatment strategy for breast cancer. Breast cancer provides fertile ground for testing these new agents. While initially highly sensitive to cytotoxic agents, cures are very hard to come by, and new approaches are needed to consolidate the initial cytoreduction achieved with intensive chemotherapy. Particularly in Stage II, III, and IV breast cancer patients who are clinically free of disease following chemotherapy, radiation and surgery, follow-up therapy is needed to prevent the relapses that are destined in the majority of patients. The Medical Breast Cancer Section is working closely with collaborators in the Radiation Oncology and Nuclear Medicine Departments to develop this new therapeutic strategy.

Our first clinical trial will be a Phase I study of intravenously administered murine CC-49 radiolabelled with the beta emitter Lutecium-177 in patients with metastatic breast cancer. We anticipate that this trial will begin in early 1992. CC-49 is a monoclonal antibody against the purified pancarcinoma antigen TAG-72. TAG-72 is expressed on the surface of 70 to 80% of human breast carcinomas. It has recently been demonstrated that CC-49 binds to and is able to image sites of metastatic disease in patients with colon cancer. Lutecium-177 is an interesting new beta emitter with a radiation path length of approximately 500 cell diameters. Unlike iodine-131, a gamma emitter which must be administered to patients in the hospital due to radiation safety concerns, the short path length of Lutecium-177 will likely allow it to be administered to outpatients, at least until the higher dose levels are reached. Lutecium-177 also has some weak gamma emissions which will allow imaging of patients' tumors. We anticipate the need to increase the dose of the Lutecium-177 delivered to very high levels, levels that may require hematopoietic stem cells support. The Medical Breast Cancer Section anticipates a multi-year commitment to the development of these novel therapeutics.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06736 01 M

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): The Clinical Therapy of Ovarian Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eddie Reed	Senior Investigator	MB, COP, DCT, NCI
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	Mace Rothenberg	Senior Investigator	MB, COP, DCT, NCI
	Debra Adamo	Research Nurse Specialist	MB, COP, DCT, NCI
	Patricia Davis	Research Nurse Specialist	MB, COP, DCT, NCI

COOPERATING UNITS (if any)

Clinical Pharmacology Branch, COP/DCT/NCI; Radiation Oncology Branch, COP/DCT/NCI; Division of Cancer Biology and Diagnosis, NCI

LAB/BRANCH: Medicine Branch

SECTION: Section of Genitourinary Malignancy

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5	5	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This unit has focused on the development of new treatment strategies for patients with advanced stage ovarian cancer, using as a primary resource patients with recurrent/refractory disease. In patients who have not received prior therapy,we have focused on the utility of "dose intense" platinum therapy in the ability to induce complete response, as well as possibly enhancing the duration of that response.

Project Description

During this year we have conducted the following studies:

1) Phase I study of Taxol and G-CSF in patients with refractory ovarian cancer. We have shown that with G-CSF support, taxol can be safely given at a dose of 250mg/m^2/q 3 wks. At 300mg/m^2/q 3 wks, peripheral neuropathy is the dose-limiting toxicity. The manuscript for this study has been submitted for review. As a result of these findings, we are now conducting a phase two study of this combination.

2) Several studies have been completed in refractory disease:

a) 5FU and Leukovorin -- 29 patients were treated with this combination at 375 and 500mg/m^2 respectively, qdx5, repeated every 3-4 wks. Only two patients experienced an objective response, however 13 patients experienced stable disease for time periods ranging from 4 to >24 months.
b) Suramin in ovarian cancer -- 9 patients were treated in a fashion that is associated with good patient response in prostate cancer. There were no objective responses. Three patients experienced stable disease with a greater than 50% drop in the CA125 level. These three patients showed higher drug levels and reduced drug clearance values that were statistically significant from patients who had progressive disease. Manipulation of the drug's pharmacology in this disease is being considered.
c) In collaboration with Thomas Delaney of ROB,DCT,NCI, we are assisting in the development of a phase I study of intraperitoneal Pseudomonas exotoxin conjugated to the OVB3 antibody. A report of that study has been accepted for publication.
d) In collaboration with Ira Pastan of DCBD/NCI, we assisted in the execution of a phase I study of intraperitoneal Pseudomonas exotoxin conjugated to the OVB3 antibody. A report of that study has been accepted for publication.

3) Tetraplatin is a third generation platinum analog that has exciting pre-clinical activity in tumors that are resistant to cisplatin or carboplatin. We are now conducting a phase I study with this agent, and have written a preliminary report on the early findings.

4) Concurrent with the clinical aspects of these studies, we have studied platinum drug resistance as well as parameters of renal function in patients. These studies have helped to further our understanding of clinical resistance to platinum compounds, and have assisted in developing strategies for practical tissues regarding patient management.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03024-22 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Treatment of Extensive Stage Small Cell Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	John C. Phares, MD	Head, Oncology Branch	NNMC
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, Ph.D.	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

COOPERATING UNITS *(if any)*
Radiation Oncology Branch; Biostatistics & Data Management Section; Surgical Oncology Division and Hematopathology Branch, National Naval Medical Center, Bethesda.

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although a dose-response curve clearly exists for alkylating agents in the initial chemotherapy of small cell lung cancer, the therapeutic benefit of higher than standard doses of the more recently introduced regimen of etoposide/cisplatin (VP16/PLAT) is uncertain. We randomized at least partially ambulatory patients with extensive stage SCLCand without major organ dysfunction to receive either VP16 80 mg/m squared + PLAT 27 mg/m squared Days 1-5 q 3wks or VP16 80 mg/m squared Days 1-3 + PLAT 80 mg/m squared Day 1 q 3 wks for the first 6 wks of therapy. Nonambulatory patients and those with organ dysfunction were assigned standard dose treatment. All patients received the standard dose regimen during wks 7-12. From wks 13-24, patients in complete response (CR) continued standard dose VP16/PLAT, while all other patients received a new 3-drug regimen that led to further improvement in response in only 5 cases. CR's were given prophylactic cranial irradiation. One hundred and eight patients have been entered (88 of whom were randomized). With a median follow-up of 55 mos, preliminary results are:

	N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt
High	40	25%	85%	12 mos	1,600	53,000
Standard	43	21%	81%	11 mos	2,500	161,000
Nonrand	25	4%	72%	6 mos	1,800	89,000

CR rates (p=0.86) and survival (p=0.93) were similar in patients randomized to high and standard dose therapy. There were 2 treatment-related deaths in the high and one in the standard dose arm. We conclude 1) standard dose VP16/PLAT is at least as active as any regimen we have ever utilized for for extensive stage SCLC and produces only modest myelotoxicity, and 2) there is no evidence of superior efficacy when planned drug doses are increased by 67% during the first 6 wks.

PROJECT DESCRIPTION

Treatment of Extensive Stage Small Cell Lung Cancer

Professional Staff:

PI:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	John C. Phares, MD	Head, Oncology Branch	NNMC
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, PhD	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

Collaborating Branches:

Eli Glatstein and Catherine Salem, Radiation Oncology Branch; Seth M. Steinberg, Biostatistics & Data Management Section; Bimal Ghosh, Surgical Oncology Division, National Naval Medical Center, Bethesda; James Cotelingham, Hematopathology Branch, National Naval Medical Center, Bethesda.

Objectives, Rationale, and Background:

This trial has several objectives. We wished to determine in a prospective randomized fashion whether high doses of etoposide (VP16) and cisplatin (PLAT) given during a six-week induction period would produce higher complete response rates and better survival than standard doses of the same drugs in patients with extensive stage small cell lung cancer (SCLC). We also wished to assess the feasibility and value of individualized chemotherapy selection based upon in vitro drug testing of tumor cell lines derived from pre-treatment patient tumor specimens. Objectives of this portion of the study were to determine the frequency with which tumor-containing specimens can be obtained from unselected patients with extensive stage SCLC, the frequency of successful cell culture and drug sensitivity testing, the degree of heterogeneity of drug sensitivity among different cell lines the correlation between in vitro drug sensitivity and clinical response, and the clinical utility of individualized drug selection based upon in vitro data.

The introduction of combination chemotherapy into the management of SCLC has led to four- to five-fold improvement in median survival and five-year disease-free survival in a small fraction of patients. Although median survival is improved to approximately the same degree compared to untreated patients in limited stage and extensive stage disease, survival of two years or more only rarely occurs in patients with extensive disease, defined as tumor extending beyond the hemithorax of origin and the regional lymph nodes. Furthermore, chest irradiation has never been suggested to yield any survival benefit in extensive stage patients. Therefore, virtually all patients with extensive SCLC are suitable subjects for investigational chemotherapy studies.

Methods employed:

Moderately aggressive chemotherapy which produces leukopenia in the range of 1,000/mcl has been shown to be superior to less intensive treatment that is virtually never associated with leukopenic fever in both randomized and non-randomized studies. However, even more intensive initial (or induction) therapy which is so myelosuppressive that hospitalization of all patients is required has not been demonstrated to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been restricted to cyclophosphamide, doxorubicin, and VP16. VP16/PLAT has been shown to be a highly synergistic combination regimen in treatment of murine leukemia and in early studies appears to be as active as most three- or four-drug combinations in patients with SCLC. VP16/PLAT is also more active than VP16 alone as a salvage regimen in this tumor. PLAT in higher than conventional doses appears to have increased activity in testicular and perhaps ovarian cancer. Although higher than standard doses of VP16/PLAT have been employed in small studies in SCLC, the issue of dose-response with this combination has not been addressed in a prospective randomized trial. We therefore initiated such a study. The first four patients randomized to the high dose regimen received VP16 120mg/m2 x 5 and PLAT 40mg/m2 x 5. Two died of infection before Day 21 without recovery from myelosuppression, and the doses of drugs on the high dose arm were subsequently reduced to VP16 80mg/m2 x 5 and PLAT 27mg/m2 x 5. Throughout the trial, doses on the standard arm have been VP16 80mg/m2 x 3 and PLAT 80mg/2 x 1.

Since a significant minority of extensive stage SCLC patients are not candidates for a very myelosuppressive regimens, such patients (deemed "poor risk") are not randomized but rather assigned to standard dose therapy.

For the past 10 years, the human tumor stem cell assay of Hamburger and Salmon has been most commonly employed for in vitro drug testing of human cancer. In applying this test to fresh tumor specimens from our SCLC patients, however, we found that sufficient tumor colonies for adequate in vitro testing of even a single drug were present only 23% of the time. Clearly, different approaches were needed to apply in vitro drug testing to a large fraction of patients. Since our laboratory has considerable experience in establishing permanent cell lines of SCLC, we decided to utilize cell lines rather than fresh tumors for drug testing. Compared to fresh tumors, cell lines provide tumor cells that are free of contaminating stromal cells and can be subjected to repeated testing. The time from specimen procurement to assay results, however, is delayed.

A modification of the Weisenthal dye exclusion assay was employed for drug testing because the assay is technically simple, does not require a single cell suspension, can be completed in four days, and can be applied to many tumors and most cell lines. Reading the assay, however is labor intensive and subjectiveand can be confounded by cell clumping.

Major Findings:

One hundred and eight patients have been entered. Median follow-up from time of patient entry is approximately 55 months. Twenty-five of the 108 patients were assigned standard dose therapy because of poor performance status, brain, lung or cardiac dysfunction, or refusal to be randomized. The remaining 83 were randomized to receive high or standard dose VP16/PLAT for the first 6 weeks of therapy.

On the high dose arm, 34 (85%) of 40 have responded to therapy, including 10 (25%) complete responders, and actuarial median survival is 12 months. On the standard dose arm, 35 (81%) of 43 patients responded, including 9 (21%) complete responders, and actuarial median survival is 11 months. There is no significant difference between the two groups in complete response rate ($p = 0.86$) or overall survival by the logrank test ($p = 0.93$). As expected, the response rate (4% complete, 72% complete plus partial) and survival (actuarial median 6 months) are inferior in patients judged not suitable for randomization. Among all 108 patients, performance status and number of distant organ systems involved with metastatic disease (0-2 vs. 3-7) are significant predictors of survival ($p < 0.001$ and $p = 0.005$, respectively).

Hematologic toxicity is significantly worse on the high dose induction program (median nadir WBC count 1,600/mcl and platelet count 53,000/mcl) compared with the standard dose induction (median nadirs 2,500 and 161,000, respectively). Among the poor risk nonrandomized patients, median nadir WBC count has been 1,800/mcl and median nadir platelet count, 89,000/mcl. Although only 43 patients have been treated, the standard dose regimen yields results are at least as good as our historical experience in good risk extensive stage SCLC with considerably less hematologic toxicity, suggesting it may have a superior therapeutic index.

A total of 141 pre-treatment staging specimens have been submitted for cell culture from the first 80 patients (1.8/patient). Seventy-eight specimens (55%) contained tumor cells. Twenty-eight cell lines, defined as sufficient in vitro amplification of tumor cell number to allow testing of multiple drugs in duplicate at three concentrations, have been obtained. The largest numbers of positive specimens and cell lines were derived from bone marrow, peripheral lymph nodes, and pleural effusions. Procurement of only five specimens required administration of general anesthesia, but three of these five procedures were performed for diagnostic purposes. Among the 80 patients with a minimum six-month follow-up, at least one staging specimen reached the cell biology laboratory in 79 (99%), and a tumor-containing specimen was procured from 60 (75%) of these previously untreated patients. A cell line was obtained from 26 (33%), or 43% of patients from whom a tumor-containing specimen was available. In addition, tumor-containing specimens have been obtained from 17 of these patients after tumor progression on chemotherapy, and a cell line has been successfully grown from eight.

Actuarial median survival of patients from whom a tumor cell line was successfully grown, patients from whom a tumor-containing specimen was obtained but did not grow in vitro, and patients from whom no tumor-containing specimen could be procured was 8, 11, and 15 months respectively. Patients with no tumor specimen had superior survival by the logrank test ($p < 0.02$). The survival of patients whose tumor specimens were or were not successfully cultured was not significantly different ($p = 0.60$). Thus, whether a patient had sufficient tumor dissemination that a biopsy specimen could be relatively easily obtained was of greater prognostic import than whether a cell line could be established from a positive biopsy specimen.

In vitro drug testing has been completed on tumor cell lines derived from 24 previously untreated SCLC patients. In vitro drug sensitivity of these cell lines correlated extremely well with response to therapy to VP16/PLAT. In 14/15 (93%) lines from patients with complete or partial response at 12-week restaging, two or more drugs were "active." Sensitivity patterns were strikingly different in the six lines from patients who never responded to VP16/PLAT or had progressed by Week 12. In none of these lines were two or more "active" drugs identified. For each of the seven drugs considered individually, lines from responding patients always exhibited a lower mean cell survival at the reference concentration than lines from non-responding patients.

Evaluation of these differences with the 2-sample rank yielded p values of less than 0.05 for VP16, doxorubicin, vincristine, and mechlorethamine, and less than 0.10 for methotrexate.

Complete response rates to the first chemotherapy regimen given after VP16/PLAT were compared in patients receiving an "in vitro best regimen" based on in vitro drug testing, or in those receiving vincristine/doxorubicin/cyclophosphamide (VAC) when in vitro drug testing results were not available for whatever reason. Thirty-five patients were treated with VAC after failure to achieve complete response by Week 13, and eight after relapse from complete response induced by VP16/PLAT. In these 43 patients, there were three complete responses (7%). Among the 16 patients who received their "in vitro best regimen," 13 had failed to achieve complete response at Week 13, and three had relapsed. Four patients (25%) attained complete response to their chemotherapy program based on in vitro drug testing ($p = 0.16$, Fisher's exact test).

Significance to Biomedical Research and the Program of the Institute:
Thus far, there is no indication from this study that a high dose regimen of VP16/PLAT (67% higher doses of each drug, 46% higher doses/unit time actually administered) is in any way superior to standard doses of this two-drug regimen.

On the other hand, the standard dose program is well tolerated and may be as effective as any other SCLC regimen, based on this data and that of others. Given the low complete response rate to any of the drug programs given to partial or non-responders at Week 13, it is likely that most or all of the survival benefit our patients received from therapy was produced solely by VP16/PLAT.

The interim results of this trial serve to emphasize several problems that arise in implementing a program of individualized chemotherapy selection with our current technology and study design. First, procurement of tumor specimens, establishment of cell lines, and drug testing are extremely labor intensive and time consuming. More efficient assay techniques and better understanding of the relationship between in vitro and in vivo pharmacokinetics would be valuable. Second, drug testing has been possible in only one-third of patients, and improved methods of cell culture are still needed. We believe these interim results justify the more frequent employment of major surgical procedures to procure larger, more rapidly grown tumor specimens in good risk consenting patients, and have already begun such a program in limited stage patients, who would be expected to more frequently be able to tolerate elective general anesthesia. And third, with the time required to establish and perform drug testing on cell lines, treatment based on in vitro testing can often be given only 10 to 12 weeks after a tumor specimen is obtained and may not be relevant to the in vivo drug sensitivity pattern present in residual tumor cells present at that time. Procurement of larger tumor specimens could help to alleviate this problem and allow more rapid drug testing and quicker administration of "individualized" chemotherapy.

Proposed Course:
In a statistical analysis done one year ago, when 77 patients with follow-up had been randomized, 95% confidence limits for differences in 12-month survival ranged from favoring the high dose arm by as much as 15% to favoring the standard dose arm by as much as 9%. We plan to continue accrual to this study until the pre-planned number of 90 patients, which would allow detection of a doubling of complete response rate or a 50% increase in median survival, have been randomized.

Despite these problems and the preliminary nature of our results, we believe several conclusions are justified. First, results of drug sensitivity testing of tumor cell lines are highly correlated with response to initial chemotherapy.

Second, preliminary results utilizing in vitro drug testing for individualized selection of chemotherapy regimens suggest modest potential for therapeutic benefit. Third, the close correspondence between in vitro and in vivo response to drugs provides justification for the use of human cancer cell lines in screening for new chemotherapeutic agents. And finally, the availability of multiple SCLC tumor cell lines from patients whose clinical course is well characterized, including some paired lines from patients before and after in vivo chemotherapy, may prove useful in helping to elucidate the basis for drug resistance and other biologic properties of this tumor.

Publications:

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2. Longo DL, DeVita VT, Duffey PL, Wesley MN, Ihde DC, Hubbard SM, Gilliom M, Jaffe ES, Cossman J, Fisher RI, Young RC. Superiority of ProMACE-CytaBOM over ProMACE-MOPP in the treatment of advanced diffuse aggressive lymphoma: Results of a prospective randomized trial. J. Clin Oncol 1991; 9:25-38.

3. Brennan J, O'Connor T, Makuch RW, Simmons AM, Russell E, Linnoila RI, Phelps RM, Gazdar AF, Ihde DC, Johnson BE. myc family DNA amplification in 107 tumors and tumor cell lines from patients with small cell lung cancer treated with different combination chemotherapy regimens. Cancer Res 1991; 51:1708-12.

4. Ihde DC. Approach to the management of lung cancers. In, Textbook of Internal Medicine, 2nd Ed., Kelley WN (ed). Philadelphia, J.B. Lippincott, in press.

5. Ihde DC. Primary lung cancer. In, Conn's Current Therapy 1991, Rakel RE (ed). Philadelphia, W.B. Saunders, 1991, pp. 139-44.

6. Ihde DC. Non-small cell lung cancer. In, Manual of Oncologic Therapeutics 1991/1992, Wittes RE (ed). Philadelphia, J.B. Lippincott, in press.

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9. Ihde DC, Minna JD. Non-small cell lung cancer Part 1: Biology, diagnosis, and staging. Curr Probl Cancer 1991; 15:61-104.

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12. Viallet J, Ihde DC. Small cell carcinoma of the lung: Clinical and biologic aspects. Crit Rev Oncol/Hematol, in press.

13. Klastersky J, Burkes R, Choi N, Dombersky P, Darwish S, Ginsberg RJ, Gregor A, Ihde DC, Kocha W, MacBeth F, Mirimanoff RO, Mornex F, Nemec J, Opalka P, Payne D, Pujol JL, Stout R, van Zandwijk N. Induction therapy for NSCLC: A concensus report. Lung Cancer 1991; 7:15-17.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06579-08 NMOB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I.R. Kirsch	Senior Investigator	NCI-NMOB
Others:	V. Bertness	Biologist	NCI-NMOB
	K. Nakahara	Biologist	NCI-NMOB
	P. Aplan	Medical Staff Fellow	NCI-NMOB
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	V. Bier	Guest Researcher	NCI-NMOB
	F. Moghadam	Guest Researcher	NCI-NMOB
	K. Tchorz	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

None

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NCI-Navy Medical Oncology Branch

SECTION

Acquired Gene Rearrangements

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	2

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in the mechanism(s) of chromosomal rearrangements because they represent such profound examples of genomic instability. We also utilize the recognition of chromosomal rearrangements as useful tools in the diagnosis, staging, treatment planning, and risk assessment of individuals or populations predisposed to the development of cancer (see separate project). But the cloning and characterization of chromosomal abnormalities is also, for us, a starting point in the investigation of genes which play crucial roles in the growth and/or development of the cells in which the aberration occurs. That this be so is based on the concept that aberrations are more likely to occur in chromatin regions that are "open", active, and accessible. This premise has been the foundation of a successful program of gene identification and characterization within our laboratory. This strategy has led us to the discovery of four interesting and important human genes. Three of these genes are members of the basic domain-helix-loop-helix (bHLH) family of transcription factors, a family known to act in nodal points of tissue specific developmental processes. One of these genes, SCL, appears to play a role in early hematopoietic development, the other two are more likely to be active in early human nervous system development. We have also identified a gene, SIL, which may be the first known tissue specific topoisomerase, and which forms a fusion message with SCL subsequent to an interstitial deletion of chromosome 1 in approximately 20% of children with T-cell acute lymphoblastic leukemia.

PROJECT DESCRIPTION

Objectives for the Future

Long Term

1. To define the necessary and/or sufficient features for chromosomal breakage and rejoining in different cell types.

2. To use the occurrence of cell-type specific chromosomal aberrations as an in-road to the exploration of differential gene activation during development.

3. To contribute to the understanding of how gene rearrangements mediated by chromosomal aberrations alter the regulation of the affected loci.

Short Term

1. To define the protein partners with which the putative hematopoietic transcription factor SCL dimerizes. To define the specific DNA sequence(s) to which the SCL heterodimer binds.

2. To determine the functional role of SCL via the enhancement or elimination of SCL in appropriate cell types, infection of SCL into bone marrow stem cells, and purification of SCL expressing stem cells from peripheral blood for expansion and differentiation studies

3. To characterize the mRNA and genomic structures and patterns of expression of two newly identified SCL related basic domain helix-loop-helix genes.

Major findings:

Within the past year we have finished the complete structural characterizaton of the SCL gene in terms of its genomic structure, mRNA, encoded protein, and 5' regulatory region. The result of this analysis has led us to the realization that SCL is a DNA binding transcription factor expressed early in hematopoietic development which can contribute to T-cell malignant transformation when it is aberrantly expressed in these cells. One of the ways in which this occurs is by chromosomal translocation between SCL and the T-cell receptor delta locus. A more common way of effecting this aberrant expression is by an interstitial deletion which places transcriptional control of SCL under the influence of the SIL (scl interrupting locus). We have now also finished the complete structural characterization of the SIL gene as well as investigation of its pattern of expression and submitted the result of this work for publication. One interesting feature of the SIL gene is the appearance within its primary amino acid sequence of a eukaryotic topoisomerase I motif. This motif has been observed in every eukaryotic topoisomerase but has not been seen in any other protein found in the current protein databanks. Thus SIL may have a rare cell-type specific topoisomerase activity. This possibility is currently being investigated. In collaboration with Dr. John Coligan of the Biological Resources Branch of NIAID we have raised polyclonal antisera to both the SCL and SIL proteins. These are proving to be useful reagents for both diagnostic and functional studies.

We have constructed a number of SCL containing vectors in order to explore the function of the SCL gene in a variety of in vitro and in vivo systems. These studies include the generation of transgenic mice directly or via the introduction of SCL transfected embryonic stem cells, knocking out SCL expression in embryonic stem cells or via "dominant negative" mutants or "antisense" RNAs, and the infection of bone marrow stem cells via a murine retroviral SCL construct. Recently we have screened an expression cDNA library with labelled SCL fusion protein (using a construct which fuses the SCL bHLH motif to the amino terminus of glutathione S-transferase) and obtained a clone which could be one of the dimerization partners for the SCL protein. Work on the characterization of this clone is proceeding.

The cloning, sequencing, and construction projects that have been necessary for a functional analysis of SCL led to the discovery of two SCL like bHLH genes. The structure and pattern of expression of these two genes is quite distinctive and interesting. Though related to SCL at the nucleotide and amino acid level they do not appear to have predominant expression in hematopoietic tissue. Rather, their primary site of expression appears to be in the murine and human early developmental nervous system, with a temporal and spatial overlap, yet distinctiveness in terms of each of their particular cell-type specific expression. We have now cloned the genomic and cDNA versions of these genes from the mouse and the human and are actively engaged in their detailed elucidation.

Publications:

1. Aplan PD, Begley CG, Bertness V, Nussmeier M, Ezquerra A, Coligan J, Kirsch IR: The SCL gene is formed from a transcriptionally complex locus. Mol Cell Biol, 10:6426-6435, 1990

2. Aplan PD, Lombardi DP, Ginsberg AM, Cossman J, Bertness VL, Kirsch IR: Disruption of the SCL locus by "illegitimate" V(D)J recombinase activity. Science, 250:1426-1429, 1990.

3. Begley CG, Visvader J, Green AR, Aplan PD, Metcalf D, Kirsch IR, Gough NM: Molecular cloning and chromosomal localization of the murine homolog of the human "helix-loop-helix" gene SCL. Proc Natl Acad Sci USA, 88:869-873, 1991.

4. Aplan PD, Lombardi DP, Kirsch IR: Structural characterization of SIL, a gene frequently disrupted in T-cell acute lymphoblastic leukemia. (submitted)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06581-08 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetics of Differentiation and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Leif Bergsagel, MD	Medical Staff Fellow	NCI-NMOB
	Carol Kobrin, PhD	Staff Fellow	NCI-NMOB
	Agnes Cuddihy, PhD	Visiting Fellow	NCI-NMOB
	Leslie Brents	Biologist	NCI-NMOB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology and Immunology

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our work continues to focus on two approaches to hematopoietic differentiation. First, we demonstrated previously that expression of a c-myc or c-myb transgene reversibly blocks terminal differentiation of a mouse erythroleukemia cell line. We are now using this system to test mutated c-myb transgenes so that we can begin to understand how c-myb affects proliferation and differentiation. Our long term goal is to understand the mechanisms that are responsible for the apparent inability of most hematopoietic tumors to differentiate. Second, we have developed a novel method for subtractive cloning by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. We have used this novel methodology to identify genes which are expressed in most murine plasmacytomas but rarely in B lymphomas. Thus far, we have identified two classes of genes having this property: 1) two genes are expressed in most plasmacytoma and pre-B lymphoma cell lines; and 2) five genes are expressed in most plasmacytoma cell lines but not in pre-B lymphoma lines. The predicted coding sequences and expression patterns in normal tissues and other cell lines suggest that some of these genes are involved in the phenotype of the normal plasma cell, whereas other genes may be involved in the tumorigenic process since they are not expressed in normal plasma cells. Our long term goal is to identify the genes which determine the phenotypes of plasmacytomas and terminally differentiated plasma cells.

Molecular Genetics of Differentiation and Transformation

Overall Objectives:

1. To clarify the cellular and molecular mechanisms which determine and regulate hematopoietic differentiation.

2. To clarify the relationship between differentiation and malignancy.

Species Studied: Mice and humans.

A. Role of c-myc and c-myb oncogenes in hematopoietic differentiation.

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Agnes Cuddihy, PhD	Visiting Fellow	NCI-NMOB
	Leslie Brents	Biologist	NCI-NMOB

Endogenous c-myc and c-myb levels decrease biphasically when murine erythroleukemia (MEL) cells are induced to differentiate with various chemical inducers. By introducing a vector with an inducible metallothionein promoter and either a c-myc or c-myb cDNA coding region into MEL cells we are able to reversibly block differentiation by addition (and subsequent removal) of nontoxic levels of $ZnCl_2$ to the medium. The results are identical for both nuclear oncogenes. We are currently pursuing three approaches to better understand this system:

1) We are using these conditional c-myb and c-myc blocked transfectants in an attempt to identify whether either of these genes acts by altering the expression of genes which might regulate the terminal differentiation process, e.g. Id, Myn, Myc, Myb, SCL, a poly-adenylated variant of histone H1. As part of this approach, we are collaborating with Dr. I. Kirsch in attempting to determine if a transfected SCL gene can reverse the c-myb or c-myc mediated block of terminal MEL differentiation.

2) We have constructed 8 mutated versions of c-myb, and tested them for biological activity in blocking terminal differentiation of MEL cells. Our results indicate that the DNA binding region and transcription activation regions of c-myb are necessary and possibly sufficient for blocking terminal differentiation in the MEL system. The negative regulatory region and other conserved sequences in the c-myb gene are not necessary for this activity. Curiously, a mutated c-myb gene which contains the DNA binding region but lacks the transcription activation region, negative regulatory region, and other conserved sequences causes significant spontaneous differentiation (i.e. as high as 30-40% of the cells become benzidine positive) when its expression is up-regulated in MEL transfectants. This apparent "dominant negative" activity suggests that interference with c-myb activity is sufficient to activate terminal differentiation.

3) In collaboration with J. Ting's laboratory at the University of North Carolina, we have shown that stable or transient transfection of c-myb into certain cell lines can up-regulate c-myc expression. It appears that increased expression of c-myb trans-activates c-myc transcription. Within 1 kb upstream of the murine c-myc promoter, there are 3 pairs of c-myb recognition elements

(i.e. MREs). Studies are in progress to demonstrate that c-myb directly increases transcription by interacting with one or more of these MREs. In addition, the c-myb mutants are being assayed in this system.

Our long term goal is to determine how c-myc and c-myb regulate proliferation and differentiation in normal and malignant hematopoietic cells.

Publications:

1. Evans, J.L., T.L. Moore, W.M. Kuehl, T. Bender, and J. Pan-yun Ting, "Functional analysis of c-myb protein in T-lymphocytic cell lines shows that it trans-activates the c-myc promoter," Molec. Cell. Biol. 10 (1990), p. 5747-5752.

B. Identification of genes involved in differentiation by subtractive cDNA cloning.

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Leif Bergsagel, MD	Medical Staff Fellow	NCI-NMOB
	Carol Kobrin, PhD	Staff Fellow	NCI-NMOB
	Leslie Brents	Biologist	NCI-NMOB

We have developed a novel and general method for subtractive cloning by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. Using this novel method of subtractive cDNA cloning, we have prepared a mouse plasmacytoma (MPC11) minus mature B lymphoma (A20.2J) subtractive cDNA library. Random selection of 115 clones has identified 16 quantitatively subtractive and 39 qualitatively subtractive clones. From these clones, we have selected 8 potentially interesting genes. One quantitatively subtractive clone (315) identifies an mRNA that is expressed in most plasmacytoma cell lines, but is expressed at an approximately 5-10-fold lower level in a small fraction of B and pre-B lymphoma cell lines. The other 7 genes were classified as qualitatively subtractive, and appeared to be expressed in none or one of 8 B lymphoma lines: 1) two genes (70 and 260) are expressed in most plasmacytomas and pre-B lymphoma cell lines; and 2) five genes (251, 289A, 289B, 326, and 291) are expressed in most plasmacytoma cell lines, but not in any of the ten pre-B lymphomas examined. The five genes described above represent the first known genes which are expressed in plasmactyomas but not in B lymphoid tumors representing earlier stages of differentiation. As a first step in characterizing these genes, we have completely sequenced three of the plasmacytoma specific genes plus the one quantitatively subtractive gene mentioned above, and have focused on further characterization of these four genes, as described below:

1) Clone 315 - This clone detects approximately equivalent amounts of 2 kb and 7 kb mRNA species in murine lymphoid cell lines. Structural studies suggest that the two forms of mRNA have identical coding capacity, but differ in the 3' untranslated region as a consequence of alternative polyadenylation. The coding sequence of this gene revealed no significant homologies in computer searches of various data bases. Additional expression studies indicate that normal murine spleen cells contain mainly the 7 kb form of mRNA, whereas LPS stimulated cultures of spleen cells (in which plasma cells comprise 60% or more of the viable cells) express similar amounts of the 7 kb and 2 kb mRNA species.

2) Clone 289A - This clone identifies the murine equivalent of an 314 amino acid pan-epithelial membrane glycoprotein which had been identified in human epithelial cells, but was not thought to be expressed in human hematopoietic cells. We find this gene to be expressed in murine tissues containing epithelial cells but not in normal mouse spleen. However, LPS stimulated cultures of spleen cells clearly express significant levels of the 2 kb mRNA detected by this clone, consistent with its expression in normal plasma cells. We also found that this gene is expressed in one of two human myeloma cell lines, but not in Burkitt's lymphoma or lymphoblastoid cell lines. Since the structure of the glycoprotein shows homology to nidogen, an extracellular adhesion factor, it seems reasonable to speculate that it may be involved in the communication between epithelial cells and plasma cells that is necessary for transport of secretory immunoglobulin into lumenal spaces, e.g. gut, lung, etc.

The human form of this protein, although expressed in virtually all normal epithelial cells, is being studied by a number of groups as a potential antigen for immunotherapy. We are planning to enter into a collaboration with a group that is trying to set up a mouse model to test this therapeutic approach.

3) Clone 251 - Sequence of murine cDNAs detected by this clone has identified this gene as an apparently new member of the hematopoietic growth factor receptor family (i.e. distantly related to erythropoietin receptor, the beta chain of the IL2 receptor, and IL6 receptor). This gene is expressed in most murine plasmacytomas, but in no other murine cell line examined, including 8 B lymphomas and 10 pre-B lymphomas. To our surprise, however, it is expressed at similar levels in virtually all normal mouse tissues examined. Overall, the expression pattern is consistent with the hypothesis that this gene is expressed in non-dividing cells, the one exception being murine plasmacytoma cell lines.

4) Clone 326 - Sequence of murine cDNAs detected by this clone predict an approximately 83,000 Dalton protein with 3 notable structural features: 1) two small region containing high proportions of acidic amino acids; 2) a unique 20 amino acid sequence which is repeated 7 times in this protein but is not homologous to sequences found in the computer data bases; and 3) a different sequence of 40 amino acids which is homologous to a sequence repeated several times within a number of other proteins, many of which have been shown to function as beta subunits of a trimeric G protein complex. Surprisingly, we cannot detect expression of this gene in normal tissues, with the possible exception of mouse testes. Although mouse cell lines (with the exception of mouse plasmacytomas) do not express this gene, it is expressed in at least 6 of 8 Burkitt's lymphomas and 1 of 2 human myeloma cell lines that have been examined. Since this gene does not seem to be expressed in normal mouse plasma cells, it appears that its expression may be linked to the tumorigenic process which generates murine plasmacytomas.

In addition to further characterization of the genes described above, we are continuing to search for other genes expressed uniquely in plasmacytomas. Our long term goal is to identify the genes which are critical in determining the plasmacytoma phenotype. This may include some genes which are important in determining the terminally differentiated plasma cell phenotype and perhaps other genes which are required for the malignant transformation to plasmacytoma cells.

Publications:

1. Timblin, C.R., P.L. Bergsagel, and W. M. Kuehl, "Identification of consensus genes expressed in plasmacytomas but not B lymphomas." _Current Topics Microbiol Immunol.166 (1990), 141-147.

2. Kuehl, W. M. "Identification and characterization of genes expressed in murine plasmacytomas but not pre-B or B lymphomas" In: Mechanisms of B Cell Neoplasia. Melchers, F. and Potter, M. (eds.) The Basel Institute of Immunology, In Press.

3. Kuehl, W. M.and J. Battey, "Generation of subtracted cDNA with PCR amplification", chapter in volume entitled Polymerase Chain Reaction: Selected Methods, Editor, B.A. White, from Methods in Molecular Biology series, In Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06587-07 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Rearrangements as Tumor Specific Markers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Stan Lipkowitz, MD	Medical Staff Fellow	NCI-NMOB
	Donald P. Lombardi, MD	Medical Staff Fellow	NCI-NMOB
	Verena Bier, MD	Guest Researcher	NCI-NMOB
	Nita Seibel, MD	Guest Researcher	NCI-NMOB
	Kenneth Nakahara	Biologist	NCI-NMOB

COOPERATING UNITS *(if any)*

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.0	1.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structural alterations and expression of immunoglobulin (Ig), T-cell receptor (TCR) and various growth affecting genes are studies in normal, "premalignant," and malignant tumors and cell lines.

A. We have shown that hybrid genes are formed by site specific recombination between variable segments from one immune receptor locus and joining segments from another. We have demonstrated that such events occur in the peripheral T-cells of all normal individuals but are 100 times more frequent in the peripheral T-cells of patients with ataxia-telangiectasia (AT). These hybrid genes 1) affect and alter the repertoire of immune receptor diversity, 2) suggest that an underlying defect in AT may be chromatin "hyperaccessibility," and 3) provide a possible screening test for people at an increased risk for the development of lymphoid specific chromosomal translocations, and therefore lymphoid malignancy. We have recently completed a pilot study of individuals involved in the agriculture industry in which we have demonstrated an acquired transient "AT-like" picture in individuals exposed to a variety of pesticides and herbicides. These individuals are the same population for which epidemiological studies have suggested an increased risk of leukemia and lymphoma.

B. We have identified a gene, SCL, involved in a nodal point in hematopoietic development. In collaboration with the Children's Cancer Study Group (CCSG) and the Southwest Oncology Group (SWOG) we have used the SCL probe in tumor genotyping studies on patients with lymphoid disorders and found SCL disruption to occur in 20-30% of childhood T-cell ALL pronounced SCL expression in M7 AML and CD34+ CML blast crisis.

Gene and transcript mapping. We have localized numerous genes of interest to specific regions of human chromosomes. Most recently using biotinylated probes we have mapped a putative neurogenic gene to human chromosomes 1q21. Furthermore, we are using RNA tissue in situ hybridization as a means of detecting transcripts of interest in individual cells. We are also engaged in a protocol to assess the utility of an SCL based PCR assay to determine and follow minimal residual disease in a subset of CCSG patients.

PROJECT DESCRIPTION

OBJECTIVES:

Long Term

1. To develop, master, and refine techniques based on molecular genetics which are of direct current application in the prevention, early diagnosis, classification, and staging of patients with cancer.

2. To demonstrate the usefulness of these techniques in pilot studies.

3. To promote the adoption of these techniques by service oriented laboratories, and supervise the implementation of such techniques in a standardized, quality controlled fashion for comprehensive, prospective, best available therapy protocols and epidemiological studies.

4. To participate in the human genome mapping effort by the localization of the genes that we discover to chromosomal subbands within the human genome.

Short Term

1. To determine the frequency and cell type distribution of inversions and translocations of human chromosomes 7 and 14 in normal, "premalignant", and malignant conditions and explore whether there is evidence for selective or random associations between particular breakpoints and particular transformed or proliferative states.

2. To determine the relevance of SCL rearrangement and expression in hematopoietic malignancy.

3. To use the fusion of the SIL (see Project Z01 CM 06579-07 NMOB) and SCL genes as a tumor specific marker for the determination of minimal residual disease and its relevance in patients with T-cell ALL.

4. To map a second putatively neural specific transcription factor to a human chromosomal subband.

Major Findings:

Hybrid Gene Formation

There is a leukemia/lymphoma "belt" in Southern Minnesota and Iowa that has been studied by epidemiologists. Among agricultural workers in this area the frequency of lymphoid malignancy is increased although in general the life expectancy is greater than the general population. Numerous studies now suggest that some environmental exposure may be contributing to the increased reisk of development of lymphoid malignancy. We had previously studied individuals with the genetic disease ataxia-telangiectasia, an illness of protean manifestations including progressive cerebellar degeneration, oculocutaneous telangiectasia, immunodeficiency, radiosensitivity, chromosomal instability, and a predisposition to lymphoid malignancy. We established that these individuals demonstrated an approximately 100 fold increase in their frequency of formation of hybrid TCR gamma/beta immune receptors (consistent with an inversion of chromosome 7). The

mechanism of V(D)J recombination which mediates this rearrangement and the mechanism by which certain malevolent lymphoma-associated chromosomal translocations (such as the t(14;18) in follicular lymphoma) occur are likely to be variations on a theme. This would be consistent with the finding that an increased frequency of hybrid gene formation is accompanied by an increased frequency of lymphoma in this population. We therefore studied a population of agricultural workers potentially at increased risk of lymphomagenesis and found their frequency of hybrid gene formation to be significantly greater than normal and only slightly less than a population of patients with AT. The frequency of hybrid gene formation may correlate with the intensity of exposure of these individuals to a variety of pesticides and herbicides. We are now engaged in three separate collaborations with epidemiologists to verify, extend, and refine this initial observation. We have applied for a patent for our PCR based assay as a screening test for increased risk of lymphomagenesis in AT and certain normal populations. We have also considered this assay as a potential in vitro test of potentially lymphomagenic compounds.

B. SCL/SIL We have discovered, cloned and characterized the genes, SCL and SIL. Each gene is interesting in itself (see separate project description) but, in addition, they are brought together by an interstitial deletion of chromosome 1. The result of this deletion, interestingly mediated by V(D)J recombinase is the generation of a fusion mRNA that substitutes the 5' SIL untranslated region for the 5' SCL untranslated region. A full SCL protein is therefore still prduced but in a dysregualted state. WE have completed a study of hematopoietic malignancies in collaboration with the CCSG and SWOG. We find SCL disruption to occur in 20-30 % of patients with T-cell ALL. The majority of this disruption is due to the SCL/SIL fusion. We also find a relatively high level of expression of SCL in CD34+ CML blast crises and in AML M7. The SCL/SIL fusion has not been observed in normal tissues and therefore lends itself to the develpment of a PCR based assay for following a subset of patients with T-cell ALL. A proposal has been submitted and accepted by CCSG for this particular study.

Publications:

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3. Smidt M, Kirsch IR, Ratner L: Deletion of alu sequences in the 5th c-sis intron in individuals with meningiomas. J Clin Invest, 86:1151-1157, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06589-07 NMOB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology, Growth and Chemosensitivity.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
Others:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Daniel Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Bruce E. Johnson, MD	Acting Chief	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

Medicine Branch, Surgery Branch, Radiation Oncology Branch, Laboratory of Molecular Biology

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. There appears to be an increase in the incidence of adenocarcinoma subtype of NSCLC in the USA. In particular, tumors with features characteristic of bronchiolo-alveolar carcinomas appear to be increasing. The relatively large number of cell lines that we have established that have ultrastructural and biochemical evidence of arising from peripheral airway cells (Clara or Type II pneumocyte) confirms these findings. Markers for differentiation in lung cancers include expression of Clara and surfactant genes (in adenocarcinomas) and N-cam in neuroendocrine tumors. In addition, expression of CEA is much higher in neuroendocrine lung cancers than in others.

B. SCLC cell lines retain their chemosensitivity patterns for many years, and in vitro testing is predictive of patient response and survival. Thus, panels of cell lines (SCLC, NSCLC, colorectal and gastric carcinomas) are useful reagents to screen putative new phase I and II drugs using the MTT tetrazolium dye assay.

E. Mutations of ras genes are an important negative prognostic factor in NSCLC, and occur independently of p53 gene mutations.

Objectives:

One of the major objectives of this Branch is to develop newer, more rational therapies for the cancer types representing our major clinical interests. By studying the biology of specific cancers in depth, new ideas for cancer control are generated, tested in vitro and brought to clinical trial. We presume that such an approach is more likely to advance in the therapy of refractory tumors such as colon and non-small cell lung cancer (NSCLC) than the development of new non-targeted cytotoxic agents. Further, a comprehensive knowledge of the biology of a cancer type can aid the physician in interpreting certain clinical phenomena such as hormone secretion, tumor progression, etc. Finally, identification of tumor markers may aid diagnosis, staging, detection of relapse, imaging, sub typing, and monitoring response to therapy.

Three of the currently active clinical protocols for the therapy of SCLC and NSCLC depend on the selection of individualized patients' chemotherapy by in vitro drug sensitivity testing. Thus one of the major objectives of the Branch is to develop methods to 1) amplify tumor cells so that adequate numbers are available for testing; 2) develop and apply rapid, accurate, reproducible testing procedures; 3) demonstrate the clinical relevance of the testing procedures; and 4) utilize in vitro testing for biological and preclinical studies.

Major Findings:

Differentiation in Lung Cancer Tumors and Cell Lines

We have noted previously an increased incidence of adenocarcinoma in our non-SCLC (NSCLC) protocol patients (63% of all NSCLC). We also noted that peripheral adenocarcinomas with some or all of the features of bronchioloalveolar (BAC) carcinomas appeared to be common (about 50% of all adenocarcinomas, or about 30% of all NSCLC tumors).

N-CAM, an important neural adhesion molecule, is expressed concordantly in lung cancer cell lines with neuroendocrine properties. All N-CAM positive lines lack substrate adherence, and grow as floating cell aggregates, a feature characteristic of several neuroendocrine and neural cell lines (Gazdar, Linnoila, Carbone).

We studied lung cancer tumors and cell lines for expression of CEA and the related genes NCA and BGP. Normal lung has abundant NCA, but relatively little CEA or BGP. All three genes are expressed, but discordantly, in lung cancer cell lines. Cell

lines expressing neuroendocrine features have a much higher expression of CEA RNA and protein than other lung cancers. Identification of the precise family member expressed in lung cancers may be of diagnostic importance (Kim, Kaye, Gazdar).

In Vitro Drug Sensitivity Testing and Clinical Correlations

The Weisenthal dye exclusion assay is used to test clinical specimens from patients entered onto therapeutic trials for lung cancer. The largest and best studied base currently available is from the Extensive Stage small cell lung cancer protocol, #83 13 (also see report by Dr.D.Ihde). We have extended these studies to permanent cell lines established from patients on this study, suing the MTT dye assay. Even after a mean culture time of 29 months, in vitro testing was predictive for clinical response and survival. Neuroendocrine differentiation in NSCLC cell lines is associated with relative chemosensitivity, suggesting that such tumors may represent a chemoresponsive subset of NSCLC. NE differentiation is present in about 15% of NSCLC tumors and cell lines.

The Role of the Topoisomerase Genes in Human Cancers

We correlated expression of topoisomeraseI and II genes and drug resistance in a panel of 20 lung cancer cell lines. All cell lines expressed both genes, but there were considerable variations between individual lines. Approximately 10% of the lines had rearrangements of one of the genes. In 7/8 lines studied in greater detail, there was an excellent correlation between in vitro chemosensitivity and topoII expression, but not with topoI expression (Giaccone, Gazdar).

The role of ras gene mutations in lung cancer

ras genes in primary lung cancers (mainly K-ras at codon 12) have been associated with a subset of adenocarcinomas having a poor prognosis. We investigated 105 lung cancer cell lines, and found codon 12 ras mutations (all K-ras) in about 20% of lung cancer lines. Unlike other studies, the mutations were not limited to adenocarcinoma, but occurred with equal frequency in all forms of adenocarcinoma. There were similar incidences in cell lines initiated from primary or metastatic tumors. No mutations were found in any of 37 small cell lung cancer cell lines. Mutation of K-ras at codon 12 define a subset of non-small cell lung cancer, but not small cell lung cancer. Cell lines having ras mutations did not have different chemosensitivity profiles than cell lines lacking mutations. Thus, ras mutations in NSCLC are not associated with metastases or with increased chemoresistance. (Mitsudomi, Viallet, Minna, Gazdar). ras gene mutations are an

important, independent negative prognostic factor for all stages of NSCLC. p53 gene mutations appear to occur independently of ras mutations. However, p53 gene mutations in lines also containting ras mutations cluster in exons 5 and 8, suggesting that there may be some association between these two molecular changes in NSCLC.

Establishment and characterization of a steroid secreting adrenocortical carcinoma cell line

A unique human cell line has been established from a patient with adrenocortical carcinoma. Mass spectrometry studies indicate that even after 7-10 years in culture the cell line continues to secrete about 35 steroid hormones, representing all of the major pathways of adrenal steroid production (glucocorticoids, mineralocorticoids and sex hormones). These studies indicate that all of the important p450 enzymes involved in adrenal steroid synthesis are present. The line, which is being patented, should be invaluable for studying steroid hormone synthesis and its regulation (Gazdar, Oie). The cell line has FGF receptors, but lacks those for ACTH. FGF appears to regulate the growth of the cells. We are investigating whether FGF is a negative autocrine growth factor.

Mutations of the p53 gene in Gastric Cancers

We found mutations of the p53 gene in 1/18 gastric tumors (6%) and 3/7 (43%) gastric cell lines. Because the tumors were primary lesions while most of the cell lines were from metastatic lesions, mutations of p53 may be associated with metastases in gastric cancer (Kim, Takahashi, Minna, Gazdar).

Future Studies

We are performing an analysis of the typing of NSCLC worldwide. Sites to be analyzed will include co-operating institutions in North America, Europe and Japan. These studies will be complemented by more limited correlations between histopathology and ultrastructure and immunohistochemistry. By these techniques we will be able to confirm our light microscopic observations regarding the increase in adenocarcinomas, and its BAC subtype.

We will continue to develop and evaluate molecular and biochemical markers for differentiation in all forms of lung cancers, for diagnostic, prognostic and therapeutic applications.

We will continue our current clinical protocols for SCLC and NSCLC based on in vitro selected therapy. Data will be correlated with the patients' responses. Preclinical studies

will include testing of phase I and II drugs and correlating *in vitro* predictions with clinical results.

As amplification and over-expression of the *myc* gene family are relatively common in lung cancer, especially previously treated SCLC, we will continue to study the relationship between oncogene expression and in vitro chemosensitivity and radioresistance. We will test both lung cancer cell lines as well as rat cell lines transfected with various oncogenes. We will extend our studies with ras genes to tumor samples from three continents, and correlate the data with clinical findings.

Disease oriented panels of cell lines will be used to test potential and actual phase I and phase II agents, both for correlation of in vitro results with clinical response and for the selection of agents to test in future phase I trials in NSCLC.

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2. Brennan J, O'Connor T, Makuch RW, et al. myc family DNA amplification in 107 tumors and tumor cell lines from patients 11 lung cancer treated with different combination chemotherapy regimens. Cancer Res 1991;51:1708-1712.

3. Deftos LJ, Gazdar AF, Hogue-Angeletti R, Mullen PS, Burton DW. Distinct patterns of chromogranin A-related species can be demonstrated in endocrine cells. Bone Miner 1990;9:169-178.

4. Dmitrovsky E, Seifter EJ, Gazdar AF, et al. A phase II trial of carboplatin (CBDCA) in small-cell and non-small-cell lung cancer with correlation to in vitro analysis of cytotoxicity. Am J Clin Oncol 1990;13:285-289.

5. Fridman R, Giaccone G, Kanemoto T, Martin GR, Gazdar AF, Mulshine JL. Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. Proc Natl Acad Sci U S A 1990;87:6698-6702.

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9. Gazdar AF, Mulshine JL, Kramer BS. Biological, molecular and clinical markers for the diagnosis and typing of lung cancer. In: Herberman RB, Mercer DW, eds. Immunodiagnosis of Cancer. New York: Marcek Dekker, 1990:453-468.

10. Gazdar AF, Oie HK, Shackleton CH, et al. Establishment and characterization of a human adrenocortical carcinoma cell line that expresses multiple pathways of steroid biosynthesis. Cancer Res 1990;50:5488-5496.

11. Gazdar AF, Steinberg SM, Russell EK, et al. Correlation of in vitro drug-sensitivity testing results with response to chemotherapy and survival in extensive-stage small cell lung cancer: a prospective clinical trial. J Natl Cancer Inst 1990;82:117-124.

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13. Ihde DC, Grayson J, Woods E, et al. Twice daily chest irradiation as an adjuvant to etoposide/cisplatin therapy of limited stage small cell lung cancer. In: Salmon SE, ed. Adjuvant Therapy of Cancer: Proceedings of the Sixth International Conference. Philadelphia: W.B. Saunders, 1990:162-165.

14. Jensen SM, Gazdar AF, Cuttitta F, Russell EK, Linnoila RI. A comparison of synaptophysin, chromogranin, and L-dopa decarboxylase as markers for neuroendocrine differentiation in lung cancer cell lines. Cancer Res 1990;50:6068-6074.

15. Mor O, Messinger Y, Rotman G, et al. Novel DNA sequences at chromosome 10q26 are amplified in human gastric carcinoma cell lines: molecular cloning by competitive DNA reassociation. Nucleic Acids Res 1991;19:117.

16. Noonan KE, Beck C, Holzmayer TA, et al. Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci U S A 1990;87:7160-7164.

17. Park JG, Frucht H, LaRocca RV, et al. Characteristics of cell lines established from human gastric carcinoma. Cancer Res 1990;50:2773-2780.

18. Tsai CM, Gazdar AF, Allegra C, Perng RP, Kramer BS. Enhancement of fluorinated pyrimidine-induced cytotoxicity by leucovorin in human lung cancer cell lines. Int J Cancer 1990;46:101-105.

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20. Tsai CM, Ihde DC, Kadoyama C, Venzon D, Gazdar AF. Correlation of in vitro drug sensitivity testing of long-term small cell lung cancer cell lines with response and survival. Cell 1991;26:1148-1152.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06594-06 NMOB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Molecular Genetic Events in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce E. Johnson, MD	Acting Chief	NCI-NMOB
Others:	Adi F. Gazdar, MD	Section Chief	NCI-NMOB
	Daniel C. Ihde, MD	Deputy Director	NCI
	James Mulshine, MD	Section Chief	NCI-NMOB
	Yoshi Ohsaki, MD	Guest Researcher	NCI-NMOB
	Gary Richardson,MD	Clinical Associate	NCI-NMOB

COOPERATING UNITS *(if any)*

Hao-Chia Chen, Ph.D., Section Chief, Endocrinology and Reproduction Research Branch, NICHHD

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Oncopeptides

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.6	3.6	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

A. We have recently reported atrial natriuretic factor mRNA expression and immunoreactivity in tumor and tumor cell lines from small cell lung cancer patients with hyponatremia who did not produce arginine vasopressin. High pressure liquid chromoatography (HPLC) analyses of the tumor cell lines and tumors from patients with hyponatremia and mRNA expression of atrial natriuretic factor have revealed that intracellular and extracellular peptide appears to be the 28 amino acid form of atrial natriuretic peptide, the form that normally circulates in human plasma. These studies are the first to characterize the ectopic production of atrial natriuretic peptide in small cell lung cancer patients and may have identified the third factor (natriuretic factor) that has been hypothesized in the syndrome of inappropriate antidiuretic hormone (SIADH). The receptor for atrial natriuretic factor is also present on small cell lung cancer cells and the cells respond to exogenously added atrial natriuretic factor with an increase in intracellular cGMP, similar to the normal receptors on vascular smooth muscle cells. Therefore, there appear to be functional ANF receptors on the surface of small cell lung cancer cells.

B. We reviewed the clinical course of 234 lung cancer patients. In contrast to none of the 123 non-small cell lung cancer (NSCLC) patients, 18 of 111 (16%) small cell lung cancer patients had hyponatremia . Ten of these 18 had tumor cell lines available and 8 expressed ANF mRNA, 8 expressed AVP mRNA, and 6 of 10 cell lines produced both ANF and AVP mRNA. All of the 10 cell lines produced ANF mRNA, AVP mRNA, or both. Studies of 10 tumor cell lines from the 93 SCLC patients without hyponatremia showed 9 produced ANF mRNA and one produced AVP mRNA. From these studies we have observed that all tumor cell lines studied from SCLC patients with hyponatremia produce ANF mRNA or AVP mRNA, or both. Atrial natriuretic peptide may be the previously hypothesized third factor and play an important role in the pathogenesis of hyponatremia in some patients with SIADH.

PROJECT DESCRIPTION

Molecular Genetic Events in Lung Cancer

Professional Staff:

PI:	Bruce E. Johnson, MD	Acting Chief	NCI-NMOB
Others:	Adi F. Gazdar, MD	Section Chief	NCI-NMOB
	Daniel C. Ihde, MD	Deputy Director	NCI
	James Mulshine, MD	Section Chief	NCI-NMOB
	Yoshi Osaki, MD	Guest Researcher	NCI-NMOB
	Gary Richardson, MD	Clinical Associate	NCI-NMOB

Collaborating Branches:

Hao-Chia Chen, Ph.D., Section Chief, Endocrinology and Reproduction Research Branch, NICHHD

Objectives:

1. Study tumor cell lines and tumors from patients with SCLC and hyponatremia for evidence of ectopic AVP or ANF production and correlate this with the patients fluid and electrolyte status.

2. Study tumor cell lines and tumors from patients with small cell lung cancer and hyponatremia that express ANP mRNA to determine the peptide structure and bioactivity.

3. Study tumor cell lines and tumors from patients with small cell lung cancer for expression of atrial natriuretic factor receptor mRNA, atrial natriuretic factor receptor binding, and intracellular cyclic GMP levels after atrial natriuretic factor addition.

Major Findings:

Tumors and Tumor Cell Lines from Patients with Small Cell Lung Cancer and SIADH Produce Ectopic Atrial Natiruretic Factor and Express the Atrial Natriuretic Factor Receptor

Atrial Natiurectic Peptide Expression and Peptide Studies

We studied mRNA expression and the atrial natriuretic factor production in tumors and tumor cell lines from patients with small cell lung cancer. RNase protection assays of the tumor and tumor cell line (NCI-H1284) mRNA from a small cell lung cancer patient with hyponatremia demonstrated a protected species of ANF mRNA identical to that seen in the human heart mRNA. Reverse phase high performance liquid chromatography characterization of atrial natriuretic factor of the tumor cell line (NCI-H1284) demonstrated immunoreactivity in both the cells and supernatant in the same fraction as the 28 amino acid ANF-(99-126). No ANF mRNA expression or immunoreactivity was detected in the cells or supernatant from the cell line (NCI-H526) established from the small cell lung cancer patient who did not have hyponatremia. We conclude that

small cell lung cancer cells can produce and secrete atrial natriuretic factor similar to the bioactive form found in the plasma.

Atrial Natriuretic Factor Receptor Studies

We studied atrial natiuretic factor receptor mRNA production in small cell lung cancer cell lines and Hela cells. ANF receptor mRNA from small cell lung cancer cell lines was detected by RNase protection assays and polymerase chain reaction analyses using probes and oligonucleotide primers derived from both the intracellular and extracellular domain of the atrial natriuretic factor receptor. In addition, exogenous addition of atrial natriuretic factor stimulated the generation of cGMP more than 40 fold over basal levels in the small cell lung cancer cell line, NCI-H82. We conclude that atrial natriuretic factor receptors are present on small cell lung cancer and have an appropriate physiologic response to the exogenous addtion of atrial natriuretic factor. Studies are underway to determine if the the addition of exogenous atrial natriuretic factor can inhibit the growth of the small cell lung cancer cells.

Studies of Patients with Lung Cancer.

In order to extend our observations to a large group of lung cancer patients, we retrospectively reviewed the records of 234 lung cancer patients treated at the NCI-Navy Medical Oncology Branch from November 1983 to July 1988. Eighteen of 111 (16%) SCLC patients had hyponatremia (serum sodium $\leq$ 130 mmol/L), compared to 0/123 (0%) non-small cell lung cancer (NSCLC) patients. Of the 18 SCLC patients with hyponatremia, 10 had tumor cell lines available for RNAse protection assays and radioimmunoassays for ANF and AVP. Eight expressed ANF mRNA, 8 expressed AVP mRNA, and 6 of 10 cell lines produced both ANF mRNA and AVP mRNA. All of the 10 cell lines produced ANF mRNA, AVP mRNA, or both. We selected 10 tumor cell lines from the 93 SCLC patients without hyponatremia to serve as controls. Nine of the 10 cell lines produced ANF mRNA, and one produced AVP mRNA. We also selected 10 tumor cell lines from the 123 NSCLC patients. Four of the 10 cell lines produced ANF mRNA, and none produced AVP mRNA. We have performed ANF and AVP radioimmunoassays on the cell pellets from 18 of these 30 lung cancer cell lines. The radioimmunassays of the peptides, AVP and ANF comfirmed the mRNA expression data in 30 of the 36 assays from 18 small cell and non-small cell lung cancer cell lines. From these studies we have observed that all tumor cell lines studied from SCLC patients with hyponatremia produce ANF mRNA and peptide or AVP mRNA and peptide, or both. Tumor cell lines from SCLC patients without hyponatremia may produce ANF mRNA and protein, tumor cell lines from NSCLC patients do not commonly produce ANF mRNA or peptide and AVP mRNA and peptide.

Publications:

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4. Graziano SL, Pfeifer AM, Testa JR, Mark GE, Johnson, BE, Hallinan EJ, Pettengill OS, Sorenson GD, Tatum AH, Brauch H, Zbar B, Ehrlich GD, and Poiesz BJ. Involvement of the RAF1 locus, at band 3p25, in the 3p deletion of small cell lung cancer. Genes Chromosomes and Cancer 1991, In Press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06595-05 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinically Relevant Immunohistochemical Markers in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*
Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Human Tumor Biology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20889

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal is to define immunohistochemical markers that will best type lung cancer for diagnosis, prognosis, and selection of therapy. Small cell lung cancer (SCLC), characterized by neuroendocrine (NE) features, is responsive to chemo- and radiotherapy. Some non-SCLC also express NE features. The hypothesis is that these tumors might be more responsive to cytotoxic treatment than other non-SCLC.

A. Characterization of markers. In a retrospective study a comprehensive group of 113 lung cancers were tested for the immunohistochemical expression of 17 antigens using a sensitive avidin-biotin-peroxidase technique. Logistic regression analysis was used to separate tumors into the proper categories (SCLC and carcinoid tumors versus NSCLC) based on the immunohistochemical markers. As a result 95% of the tumors were correctly predicted using the cell counts and staining intensities of only six markers. The results suggested that 1) individual marker counts are not useful in tumor classification, 2) "specific" NE markers such as serotonin and neuropeptides bombesin, calcitonin, ACTH, vasopression, neurotensin are not useful, 3) the best NE markers are a panel of "general" NE markers (Chromogranin A, Leu 7, NSE) which are present in NE cells throughout the body.

B. Clinicopathologic correlation. This panel of "general" NE markers was applied to the non-SCLC cases on protocol 83-15 in our branch. Although the numbers were small, the response rate to chemotherapy was 50% (4/8) in the patients whose tumors were positive for NE markers versus 16% (6/38) in those with negative NE markers. Moreover, patients with NE positive tumors developed metastases significantly earlier ($p2 < 0.027$). The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. Immunohistochemistry provides a highly effective and specific technique to achieve this goal.

PROJECT DESCRIPTION

Clinically Relevant Immunohistochemical Markers in Lung Cancer

Professional Staff:

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

Objectives:

There are four major histological types of lung cancer, namely small cell lung cancer (SCLC) (25%), adenocarcinoma (25%), squamous cell carcinoma (30%) and large cell carcinoma (15%). For a number of biological and clinical reasons, these lung carcinomas may be divided into SCLC and non-SCLC (NSCLC) tumors. SCLC and the rare bronchial carcinoid express many neuroendocrine (NE) features including dense core granules by electron microscopy, high levels of the key amine producing enzyme L-dopa decarboxylase, and the glycolytic isoenzyme neuronspecific enolase (NSE) and hormone or neuropeptide production. SCLC unlike NSCLC is extremely sensitive for chemotherapy and radiation, and there are scattered reports on favorable responses to chemotherapy by "atypical endocrine" tumors of the lung. This knowledge together with the recently established NE markers has prompted us to explore if 1) the expression of neuroendocrine markers in NSCLC is associated with favorable response to chemo- or radiotherapy, and 2) if the degree of expression of neuroendocrine markers in SCLC correlates with clinical outcome. Immunohistochemical technique provides a readily applicable tool for this.

Methods Employed:

1. Tumors. A comprehensive group of 113 primary lung cancers was chosen from the archives of the departments of pathology at the Bethesda Naval Hospital and National Cancer Institute. In addition, tumor material was obtained also from the patients on NCI protocol 83-15. Serial sections from routinely processed paraffin blocks were used.

2. Antibodies. The application of immunologic techniques that use hormone markers has been hampered by the fact that tumors with similar histologic and cytologic features may produce a variety of immunoreative substances, and some tumors may synthesize more than one hormone. Recently, a mouse monoclonal antibody LK2H10 produced against human pheochromocytoma has been shown to be directed against chromogranin A (ChrA) a constituent of secretory

granules in most peptide producing endocrine cells. The demonstration of chromogranin in lung tumors serves as a useful marker for a broad spectrum of lung tumors with NE features including SCLC and the rare bronchial carcinoid. Other general immunohistochemical markers for NE differentiation include monoclonal antibody Leu-7 (HNK-1). Leu-7 reactivity was originally identified in subpopulation of lymphocytes called natural killer cells and later noted to be present also in nerves and wide variety of endocrine cells. Antibodies to NSE also react with nerves and cells of the diffuse NE system and its tumors. The advantage of applying such general NE markers in that they provide a more uniform recognition for multiple NE tumors that may in turn synthesize a variety of specific products such as different hormones.

3. Immunohistochemical Staining. Staining was performed using the avidinbiotin peroxidase technique. Appropriate positive and negative controls were included in each assay. Results of the immunostaining were reviewed scoring both for the intensity of the staining and number of positive cells.

Major Findings:

1. Characterization of Markers. We were able to demonstrate that the majoritiy of cells in most SCLC and all carcinoid tumors were positive forthe general NE markers and many hormones. Logistic regression analysis was used to separate tumors into the proper categories on the basis of markers and 95% the tumors were correctly classified applying a model created from staining indeces of general NE markers (ChrA, Leu 7, NSE). Evaluation of the expression of multiple markers revealed that 7/77 NSCLC had a staining pattern indistinguishable from SCLC.

We have concluded that 1) Application of the general NE markers produces acceptable classification of lung tumors; 2) Most but not all SCLC and carcinoids express multiple NE markers in a high percentage of tumor cells; 3) Occasional NSCLC show staining patterns indistinguishable from SCLC; 4) Many NSCLC contain a small subpopulation of cells expressing NE markers.

2. Clinicopathologic Correlation. The panel of "general" NE markers (ChrA, Leu7, NSE) was applied to the non-SCLC cases on protocol 83-15 ("Treatment of Non-Small Cell lung Cancer Utilizing In Vitro Drug Sensitivity"). Based on a detailed histopathological evaluation of tumor specimens of the patients already entered in the protocol it appears than in over 80% of the cases such an immunohistochemical analysis on untreated patient specimens can be performed. Currently we have stained 101 of the 133 cases entered and in 20/98 (20%) non-SCLC at least two out of the three general NE markers were positive. The results of the first 80 cases are summarized in the following table as an example:

GENERAL NE MARKERS IN NSCLC BY HISTOLOGICAL TYPE
(80 CASES ON PROTOCOL 83-15)

(% positive)	Chr A	Leu 7	NSE
Adenocarcinoma	2/45 (4)	10/45 (22)	22/45 (49)
Large cell	6/19 (32)	3/19 (16)	9/19 (47)
Epidermoid	0/11 (0)	2/11 (18)	4/11 (36)
Other	0/2 (0)	0/2 (0)	1/2 (50)
TOTAL NSCLC	8/77 (10)	15/77 (19)	35/77 (45)
Carcinoid	3/3 (100)	3/3 (100)	3/3 (100)

The updated analysis of the response rate to chemotherapy in those 122 non-SCLC patients on protocol 83-15 in correlation with the results of immunohistochemistry revealed a rate 50% (4/8) in the patients whose tumors were positive for at least 2 out of 3 general NE markers versus 16% (6/34) in those with negative NE markers. There was also a strong correlation of the expression of immunohistochemical NE markers with other biochemical markers for NE differentiation, such as L-dopa decarboxylase levels in tumors. While there was no difference in survival between patients whose tumors were NE positive and other non-SCLC, patients with NE positive tumors developed metastases significantly earlier ($p2 < 0.027$).

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. There are at least 150,000 new cases of lung cancer (75% of which are non-SCLC) discovered annually. Our preliminary results support our hypothesis that non-SCLC which express NE features might be more responsive to cytotoxic treatment than other non-SCLC. Immunohistochemistry provides a highly effective and specific manner to screen for these tumors.

An important, practical aspect of this study is that it will provide a valuable archive of large patient material with well characterized clinical data. This enables statistically meaningful correlations allowing systematic evaluation of the biological significance of defined markers.

Proposed Course:

1. The expression of markers will be correlated to the clinical data including performance status, best response, and survival. At the end of the protocol 83-15 we should have accumulated results on 120 patients, if 150 patients are accrued as planned. This will provide a basic correlation.

2. Based on our initial observations we expect that 10-20% of non-small cell lung cancers express neuroendocrine markers. In order to extend the analysis and reach meaningful clinical correlations we have initiated a collaboration with the ECOG (Eastern Cooperative Oncology Group) and LCSG (Lung Cancer Study Group). ECOG and LCSG have full clinical response and survival

information on nearly 2,800 treated patients. A large number of these have pretreatment tumor samples available for analysis. We plan to study the expression of general NE markers chromogranin, Leu 7 and NSE, as well as selected other markers, and relate this to tumor type, response to therapy, and survival. At the present time, we have stained 400 cases, and the interim analysis revealed that 12-16% of the cases were positive for at least two out of the three general NE markers, thus confirming our initial findings in independent tumor sets.

Our preliminary analysis of other common immunohistochemical tumor markers in the LCSG cases revealed that patients whose tumors lacked mucin, which is a secretory product of many adenocarcinomas, had an average 5 year recurrence free survival, while the most mucin positive cases had an average recurrence free survival of about one year. Also, patients with elevated tissue staining for carcinoembryonic antigen, CEA, in their tumors had a more favorable outcome.

Publications:

1. Bliss DP, Jr., Battey JF, Linnoila RI, Birrer MJ, Gazdar AF, Johnson BE. Expression of the atrial natriuretic factor gene in small cell lung cancer tumors and cell lines. J Natl Cancer Inst, 1990, 82:305-310.

2. Jensen SM, Gazdar AF, Cuttitta F, Russell EK, Linnoila RI. A comparison of synaptophysin, chromogranin, and L-dopa decarboxylase as markers for neuroendocrine differentiation in lung cancer cell lines. Cancer Res, 1990, 50:6068-6074.

3. Travis WD, Linnolia RI, Tsokos MG, Hitchcock CL, Nieman L, Cutler G, Chrousos G, Pass H, Doppman J. Neuroendocrine tumors of the lung: Immunohistochemical, ultrastructural and flow cytometric study of 35 cases. Am J Surg Path, 1991, 15:529-553.

4. Gazdar AF, Kadoyama C, Venzon D, Park J-G, Tsai C-M, Linnoila RI, Mulshine JL, Ihde DC, Giaccone G. The association between histological type, neuroendocrine differentiation and drug sensitivity of lung cancer cell lines. In press, J Natl Cancer Inst.

5. Linnoila RI, Gazdar AF. Non-small cell lung carcinoma with neuroendocrine features. (AP-195) Check Sample, Continuing Education Program by the American Society of Clinical Pathologists. Anat Pathol, 1990, 18:3:1-5.

6. Mulshine JL, Magnani JL, Linnoila RI. Applications of monoclonal antibodies in treatment of solid tumors. DeVita V, Jr., Hellman S, Rosenberg S (Eds), Biologic Therapy of Cancer, Lippincott, Philadelphia, 563-588.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 CM 06596-05 NMOB

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Bruce E. Johnson, MD	Acting Chief	NCI-NMOB
Others:	Daniel C. Ihde, MD	Deputy Director	NCI
	Adi F. Gazdar, MD	Section Chief	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

COOPERATING UNITS (if any): Eli Glatstein, M.D., Catherine Salem, M.D. Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Food and Drug Administration

LAB/BRANCH: NCI-Navy Medical Oncology Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS: 2.6	PROFESSIONAL: 1.8	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. Thirty-eight patients have been entered onto study and 28 of 36 (78%) patients who have completed therapy have achieved a complete remission. The projected median survival is 30 months with a median potential follow-up of 27 months. One patient has died from combined modality pneumonitis.

A phase I trial using dihydrolenperone, an agent identified as being active against human lung cancer by the human tumor colony forming assay (HTCFA) has been completed. Thirty-two patients have been studied at 6 dosage levels. The principle side effects have been somnolence and hypotension in all patients. Six patients have had to stop therapy because of somnolence and none because of hypotension. There have been no objective responses to date.

In vitro testing with dihydrolenperone showed 50% inhibition of growth of non-small cell and small cell lung cancer lines at 25-165 ug/ml. Pharmacokinetic determinations show peak absorption at 3-5 hours and plasma levels were more than 100 fold less than the levels where in vitro activity against lung cancer cell lines was observed.

From this studies we conclude that the HTCFA has identified a compound with novel side effects, the maximum tolerated dose is 50 mg per square meter, and achievable plasma levels are much less than that required for in vitro activity.

PROJECT DESCRIPTION

In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

Professional Staff:

PI:	Bruce E. Johnson, MD	Acting Chief	NCI-NMOB
Others:	Daniel C. Ihde, MD	Deputy Director	NCI
	Adi F. Gazdar, MD	Section Chief	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Eli Glatstein, M.D., Catherine Salem, M.D. Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Food and Drug Administration

Objectives:

1. Determine the frequency with which adequate tumor tissue can safely be obtained and drug sensitivity data determined.

2. Determine the response rate, toxicity, and survival of limited stage small cell lung cancer patients treated with VP/PLAT, simultaneous twice a day chest radiotherapy, and chemotherapy based on in vitro drug testing or a standard regimen (VAC).

3. Determine the side effects and maximum tolerated dose of dihydrolenperone.

4. Determine the pharmacokinetics of orally administered dihydrolenperone.

5. Determine the activity of dihydrolenperone within the confines of a Phase I trial.

6. Determine the correlation between the in vitro determined activity of dihydrolenperone and the achievable plasma levels of dihydrolenperone.

Methods:

1. Small cell lung cancer patients undergo staging

2. Limited stage patients undergo surgical biopsy of tumor tissue

3. Induction with 12 weeks of VP-16/Plat with concomitant 150 RAD twice a day radiothearpy to 4500 RAD over 19 days.

4. Patients with in vitro drug sensitivity data receive an additional 12 weeks of the in vitro best regimen, patients with no in vitro data receive 12 weeks of a standard vincristine, doxorubicin, and cyclophosphamide regimen.

5. Patients are followed for survival and toxicity.

6. Small cell lung cancer patients failing conventional combination chemotherapy and non-small cell lung cancer patients for whom no curative therapies are available are identified for Phase I drug trial.

7. Patients are treated orally twice a day for 28 days with dihydrolenperone and observed for toxicity.

8. Patients with tumor tissue available have in vitro testing with DHLP

Major Findings:

The Limited Stage Small Cell Lung Cancer Trial Administering VP 16 and Cisplatin Plus B.I.D. Chest Radiotherapy has a Decreased Rate of Pulmonary Toxicity and the Preliminary Survival Data shows a Prolognation of Median Survival

Between 7/86 and 3/91, 38 previously untreated patients (pts) with LTD stage SCLC entered a combined modality study. 26 were male, 12 female; 3 were PS 0, 33 PS 1, and 2 PS 2. The median age was 58 (range 34-72). Medically fit pts were offered a surgical procedure including thoracotomy to obtain tumor tissue for in vitro DST to select the CT given in weeks 13-24. Pts were initially treated with VP 80 mg/m^2 d1,2,3,27,28,29, PT 80 mg/m^2 d1,27, and concurrent chest RT 150 cGY bid Mon-Fri d5-24. Pts then received 2 more cycles of VP/PT followed by 4 cycles of individualized CT based on in vitro DST if available or empiric vincristine, doxorubicin, cyclophosphamide (VAC). 36 pts have completed therapy and are evaluable for response. 28 had a CR (78%) and the remaining 8 a PR (22%). The median potential follow-up is now 27 months (range 1-43). The median survival is 30 months with an actuarial survival of greater than 90% at 1 year and 65% at 2 years. 17 of 38 pts (45%) underwent a biopsy procedure to obtain tumor tissue. 7 of those 17 pts (41% or 18% of the total) had adequate number of SCLC cells for in vitro DST by their 13th week of treatment. Those 7 patients who received CT selected by in vitro DST have a median survival of 39 months. The median survival of the other 10 patients with biopsies that did not yield sufficient cells for DST lived a median of 22 months, similar to the entire group. This regimen has been associated with acceptable toxicity and the survival is nearly twice as long as in our previous combined modality treatment regimens for LTD stage SCLC. The preliminary information on survival of patients whose second 12 weeks of CT was selected on the basis of in vitro DST is encouraging and warrants further investigation.

Phase I Trial of Dihydrolenpoerone, A Novel Compound Active Against Lung Cancer Identified by the Human Tumor Colony Forming Assay

Antitumor activity of the butyrophenone dihydrolenperone was identified by screening compounds against human non-small cell lung cancers using the human tumor colony forming assay. We have completed a directed phase I trial in lung cancer patients using an oral twice a day regimen for 28 days. Thirty-two lung cancer patients have completed 25 courses of therapy at doses of 10 to 60 mg/m^2 PO BID. Twenty-three males and 9 females with a median age of 55 (range 24-69) were entered. Twenty-four were PS 0, 1 and 8 were PS 2. The maximum tolerated dose was 50 mg/m^2 PO BID and the dose limiting toxicity was somnolence. Three of the 6 patients started at the next higher dose of 60 mg/m^2 discontinued the drug because of intolerable somnolence (2) or progressive disease (1). Only 3 of 13 patients chose not to complete their course of 50 mg/m^2 because of DHLP toxicity (somnolence in 2 and visual hallucinations in 1) although the side effects were not incapacitating. Of the 32 patients, 4 developed grade 2 and 14 grade 1 hypotension. There was no significant hematologic, renal, or hepatic toxicity. Twenty-two patients have completed at least one course of DHLP and were evaluable for response. Ten patients had progressive disease, 11 had stable disease, and 1 had a minor response. In vitro drug testing using the MTT assay confirmed 50% inhibition of NSCLC and SCLC cell line growth at 25-165 ug/ml. Serum DHLP levels were 100-fold less than levels at which in-vitro activity was observed. We conclude: 1) the maximum tolerated dose in our study is 50 mg/m^2 PO BID 2) the dose-limiting side effect of dihydrolenperone is somnolence 3) plasma levels of dihydrolenperone are much less than those associated with in-vitro activity.

Publications:

1.Ihde DC, Grayson J, Woods E, Gazdar AF, Edison M, Lesar M, Linnoila RI, Minna JD, Glatstein E, Johnson BE. Twice daily irradiation as an adjuvant to etoposide/cisplatin therapy of limited stage small cell lung cancer. In Adjuvant Therapy of Cancer, VI. Salmon SE, Editor. W.B. Saunders, Philadelphia. 1990; 162-165

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06597-05 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Non Small Cell Lung Cancer Therapy Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Research Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB

COOPERATING UNITS *(if any)*
Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (J. Nesbitt); Radiation Oncology Branch, Surgery Branch, (R. Deming); Clinical Oncology Program

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Biotherapy

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20889

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A primary objective of this Branch is to improve the state-of-the-art in the therapy of lung cancer. In the past, this Branch had focused this effort in the study of small cell lung cancer. With the advances both in the therapy of the small cell patients as well as in the study of small cell lung cancer biology, we decided to generalize the Branch effort to include the systemic evaluation of non-small cell lung cancer. The vehicle for this pilot study of the feasibility and value of using in vitro criteria to select therapy for patients with metastatic non-small cell lung cancer.

PROJECT DESCRIPTION

Non Small Cell Lung Cancer Therapy Project

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Research Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB

Collaborating Branches:

Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (J. Nesbitt); Radiation Oncology Branch, Surgery Branch, (K. Salem); Clinical Oncology Program

Objectives:

1. To improve therapy of non small cell lung cancer by selecting chemotherapy on the basis of in vitro analyses, both of drug sensitivity and neuroendocrine markers. To use that protocol as a vehicle for the in-depth study of small cell lung cancer biology.

2. Pilot study to evaluate if patients treated on the basis of their tumor cells' in vitro response to a panel of chemotherapeutic agents have more effective tumor cytoreduction than conventionally treated control patients or historic controls.

3. To determine if non-small cell lung cancer patients with tumors expressing neuroendocrine features characteristic if small cell lung cancer experience natural history more typical of small cell lung cancer.

4. To evaluate our ability to prospectively establish clinical specimens as long-term cell lines.

   a. Optimizing our ability to grow specimens, especially in developing serum-free media systems.

   b. Use the computerized clinical and laboratory data bases to correlate.

Methods Employed:

1. Clinical trial

2. In vitro drug sensitivity analysis

3. Immunohistochemistry

4. Biochemical Marker analysis

5. Cell culture

Major Findings:

Since this study opened in April, 1984, over 100 patients have been accrued to this study. We performed an interim analysis on the study.

As a function of protocol design, all patients had tumor tissue come to the laboratory. In several instances, the tumor tissue non-viable due to immersion in formaldehyde, but excellent cooperation between surgeons and pathologists resulted in a better than 95% yield. In order to obtain tissue from as many patients as possible, we frequently obtain tissue from patients undergoing potential curative thoracic resection. We treat only those patients with metastatic disease that is measurable or can be evaluated. Of the 35 patients who already received chemotherapy on this protocol, tumor tissue arrived in the lab was of sufficient size and condition to do at least limited in vitro drug sensitivity analysis in 29%. Some patients have relapsed and died without any chemotherapy (5 patients) and many more are still followed without any evidence of recurrent disease (39 patients). We have established continuous cell lines on 23% of the patients we have evaluated. We project that the frequency of successfully performing in vitro analysis with our current approach may increase to 40% of the total prospective cases. Further refinements of this approach will be necessary to permit this approach to be more generally applicable and we will outline some of the research directions we are pursuing to accomplish this.

These cell lines are a very useful recourse in conducting further experiments to improve the frequency of successful drug sensitivity analysis. First, the initial cell lines derived in the course of non-small cell protocol are being used in validation of another technique of drug sensitivity analysis, the semi-automatic colorimetric assay. This work will be discussed elsewhere in this document, but there are two areas in which the work with this assay impacts on the non-small cell clinical trial. First, this assay requires significantly less operator time to perform, has a more objective mode of analysis, and ultimately may require a smaller number of tumor cells for analysis. Due to the efficacy of this technique, we might also be able to achieve the goal of testing combinations instead of single agents in vitro. For these reasons, we are motivated to substitute this assay for the dye exclusion assay, after we determine the degree of comparability between the two assays. Second, we have used this assay to examine the growth factor requirement of small cell lung cancer to optimize a serum-free media system for those cells. We are now ready to extend this approach to non-small cell tumors as it is apparent from our low rate of successfully generating tumor cell lines that our current media systems are suboptimal. Both of these adjustments, a more efficient in vitro assay and a more effective media system, have the potential of improving the biggest shortcoming of this approach, this is, increasing the percentage of cases that

we can successfully test for drug sensitivity in vitro.

As discussed previously, the number of patients actually receiving the combinations of drugs selected by the assay as being most active (based on single agent activity) is small (8 patients). This number will increase since we plan to accrue another 50 patients and as more of the patients, who underwent potentially curative thoracic resection, develop recurrent disease. Nevertheless, the results of the in vitro analysis suggested their tumors would be minimally responsive. The eight predicted most active combinations resulted in only a half log of cell kill in vitro.

Seventy percent of the single agents tested with these 8 tumors were resistant by our arbitrary scale (resulting in less than 50% tumor cell kill). None of these patients had an objective tumor response, but their median survival was five months. The survival rate was equivalent to the patients who received empiric etoposide/cisplatin. Since we are still dealing with small numbers of patients, we have not evaluated the two groups for the equivalence of prognostic features, so it is too early to conclude anything about the utility of the in vitro drug selection to see if a trend emerges. This study, which is really a pilot effort, will not definitely answer the questions regarding the clinical value of in vitro drug sensitivity analysis, but it will provide a departure point for constructing subsequent clinical trials to further resolve such issues.

One of the most provocative directions explored in this study is the prospective evaluation of the fate of the subset of non-small cell lung cancer without biochemical features of small cell cancer. We have prospectively analyzed cell tissues obtained in this study for the expression of four biochemical features generally felt to be characteristic of small cell. Based on the previous retrospective work in characterizing these biochemical markers, expected this phenomenon would be present in about 15% of clear cut non-small cell lung cancers. Our hypothesis was that the patients with these tumors would respond to their treatments in a fashion similar to small cell lung cancer patients (i.e. a higher response rate). We were able to do at least one biochemical parameter on 71 of 81 adequate tumors (88%). 11% of these specimens had elevated levels of expression of at least one biochemical marker. Seven non-small cell lung cancer parts with neuroendocrine biochemical features were treated with a combination chemotherapy used extensively in the Branch for small cell lung cancer (cytoxan, methotrexate, CCNU, vincristine, adriamycin, procarbazine). The response rate has been 43% for those seven "neuroendocrine" patients versus 11% for the remaining 28 non-small cell lung cancer patients treated to date on this study with corresponding median survival rate of 9 months versus 6 months. Considerable work has been done with the in vitro characterization of these neuroendocrine non-small cell lung cancer cell lines, especially in regard to their in vitro drug sensitivity. This will be discussed elsewhere in this document.

Significance to Biomedical Research and the Program of the Institute:

Lung cancer is the leading cause of cancer mortality in our society. Non-small

cell lung cancer which comprises 75% of all lung cancer is universally fatal once it has metastasized. Despite intensive clinical research, no major improvement has occurred in the treatment of disseminated non-small cell lung cancer. To address this the NCI-Navy Medical Oncology Branch has attempted to integrate a sytemic effort to study the biology of this cancer in conjugation with an attempt

to optimize the best available treatment. This entails testing a patient's tumor tissue in the laboratory for its response to standard chemotherapy agents. Based on the in vitro result, a combination is constructed that represents the most cytotoxic single agents for a particular patient's tumor.

This approach potentially has general merit in attempting to specifically tailor available treatments to the unique biology of a patient's tumor. This approach also insures tumor tissue comes to our laboratory and is potentially available to be established as a continuous cell line. Over 30 cell lines have already been established in the course of this study and these lung cancer lines comprise an excellent model system for a variety of laboratory investigations.

Proposed Course:

Further accrual of patients to the ongoing protocol will continue. A successor protocol is being developed to replace this study. The new trial is based on an in vitro analysis of a promising new drug 10 methyl EDAM. The addition of clinically achievable levels of dipyrimadole (the auto platelet agent) significantly enhanced the antitumor activity of ID methyl EDAM used alone. If this combination is found to be promising in vivo then it would comprise an active target for consideration in adjuvant settings as it would be expected to have a favorable toxicity profile.

Independent validation of the enhanced initial responsiveness to chemotherapy of patients whose tumor empresses neuroendocrine differentiation is required to corroborate the preliminary clinical trial outcome. To accomplish this, collaborations have been developed with two cooperative groups to analyze for the expression of neuroendocrine features from tumor specimens obtained from patients already treated with chemotherapy. The goal would be examine if the correlation over neuroendocrine expression with enhanced responsiveness to chemotherapy. Further associated biological studies will also proceed.

Publications:

1. Gazdar AF, Linnoila RI, Kurita Y, Oie HK, Mulshine JL, Clark JC, Whitsett JA. Peripheral airway cell differentiation in human lung cancer cell lines. Cancer Res 1990; 50:5481-5487.

2. Stevenson H, Gazdar AF, Phelps R, Linnoila RI, Ihde DC, Ghosh B, Walsh T, Woods E, Oie H, O'Connor T, Makuch R, Kramer BS, Mulshine JL. Establishment of tumor cell lines in vitro: an independent prognostic factor for survival in non-small cell lung cancer. Ann Intern Med 11:674-770, 1990)

3. Fridman R, Giaccone G, Kanomoto T, Martin FR, Gazdar AF, Mulshine JL. Laminin stimulates the attachment, the tumorgenicity and the drug resistance of small cell lung cancer cells. Proc Natl Acad Sci 1990; 87:6698-6702.

4. Linnoila RI, Jensen SM, Steinberg SM, Mulshine JL, Eggleston JC, Gazdar AF. Peripheral Airway Cell Marker Expression in Non-Small Cell Lung Carcinoma: Association with Distinct Clinicopathologic Features. In press, Am J Clin Path, 1991.

5. Mulshine JL, Linnoila RI, Magnani JL. Applications of monoclonal antibodies in the management of lung cancer Patients: An analysis. In press, Thoracic Clin of North America, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06598-05 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James L. Mulshine, MD	Senior Investigator	NCI-DCPC-BPRB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Res Assoc Prof NCI-USUHS	NCI-NMOB
	Daniel C. Ihde, MD	Deputy Director	NCI
	Barnett Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-DCPC-EDCOP
	Ingalill Avis, RN	Biologist	NCI-NMOB
	Anthony M. Treston PhD	Guest Researcher	NCI-NMOB
	Francis Scott, PhD	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Johns Hopkins (B. Eipper, M. Tockman)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Biotherapy

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20889

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efforts of this Branch has been central to the recognition of gastrin releasing peptide as an autocrine growth factor for small cell lung cancer. Dr. Cuttitta developed a monoclonal antibody (2A11) to the active portion of that peptide and demonstrated that the immunoglobulin could block the mitogenic effect of GRP in vitro and in vivo. We have recently, in collaboration with Hybritech, Inc. (San Diego, CA), initiated a clinical trial to test whether one can control autocrine mediated malignant proliferation of small cell lung cancer using a monoclonal antibody. Our Branch has a long standing interest in the role of growth factors in cancer, so that information from 2A11 antibody clinical trial could be a foundation from subsequent anti-growth factor trials.

The phase I portion of the 2A11 antibody trial identified 250mg/m2 on the monoclonal as the optimal dose. The Phase II portion of the 2A11 evaluation has recently started. We have previously reported the diagnostic application of lung associated monoclonal antibodies derived at this Branch for use in the early detection of lung cancer. We have patented the method for this approach with collaboration from John Hopkins and in conjunction with the Lung Cancer Study group will proceed to rapidly follow up on this critical area. We have followed up on this work with several publications including a report characterizing the fine binding affinity of one of the antibodies used for early lung cancer detection.

PROJECT DESCRIPTION

Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-DCPC-BPRB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Res Assoc Prof NCI-USUHS	NCI-NMOB
	Daniel C. Ihde, MD	Deputy Director	NCI
	Barnett Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-DCPC-EDCOP
	Ingalill Avis, RN	Biologist	NCI-NMOB
	Anthony M. Treston, PhD	Guest Researcher	NCI-NMOB
	Francis Scott, PhD	Guest Researcher	NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Johns Hopkins Medical School (B. Eipper, M. Tockman)

Objectives:

1. To study the pharmakinetics of monoclonal antibody delivery, attempting to maximize delivery of antibody to tumor involved sites.

2. To study methods of radiolabeled monoclonal antibody imaging as a staging tool in evaluating patients with cancer.

3. To determine if monoclonal antibody can be used to block growth factor stimulated tumor proliferation.

4. To study tumor cells to identify other growth factors which are potential targets for immunomolecular regulation.

5. To determine the utility of monoclonal antibodies as differentiation markers which have potential utility for the early detection of lung cancer.

Methods Employed:

1. Radionuclide Imaging
2. Immunohistochemistry/immunocytochemistry
3. Radioimmunoassay
4. Radioautography

Major Findings:

A. This Branch was involved in an early monoclonal antibody therapy trials in cutaneous T-cell lymphoma to determine if the monoclonal antibody could mediate cytoreduction by enhancing immune clearance of malignant T-cell. This trial failed to demonstrate significant therapeutic benefit, but did provide a vehicle for successful diagnostic imaging studies. The initial diagnostic imaging studies were developed by Paul Bunn, M.D., and continued by Dr. Mulshine. The intravenous delivery of Inlll labeled Tl01 has resulted in the highest percent of tumor targeting achieved as of the time of its reporting. This localization efficiency was further therapeutic improved after regional delivery vial the lympahtics of subcutaneously delivered Inlll labeled Tl01. Further studies included a comparison of quality imaging Inl31 Tl01 versus lll Tl01. In this study, the Inlll conjugate was clearly superior. This work now proceeds to further analysis of specificity of targeting by using an isotopically matched Inlll Tl01-control antibody in sequential scanning studies with Inlll Tl01. Information generated in the course of these studies include en hanced understanding of the pharmacology of antibody targeting, the immunogeni city of administered mouse immunoglobulin, and the efficiency if regional delivery techniques. These studies collectively provide the basis for proceed ing with the radiolabeled Tl01 therapy trial which is discussed separately. Efforts have been productive, both in terms of published manuscripts and in developing useful collaborations with other investigators at the Clinical Center engaged in clinical research with monoclonal antibodies.

B. Small cell lung cancer has been extensively studied both at this Branch and elsewhere as a model of a neuroendocrine tumor. Small cell lung cancer has already been reported to produce over 25 different peptide hormone products. Recently, workers from our lab sequenced the gene for GRP from small cell lung cancer. A family of previously unknown peptides synthesized from open reading frames found on the GRP gene. Hetero anti-sera were developed to the three GRP gene associated peptides (G-Gap peptides). By several assays, immunologic evidence of expression of these three distinct products was documented in both small cell tumors and in fetal tissues. These facts suggest that despite the already known numbers of peptide products of small cell lung cancer, there may be a considerably larger number of small cell tumor products. With the rapid development of many areas of biotechnology, the techniques may now be available to begin systematically evaluating the total peptide synthetic capabilities of small cell.

C. We are interested in elucidating and additional new peptide products of small cell, we propose to focus on those peptide products that possess mito genic capabilities. To facilitate this, we have invested considerable time in validating a semi-automatic colorimetric assay for evaluation of growth factor effects. The parameters to evaluation for such an application are considerably different than the conditions for the assay as reported by Carmichael and others from our Branch. The advantage of this assay is that it provides the exceedingly efficient assay to monitor for growth stimulatory effects, which will be essential when screening large numbers of purified fractions generated in typical HPLC purification efforts.

D. Using the semi-automatic colorimetric assay, we have already demonstrated the mitogenic effect of insulin-like growth factor-I (IGF-I) on small cell lung cancer cell lines. We have further demonstrated that this effect can be blocked by a monoclonal antibody specific for the anti-IGF-I receptor.

We have studied the biology of IGF-I in small cell and it appears to be an attractive candidate to target for a therapy approach similar to the anti-GRP monoclonal antibody trial. In thinking about GRP and a candidate for immunotherapy, the limited role this molecule plays in normal adult physiology potentially permits one to completely block this peptide effect without lethal consequences. The situation with IGF-I may not be similar as this molecule plays a more obvious role in normal adult physiology. Although that might not prevent us from exploring the same type of anti-autocrine factor strategy we employed in the anti-GRP trial, it did provoke us to consider approaches.

E. Many investigators have suggested that cancer can be thought of as a re-expression of normal embryonic and fetal developmental processes. An extrapolation is that autocrine type stimulation may be an important developmental mechanism. If so, such autocrine proliferation should be controlled through some signaling mechanism to allow for the uniform development of a fetus. In cancer, autocrine proliferation proceeds unabated either because of the regula tory signal. We tested to see if the stimulation of small cell lung cancer mediated be IGF-I could be inhibited by glucagon, a normal antagonist of IGF-I activity. Of interest, at a concentration of 10 g/ml, glucagon inhibits the growth enhancement of exogenous effect of IGF-I in other cell lines. In addition, we are attempting to define the mechanism mediating the inhibitory effect in the cell lines responsive to glucagon.

F. Work in early lung cancer detection using on immunocytochemical analysis continues and has been described in detail in the literature. The prospective confirmatory trial is due to begin in 1990.

Significance to Biomedical Research and the Program of the Institute:

These studies have two goals: First to complete the ongoing trial which represent a first effort to establish the clinical utility of monoclonal antibody based imaging and treatment approaches; Second, we have design ongoing in vitro analysis in conjunction with the clinical trials as well as other laboratory investigations to develop second generation biological trials which lend to more effective therapeutic control of malignant proliferation.

Proposed Course:

The Phase I component of the anti-GRP trial is complete and will be extended to Phase II at the 250mg/m2 dose levels. Clinical trials with the other antibodies will also continue with the goal of moving to radionuclide conjugate therapy using monoclonal antibodies in cutaneous T-cell lymphoma and lung cancer. Further work will continue to develop a feasible approach to block IGF-I stimulation of lung cancer. Dr. Cuttitta is generating antibodies against

synthetic peptides from various portions of prepro IGF-I and the IGF-I receptor. Using either an available reagent or Branch derived product, we will do further in vivo work to block IGF-I stimulation. This work may lead us to a clinical trial in a similar fashion to the anti-GRP monoclonal antibody trial. Due to the frequent requirement of neuropeptides for alpha terminal amidation we have initiated a series of experiments to characterize the processing enzyme responsible for the event. Two of presentations at the national meetings sum marize that work. The alpha amidation processing step may comprise a very critical regulatory even in the maturation of multiple lung cancer autocrine growth factors.

As a result of careful consideration of the role of growth factors in lung cancer progression, a strategy to block these targets in early stages of cancer progression is attractive. Intervention strategies based on protracted neutralization of lung growth factor effects will be systematically explored to determine if this approach results in more effective cancer control.

Publications:

1. Mulshine JL. Sputum cytology review: Editorial. Primary care and cancer. 1991; 11:21-26.

2. Mulshine JL, Treston AM, Scott FM, Avis I, Boland C, Phelps R, Kasprzyk PG, Nakanishi Y, Cuttitta F. New approaches to the management of lung cancer. Rational strategies for early detection and intervention. Oncology 1991; 5:25-40.

3. Avis I, Kovacs TO, Kasprzyk PG, Treston AM, Bartholomew R, Walsh JH, Cuttitta F, Mulshine JL. Preclinical evaluation of an anti-growth factor monoclonal antibody to treat patients with lung cancer. In press, J Nat Cancer Inst, 1991.

4. Shaw GL, Mulshine JL. Markers of lung differentiation as biomarkers of lung cancer. In press, Cont Oncol, 1991.

5. Gazdar AF, Kadoyama C, Venzon D, Park JG, Tsai CM, Linnolia RI, Mulshine JL, Ihde DC, Giaccone G. The effects of histological type and neuroendocrine differentiation on drug sensitivity of lung cancer cell. In press, J Nat Cancer Inst, 1991.

6. Mahmoud S, Staley J, Taylor J, Bogden A, Moreau JP, Coy D, Avis I, Cuttitta F, Mulshine JL, Moody TW. Bombesin analogues inhibit the growth of small cell lung cancer in vitro and in vivo. Cancer Res 1991; 51:1798-1802.

7. Mulshine JL, Magnani J, Linnoila RI. Clinical applications of monoclonal antibodies in solid tumors. In Principles and Practices of Biotherapy. DeVita V, Hellman S, Rosenberg S, (eds), J.B. Lippincott Co., Philadelphia, PA. In press, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07250-05 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
New Drug Discovery Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-DCPC-EDCOP
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Senior Investigator	NCI-NMOB
	Daniel Ihde, MD	Deputy Director	NCI
	James Mulshine, MD	Senior Investigator	NCI-DCPC-BPRB
	Gary Sladek, MD		NCI-NMOB

COOPERATING UNITS *(if any)*

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Investigational Drug Branch, CTEP (M. Christian)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20889

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this group is to identify new agents of potential clinical use in treating solid tumors. A major effort over the past 5 years has been the use of an in vitro assay which may be helpful as a preclinical screening model for antitumor agents. The model has been used to predict the clinical activity of 7 chemotherapeutic agents against 11 human colorectal carcinoma cell lines which have been developed in this branch. Using the model, we have shown that leucovorin enhances the in vitro cytotoxicity of the fluoropyridines versus our panel of colorectal cell lines. A study was also performed to detect possible synergy between etoposide and cisplatin in a panel of 8 human bronchogenic carcinoma cell lines. Extensive analysis revealed no in vitro synergy, a finding at variance with standard feeling. Schedule dependent drug interaction has been documented between methotrexate and 5-fluorouracil. Persantine has been shown to enhance the cytotoxicity of 10-EDAM in human lung cancer cell lines. Clinical trials are planned to explore this.

At present, we are involved in several trials of new experimental therapeutic agents: a radiolabeled monoclonal antibody ^{90}yttrium-T101) in mycosis fungoides and chronic lymphocytic leukemia; 4-ipomeanol in lung cancer. A phase I trial of hepsulfam has recently been completed, and maximally tolerated schedule identified as 360 mg/m^2 i.v. every 5 weeks. Dose limiting toxicity was leukopenia.

PROJECT DESCRIPTION

New Drug Discovery Project

Professional Staff:

PI:	Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-DCPC-EDCOP
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Senior Investigator	NCI-NMOB
	Daniel Ihde, MD	Deputy Director	NCI
	James Mulshine, MD	Senior Investigator	NCI-DCPC-BPRB
	Gary Sladek, MD		NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo; Investigational Drug Branch, CTEP (M. Christian)

Objectives:

1. Identification of new compounds for the treatment of solid tumors.

2. Preclinical testing of combinations of drugs to detect synergy.

3. Validation of in vitro chemosensitivity test.

4. Testing new compounds in the clinic for lung and colon cancers.

Methods Employed:

1. In vitro chemosensitivity: MIT assay (a tetrazolium-based colorimetric test for cell viability.

2. Phase I trials of new drugs in cancer (for example, ipomeanol in lung cancer).

Major Findings:

1. 5-FU was the only one of 7 drugs tested which we predict would be effective in some of our colorectal cell lines.

2. Leucovorin enhanced the cytotoxicity of 5-FU and of FUDR in 10 of 11 colorectal cell lines tested.

3. The ipomeanol study has opened; the ^{90}yttrium-T101 study has also opened. Thirteen patients have been treated with sulfamic acid. The study was closed when the maximally tolerated dose was found to be 360 mg/m^2 i.v. every 5 weeks, with dose limiting toxicity of leukopenia.

Significance to Biomedical Research and the Program of the Institute:

New drug development is a major charge of the National Cancer Institute. The preclinical screening program of the NCI-NMOB is based upon the MIT assay. It is important to pursue innovative therapies, such as treatment with radiolabeled monoclonal antibodies directed against malignant cells (e.g. ^{90}yttrium-T101).

Publications:

1. Park J-G, Kramer BS, Lai S-L, et al. Chemosensitivity patterns and expression of human multidrug resistance (MDRI) gene by human gastric and colorectal cell lines. J. Natl Cancer Inst, 1990.

2. Tsai C-M, Gazdar AF, Perng R-P, and Kramer BS. Schedule dependent in vitro combination effects of methotrexate and 5-fluorouracil in human tumor cell lines. Proc ASCO 10:31, 1990.

3. Dearing MP, Englee-Miller MJ, Kramer BS, et al. Enhanced cell of human lung cancer lines by 10-Ethyl-10-deazouminopterin (10-EDAM) when given with dipyridamole (DPM). Proc ASCO 10:31, 1990.

4. Tsai C-M, Gazdar AF, Allegra C, and Kramer BS. Enhancement of fluorinated pyrimidine-induced cytotoxicity by leucovorin in human lung cancer cell lines. Int J Cancer, 1990.

5. Tsai C-M, Gazdar AF, Perng R-P, and Kramer BS. Schedule dependent in vitro combination effects of methotrexate and 5-fluorouracil in human tumor cell lines. Int J Cancer, 1990.

6. Carrasquillo, JA, Kramer BS, Fleisher T, et al. In-111 versus Y-90 T101 biodistribution in patients with hematopoietic malignancies. Proc Soc Nuc Med, 1991.

7. Sladek G, Dearing MP, Kramer BS, et al. Phase I clinical and pharmacokenitic study of hepsulfam. Proc Am Soc Clin Oncol 10:32, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07255-03 NMOB

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Birrer, MD, PhD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	Powel Brown, MD	Clinical Associate	NCI-NMOB
	Eva Szabo, MD	Clinical Associate	NCI-NMOB
	Dennis Sanders, MD	Clinical Associate	NCI-NMOB
	Lisa Preis	Biologist	NCI-NMOB

COOPERATING UNITS *(if any)*: University of California San Diego (Dr. Michael Karin)

LAB/BRANCH: NCI-Navy Medical Oncology Branch

SECTION: Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION: NCI, COP, DCT, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent developments in molecular biology has led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes in various tumor systems. These genes, called oncogenes, are involved in various aspects in the regulation of cell growth. It is now critical to understand the precise mechanism by which these genes function so molecular agents ultimately can be derived to alter or repress their effects.

We have chosen to explore the biologic and biochemical functions of 2 dominant (L-myc and c-jun). Transcriptional and translational products of L-myc have been characterized and are now being correlated with biologic functions. Ultimately, truncated fragments of this gene will be tested for potential transformation suppression function.

Likewise, we have recently described the transforming function of c-jun in mammalian cells and are now mapping this function by deletion mutation. Correlation of this function with other known activities of c-jun, such as transactivation will be done. Mutants of c-jun capable of inhibiting AP-1 transactivation and cellular transformation will be characterized.

PROJECT DESCRIPTION AND RESULTS

Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects

PI:	Michael J. Birrer, MD, PhD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	Powel Brown, MD	Clinical Associate	NCI-NMOB
	Eva Szabo, MD	Clinical Associate	NCI-NMOB
	Dennis Sanders, MD	Clinical Associate	NCI-NMOB
	Lisa Preis	Biologist	NCI-NMOB

Project 1 - The L-myc Proteins and Their Biologic Activities:

The protein products of the L-myc gene have been further characterized. The larger molecular weight species possibly arise from alternative translational initiation sites (including a non-AUG initiation site) and post-translational phosphorylation. These various proteins have been shown to cotransform rat embryo cells with an activated ras gene and are presently being examined for differences in their biologic activities. (Birrer in collaboration with Minna)

Project 2 - The Transforming Activity of the c-jun Proto-oncogene:

The transforming activity for the c-jun proto-oncogene was established by demonstrating the cotransforming activity of this gene in combination with an activated ras gene in rat embryo cells. Further, it was shown that c-jun can transform an immortalized rat fibroblast cell line Rat-1a as a single gene. This demonstrates that no mutational activating event is required for c-jun to transform mammalian cells. (Birrer, in collaboration and Minna)

To elucidate the mechanism of transforming activity of c-jun we have undertaken a mutation/deletion study of the gene. Presently, the transforming activity of the gene maps to two highly conserved regions in the gene, one of which contains the DNA binding domain. Preliminary experiments map these transforming domains to those required for transactivation. Further, in isolating these mutants, some non-transforming ones were found to inhibit the transforming activity of the full length gene, hence displaying a "dominant-negative" phenotype. We are presently characterizing these for their biologic and biochemical properties. (Brown, Szabo, and Birrer)

Project 3 - Identification of AP-1 Regulated Genes:

In an attempt to further elucidate the mechanisms involved in the biologic activities of c-jun we will identify downstream genes regulated by AP-1. We will use 2 approaches: 1) isolation of gene whose transcription is up-regulated by c-jun by cDNA subtractive hybridization. We will subtract a normal cell mRNA from one transformed by c-jun. 2) isolation of genes with AP-1 sites by identification of genomic clones through binding of Jun/Fos protein complex in an in vitro assay (Sanders and Birrer).

Publications:

1. Bliss DP, Battey JF, Linnoila RI, Birrer MJ, Gazdar AF, Johnson BE. Expression of atrial natriuretic factor mRNA in small cell lung cancer suggests a new mechanism for hyponatremia. J Natl Cancer Inst, 1990, 82:# 4.

2. Minna JD, Schutte J, Viallet J, Thomas F, Kaye FJ, Takahashi T, Nau M, Whang-Peng J, Birrer M, Gazdar AF. Transcription factors and recessive oncogenes in the pathogenesis of human lung cancer. Int J Cancer, 1989, Supp 4 32-34.

3. Dosaka H, Rosenberg R, Minna JD, Birrer MJ. A complex pattern of translational initiation and phosphorylation in L-myc proteins. Oncogene, 1991, 6:371-378.

4. Kim JH, Takahashi T, Chiba I, Park JG, Birrer MJ, Roh JK, Lee HD, Kim JP, Minna JD, Gazdar AF. Occurrence of p53 gene mutations in gastric carcinoma tumors and cell lines. J Natl Cancer Inst, 1991, 83:13:938-943.

5. Szabo E, Birrer MJ. The role of Jun and Fos gene family members in TPA induced hematopoietic cell differentiation. In press, Cell Growth and Differentiation.

6. Birrer MJ, Alani R, Brown PH, Preis LH, Sanders DA, Seigfried JM, Szabo E. Early events in the neoplastic transformation of respiratory epithelium. In press, J Natl Cancer Inst, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07256-03 NMOB

PERIOD COVERED
October, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Oncogene Action in Tumorigenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederic J. Kaye, MD	Senior Investigator	NCI-NMOB
Others:	Albert Lin, MD	Medical Staff Fellow	NCI-NMOB
	Greg Otterson, MD	Medical Staff Fellow	NCI-NMOB
	Eiji Shimizu, MD	Guest Researcher	NCI-NMOB
	Robert Kratzke, MD	Medical Staff Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

Duke University Medical Center, Durham, NC (J. Horowitz)
Dana-Farber Cancer Institute, Boston, MA (D. Livingston)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology, and Immunology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have undertaken a study to identify critical genetic events in the pathogenesis of human cancer. We have currently focused our research efforts on studying the mechanism and implication of inactivation of the retinoblastoma (Rb) gene in human cancer. Our recent findings are as follows: 1) essentially all small cell lung cancer tumors have absent or aberrant Rb protein products; 2) we have identified and characterized a series Rb mutants (defective in phosphorylation and oncoprotein binding); 3) we are investigating the possibility of these Rb mutants to function as transforming genes (dominant negative effect); 4) we have successfully transfected a wild-type or mutant Rb gene in a SCLC cell line to study its biological effect; 5) we are using our Rb open reading frame reagents to identify putative cellular proteins that normally interact with the Rb protein and presumably modulate its growth inhibitory effect; and 6) we have generated a large number of in vitro Rb mutant proteins to characterize different functional domains of this protein.

In addition, we continue to maintain a research effort studying mechanisms of L-myc gene activation. We have identified a 200 Kd cellular protein that binds to a specific region of the L-myc protein and we are currently attempting to clone this protein. Further we have preliminary evidence that the Rb tumor suppressor gene may also bind to the L-myc protein as well.

PROJECT DESCRIPTION

Mechanisms of Oncogene Action in Tumorigenesis

Role of the Retinoblastoma Gene in the Pathogenesis of Human Cancer

PI:	Frederic J. Kaye, MD	Asst Prof Med-NCI/USUHS	NCI-NMOB
Others:	Albert Lin, MD	Medical Staff Fellow	NCI-NMOB
	Greg Otterson, MD	Medical Staff Fellow	NCI-NMOB
	Eiji Shimizu, MD	Guest Researcher	NCI-NMOB
	Robert Kratzke, MD	Medical Staff Fellow	NCI-NMOB

We have demonstrated inactivation of the Rb gene in essentially all SCLC tumors. We now wish to address two critical questions:

A. Can we revert tumorigenicity in SCLC by reintroducing the Rb gene and can we use this information to implement preventive or therapeutic strategies?

B. What is the role of the Rb gene in normal cellular physiology and how does its inactivation result in tumorigenesis?

Question A: Transfection of the Rb gene into SCLC cell lines.

We have successfully transfected either a wild-type or mutant Rb gene in a SCLC cell line lacking endogenous Rb expression. To date we have not observed suppression of tumorigenicity of transfected cell lines when injected into nude mide. Further analysis of tumor nodule resected from mice appear to still express functional Rb protein. Additional experiments are still in progress. (Kaye, Gerster, Kratzke in collaboration with S. Segal).

Question B: Identification and characterization of mutant Rb proteins in SCLC.

Although 40% of SCLC tumors express a normal sized mRNA, we have now shown that these transcripts are defective and result in absent or mutant Rb protein.

Therefore, in excess of 90% of SCLC tumors studied to date have evidence for Rb inactivation. In collaboration with J. Horowitz and R. Weinberg (Boston, MA), we have identified several cell lines with mutant proteins and have characterized the molecular defects that generated these mutants. In addition we have identified a SCLC line with an Rb protein defective in phosphorylation. This is of great interest since Rb phosphorylation is believed to regulate cell cycle events. We have characterized this mutant protein and found a missense mutation changing a single amino acid. This analysis will help define functional domains of the Rb protein. We have now generated a series of in vitro mutants in the region to further identify phosphorylation domains and/or tertiary structure of the Rb protein.

We also have studied the potential transforming effect of these mutant proteins in rat embryo fibroblast system to determine if they might have a direct effect on growth regulation similar to that observed with mutant p53 proteins. We examined several in vivo mutant Rb protein and found that they do not function as dominant oncogenes in contrast to that observed with mutant p53.

Another key experiment is to identify cellular proteins which interact with Rb to modulate its growth inhibitory effect and these studies are ongoing in collaboration with W. Kaelin and D. Livingston, Boston, MA. (Kaye, Kratzke, Gerster, Lin). These experiments have identified about 10 cellular proteins that are capable of binding to Rb and work is in progress to clone these proteins.

We are also interested in examining the role of myc gene activation in lung cancer. The E myc protein has recently been shown to bind to a small helix-loop-helix protein designated as Max. We are conducting experiments to identify other cellular proteins that might modulate L-myc protein activity. (Shimizu, Kratzke, Kaye). We have generated a series of glutathione transferase L-myc fusion proteins and have searched for cellular binding proteins extracted from a variety of different cell types. To date we have identified a 200 Kd protein that specificalkly binds to a discrete region of the L-myc protein, distinct from the Max binding site. Similarly, we have preliminary evidence suggesting that the Rb protein may also bind to a specific domain of the L-myc ptotein.

Publications:

1. Kaye F, Kratzke R, Gerster J, Horowitz J. Mutation of a single amino acid of the retinoblastoma protein blocks phosphorylation and oncoprotein binding. Proc Natl Acad Sci USA 1990;87:6922-6926.

2. Kaye F, Kratzke R, Gerster J, Lin P. Recessive oncogenes in lung cancer. Ann Rev Resp Dis 1990;142:44-47.

3. Kaelin WG, Pallas DC, DeCaprio JA, Kaye FJ, Livingston DM. Identificatioin of cellular proteins that can interact specifically with the T/Ela-binding region of the retinoblastoma gene product. Cell 1991; 64:521-532.

4. Kratzke RA, Lin AY, Otterson GA, Kaye FJ. Phosphorylation defective retinoblastoma proteins do not function in vitro as dominant oncogenes. Submitted

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07257-03 NMOB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shoshana Segal, PhD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Matia Bar-Ner, PhD	Fogarty Visiting Fellow	NCI-NMOB
	Constance Cultraro	Biologist	NCI-NMOB
	Barbara Dunn, MD. PhD	Clinical Associate	NCI-NMOB

COOPERATING UNITS *(if any)*

Experimental Immunology Branch, NCI

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Differentiation

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD, 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cellular differentiation is a complex process for which the molecular mechanisms are poorly understood. How changes in growth potential are related to expression of the differentiated phenotype is at present unknown. We have focused our attention on questions such as the role of oncogenes in the differentiation process of murine erythroleukemia (MEL) and F9 teratocarcinoma cell lines. We were able to demonstrate that in both cell lines, high levels of expression of a transfected c-myc gene blocks HMBA, DMSO or Retinoic Acid (RA) induced differentiation.

Based on these findings and the published reports on the homology between C, N, and L-myc protooncogenes, we investigated the ability of the related L- and N-myc genes to substitute for c-myc in blocking MEL differentiation. Our results clearly indicate that constitutive high levels of transfected L- and N-myc mRNAs block inducer-mediated differentiation. These studies strongly suggest that down regulation of c-myc expression in MEL cells is a necessary event for terminal differentiation. We used a number of deletion mutants of the human c-myc gene for mapping the regions responsible for its apparent critical role in MEL and F-9 teratocarcinoma cell differentiation. In MEL cells , our results suggest that the first 40 amino acids of the c-myc protein are dispensable for blocking differentiation, the other domains of the protein are necessary for this function. In addition, we are developing a new approach for identifying proteins that interact with the c-myc protein during differentiation.

PROJECT DESCRIPTION

Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation

PI:	Shoshana Segal, PhD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Matia Bar-Ner, PhD	Fogarty Visiting Fellow	NCI-NMOB
	Constance Cultraro	Biologist	NCI-NMOB
	Barbara Dunn, MD, PhD.	Clinical Associate	NCI-NMOB

Collaborating Branch:
Experimental Immunology Branch, NCI

Objectives:

1. To study the role of C, N, and L-myc protooncogenes in growth and differentiation of MEL and F9 teratocarcinoma cells.

2. To identify and map regions on the c-myc gene essential for differentiation.

3. To study mechanisms and genes involved in hematopoietic and F9 teratocarcinoma cell differentiation.

A. Members of the Myc Family Block Chemically Induced Differentiation of MEL and F9 Teratocarcinoma Cells.

MEL and F9 teratocarcinoma cells express high levels of the c-myc protooncogene, however, shortly after the addition of inducer (HMBA, DMSO, RA) a sharp decline in c-myc mRNA occurs which is followed by a cessation of cell growth and terminal differentiation. We transfected both cell lines with a plasmid containing the c-myc gene driven by the Molony LTR. All clones obtained from the MEL cell line expressed constitutive high levels of the transgene and were blocked in their ability to differentiate in response to chemical inducers. F9 derived clones expressed high levels of the exogenous c-myc gene, but the mRNA was down regulated in a similar fashion to the endogenous gene causing only a partial block to differentiation. To further support these findings we introduced, by stable transformation, into MEL cells related myc family genes L- and N-myc. A number of studies have shown greater than 90% sequence homology between C, N, and L-myc in several discrete areas of the gene. Although MEL cells do not express normally L- or N-myc, all of the clones expressing high constitutive levels of the transfected genes fail to differentiate in response to the chemical inducer HMBA.

B. Identification of Regions in Human c-myc That are Involved in Cellular Differentiation.

The involvement of c-myc in normal and neoplastic growth makes it important to understand its function(s) and the structural basis of some of its properties.

Studies by Lee et al. have identified three areas that are essential for rat embryo cells cotransforming activity. The mapping of these areas was accomplished by the use of a large number of c-myc deletion/insertion mutants. We undertook a similar approach for identifying regions involved in differentiation.

MEL cells were transfected with deletion mutants spanning the normal c-myc coding regions (exons 2 and 3). Clones expressing the mutated c-myc gene were isolated and analyzed for HMBA induced differentiation. Results obtained from independent transfectants indicate that sequences at the 5' and 3' ends of the coding region are necessary for activity; however, short deletions at the 5' end are tolerated.

The helix loop helix (HLH) as well as the leucine zipper motifs located at the 3' end of the gene are essential for inhibition of differentiation. A large deletion in the center of the coding regions (a.a. 145-262) has an intermediate effect of differentiation.

C. Identification of Proteins Interacting with the myc Gene Products During Differentiation of MEL cells. (In collaboration with D. Segal, Experimental Immunology Branch, NCI).

I. A construct containing a single chain FV fragment from an anti DNP antibody was ligated in frame to coding region of the human c-myc gene. We plan to transfect the hybrid gene into MEL cells, obtain clones expressing the transfected gene which do not differentiate following induction with HMBA. We will make protein extracts and mix them with DNP-sepharose beads. Since the fusion protein contains anti-DNP binding activity, c-myc and its associated proteins will bind to the beads. More detailed analysis of the proteins will be done after elution from the beads.

II. To identify genes that interact specifically with c-myc in MEL cells we constructed a plasmid containing a fusion gene of glutathione-s-transferase and human c-myc. The fusion protein was incubated with protein lysates from MEL cells and we were able to identify a number of proteins which are being characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 CM 07258-03 NMOB

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Etiology of Cutaneous T-cell Lymphomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Francine Foss, MD	Asst Prof Med USUHS	NCI-NMOB
	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Ross Turner, MS	Biologist	NCI-NMOB
	Dat Nguyen, MD	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

Robert Gallo, LTCB, DCE, NCI, NIH
Edward Sausville, CPB, DCT, NCI, NIH

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Molecular Biology of Differentiation

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The cutaneous T-cell lymphomas (Mycosis Fungoides and the Sezary Syndrome) comprise a group of indolent neoplasms of mature T-cell phenotype, the etiology of which is poorly understood. The clinical spectrum of these neoplasms varies from one of chronic skin involvement to one of aggressive disease with organ infiltration and circulating malignant T-cells. Since it has been suggested that early stage skin lesions comprise a polyclonal rather than a monoclonal population, it is unclear whether the disease arises from an event in a T-cell precursor, or whether it arises out of a T-cell response to an event, or possibly a viral infection, in an accessory cell. We are attempting to address this question by determining the clonal nature of early stage skin infiltration using PCR amplification and sequencing of T-cell receptor rearrangements in the skin. We are also studying the role of suppressor gene mutation in the evolution of the disease and we have detected p53 mutations in several patients with advanced stage disease. We are exploring the hypothesis that a retrovirus may be implicated in the pathogenesis of this disease by studying patient materials for retroviral-like sequences and by culturing cells from patients and attempting to isolate retroviral activity. In addition, we have studied response to growth factors and cytotoxic activities of a number of pharmacologic agents in MF cells and in Hut 78, an MF cell line, in an attempt to derive new therapies for patients.

PROJECT DESCRIPTION

Etiology of Cutaneous T-cell Lymphomas

PI:	Francine Foss, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Ross Turner, MS	Biologist	NCI-NMOB
	Dat Nguyen, MD	Guest Researcher	NCI-NMOB

Collaborating Branches:

Edward Sausville, CPB, DCT, NCI, NIH
Robert Gallo, LTCB, DCE, NIH

Objectives:

1. To determine the origin of the malignant cells in Mycosis Fungoides by attempting to establish cell lines from various sites.

2. To study the biology of the sezary cell lines with respect to growth factor production and response, oncogenic alterations, and sensitivity to chemo therapeutic and biologic agents.

3. To determine whether very early stage skin lesions represent polyclonal or monoclonal T-cell populations both for better understanding of disease pathogenesis and for possible use in diagnosis.

4. To define the possible role of retroviruses in the etiology of the disease.

Major Findings:

Cell Culture Experiments

Previous efforts to establish long-term cultures of sezary cells have yielded only one cell line, Hut 78. Immunophenotypic studies of this cell line and of and fresh sezary cells from patients have shown that these cells represent a mature T-helper phenotype, expressing the CD4 antigen and lacking the TAC anti gen, or IL2 receptor. The cells demonstrate a moderate but variable response to T-cell mitogens. Kinetic studies reveal that the cells in the circulating compartment are largely non-proliferating, in contrast to those in lymph node and skin. We have attempted to establish cell lines from blood, bone marrow and lymph node from patients with Mycosis Fungoides and have successfully maintained cells from bone marrow in four patients and from lymph node in four. These cell lines are of two types, one being characteristic of T-cells and one bearing markers of cells of monocytoid origin. At least two of the lines derived from lymph node bear surface markers characteristic of sezary cells and further characterization of these is underway.

Retroviruses as Etiologic Agents

In collaboration with Dr. P. Browning and Dr. R. Gallo, we have been able to identify reverse transcriptase activity in cultured cells from two CTCL patients. Both of these lines represent a populations of cells which appear to be of monocytoid origin. Further analysis of these cells indicates that the reverse transcriptase activity can be isolated on sucrose gradients. However, the cells proliferate very slowly, and obtaining large volumes of cell supernatant for definitive virus isolation has been difficult. Two strategies have been evolved to address this problem in our lab. First, we have cocultivated the cells from these and other MF patients with permissive lines, such as HUT78 and A3.01 in attempt to passage virus. Several of these cocultivations have reproducibly developed reserve transcriptase activity two to four weeks after cocultivation. DNA and RNA from these cocultivations and from fresh patient tissue has been screened for retroviral reverse transcriptase-like sequences using PCR.

Our PCR-directed analysis of DNA from MF patients yielded a DNA sequence which is part of a reverse-transcriptase containing gene not homologous to any known viruses or endogenous retroviruses. Southern blot analysis and sequencing have revealed that this represents a novel human endogenous retrovirus. We are further characterizing the genomic study and expression of this gene.

Clonality of Early Stage Skin Lesions

We have developed techniques to evaluate small populations of clonal T-cells using PCR. We have evaluated lymph nodes at various stages of involvement to ascertain the sensitivity of this technique in isolating a clonal population midst a polyclonal background. We are currently cloning and sequencing rearranged T-cell receptor genes from MF patient skin in order to determine whether early stage disease is a monoclonal or polyclonal disorder and to study TCR-B-variable region utilization in MF monoclonal malignant populations.

Genotyping and Karyotypic Analysis of MF tissues

We have extensively genotyped the peripheral blood and lymph node from at least 40 MF patients with respect to rearrangements of the TCR and IG loci. We found a correlation between detection of TRC rearrangements and clinical outcome in MF lymph node tissue. We are further analyzing these tissues with karyotyping and immunophenotyping. We have studied mutations in tumor suppressor genes which have been shown to be deleted or altered in other lymphoid malignancies as possible markers of malignancies or disease progression. We have analyses the tumor suppressor p53 gene exon 4 to 8 in lymphnode tissue using RT-PCR-SSCP analysis to detect point mutations and have found mutations only in advanced stages of the disease. These mutations will be further studied by sequencing. Earlier stage tissues from these patients will be analyzed in order to study the evaluation of these mutations.

Development of New Therapies for MF

Over the past year we have attempted to evolve new therapies in the lab which could be directly applied to patient care. We have used MTT testing to determine

sensitivity of MF cells and of the MF cell line HUT-78 and other T-lymphoid cell lines to a variety of chemotherapeutic and biologic agents. We have determined in vitro that DDI, a drug thought to act by inhibition of DNA polymerase and viral reverse transcriptase, is capable of killing MF cells and other T-lymphoid cells at modest doses. We are exploring the mechanism of this cytotoxicity and are looking for synergy between DDI and other agents, including fludarabine, deoxycoformycin, and interferon. Hopefully, these studies will form the basis for new clinical trials. Our most recent clinical study, utilizing fludarabine and low does interferon, was based on demonstrated synergy in vitro.

In addition to studies of cytotoxic therapies, we have attempted to delineate the role of growth factor therapy in these patients by studying the in vitro effects of growth factors on cell viability. We have identified a possible role for IL-12 generated therapy in a subset of early stage patients who demonstrate high numbers of activated lymphocytes in their peripheral blood. Studies are underway to determine the tumor specificity of these cells and their response to IL-2 in vitro. We have currently undertaken a study utilizing an 162-diphtheria toxin conjugate and are studying the disposition of this drug in patient skin and other tissues as well as its effect on populations of lymphocytes and activated cell in skin and peripheral blood. This work is underway in collaboration with Dr. E. Sausville.

Proposed Course

A large part of our effort will be to continue to explore the possible retroviral etiology of MF. We hope to propagate patient cells in culture using transfected immortalization genes and to study their DNA and RNA for retroviral-like sequences. We hope to perform electron microscopic analysis of patient cells shortly after placing them in culture to look for retroviral particles. Our cocultivated specimens with demonstrated reverse transcriptase activity will be further evaluated by electron microscopy and by genetic analysis for presence of retroviruses.

Characterization of early stage skin lesions will continue. We have already obtained specific TCR rearranged sequences from skin in one patient and LN in another and we will attempt to perform in-situ hybridization using these specific TCR-B probes to correlate the morphologic features of the infiltrate with the clonal genetic alteration. We will continue our studies of the role of suppressor oncogenies in evolution of this disease.

We also hope that, by sequencing TCR VDJ regions from many patients, we can answer the question of whether there is selective V-region utilization in the malignant cells of MF patients. Our data suggests selective VB-8 utilization in several patients we have studies. We are hoping to screen subsets of patients with different stages of disease selective VB utilization.

In vitro drug and growth factor sensitivity studies will continue. We will attempt to apply these results to the design of new clinical trials.

Publications

1. Foss, F., Veillette, A., Sartor, O., Rosen, N., Bolen, J. Alterations in the expression of pp60c-src and p56-lck associated with butyrate-induced differentiation of human colon carcinoma cells. Oncogene Research 5:13-23, 1989.

2. Micklee, L., Bates, S., Richert, N., Foss, F., Rosen, N., Fojo, T. Modulation of the expressiuon of the MDR-1 (p170) gene by differentiating agents. J. Biol. Chem. 234 (40):188031-18040, 1989.

3. Kaye, F., Bunn, P., Steinberg, S., Stocker, J., Ihde, D., Fischmann, B., Glatstein, E., Schechter, G., Phelps, R., Foss, F., Parlette, H., Anderson, M., Sausville, E. A randomized study comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. New England Journal of Medicine 321:1784-1790, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07259-02 NMOB

PERIOD COVERED

October 1, 1990 to September 30, 1991.

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Molecular pathology of pre-malignant lung

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Branch Chief	NCI-NMOB
	Sandra Jensen	Biologist	NCI-NMOB

COOPERATING UNITS *(if any)*

Surgery Branch,NCI (Harvey Pass,MD), Anatomic Pathology, NCI,NIH (Bill Travis, MD), and Anatomic Pathology, Naval Hospital, Biostatistics and Data Management Section, Clinical Oncology Program, DCT (Seth Steinberg, PhD).

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Our goal is to define the molecular events that occur in the bronchopulmonary epithelium in the premalignant state. This involves mapping the expression of growth factors and their receptors, oncogenes and tumor suppressor genes at the cellular level in the progenitor cells of lung cancer in the non-neoplastic lung. This helps to understand the order of events leading to malignant transformation, and provides tools for early detection of cancer and cancer susceptible individuals as well as basis for the early intervention.

Characterization of the system. Surgically resected pairs of malignant and corresponding non-neoplastic lung from the same patient composed of all NSCLC types was studied by RNA-RNA in situ hybridization for the expression of myc-family oncogenes and the peripheral airway cell (PAC) cell(progenitor cells) differentiation. c-myc oncogene was overexpressed in 8 out of 17 tumors, 2 of which also expressed L-myc, while 15 out of 17 lungs showed low levels of c-myc both in airway epithelium and alveoli. N-myc levels both in tumors and lung tissue remained undetectable. The expression of PAC differentiation genes SP-a (the major surfactant associated protein) and Clara cell protein was focal in 4 tumors and restricted to type II cells in alveoli and bronchiolar cells of the lung, respectively. By immunohistochemistry 5 tumors were positive for p53 staining signifying the possible presence of mutated form of this suppressor gene. These results suggest that 1) expression of myc in NSCLC is a common event and 2) low levels are present in most cells in the lung and 3) PAC differentiation is restricted to subpopulations of bronchopulmonary cells.

PROJECT DESCRIPTION

Molecular pathology of pre-malignant lung

Professional Staff:

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Branch Chief	NCI-NMOB
	Sandra Jensen	Biologist	NCI-NMOB

Collaborating Branches:

Surgery Branch, NCI (Harvey Pass,MD), Anatomic Pathology, NCI,NIH (Bill Travis, MD), and Anatomic Pathology, Naval Hospital, Biostatistics and Data Management Section, Clinical Oncology Program, DCT (Seth Steinberg, PhD).

Objectives:

The malignant transformation of the human bronchial epithelium is an end point of the deregulation of multiple events involving the growth control which are produced by exposure to carcinogens and possible inherited predisposition to lung cancer. Our goal is to define the molecular events that occur in the bronchopulmonary epithelium in the premalignant state.

Bronchial carcinogenesis is a complex process associated with genetic abnormalities in both dominant and recessive ("tumor suppressor") oncogenes.

Dominant, classic cellular oncogenes can cause cellular transformation in selected model systems through inappropriate activation which reflects a positive deregulation of their function following a change in only one of the maternal or paternal alleles.
The dominant oncogenes implicated in the pathogenesis of lung cancer include myc family oncogenes, ras, raf, and neu. Activation can happen through several mechanisms:1) Amplification of protooncogenes has been reported in up to 21% of NSCLC in which about 90% of the amplified genes were of the myc, ras, or erbB/neu family. 2) Point mutations are characteristic of ras family genes in NSCLC. Other mechanisms include 3) gene rearrangement producing chimeric or truncated genes, and 4) rearrangement of a gene in a region outside the transcribed sequence.

In contrast to the dominant oncogenes the antioncogenes or tumor suppressor genes posses a normalizing or negative regulatory role on growth and their inactivation through deletions or mutations produce the malignant phenotype. Two genetic lesions are required for their effect; one for inactivation of the maternal allele and the other for the paternal allele, and thus they have been also called recessive oncogenes.. Lung cancer cells demonstrate numerous specific chromosomal deletions suggesting that anti-oncogenes are important in the pathogenesis of lung cancer. In addition to structural and numerical cytogenetic changes, comparison of tumor and normal tissue DNAs by means of restriction fragment length polymorphism (RFLP) probes revealed loss of heterozygosity in chromosome

regions 3p,13q, and 17p. The known recessive oncogenes implicated in the pathogenesis of lung cancer include retinoblastoma (rb) gene an the p53 nuclear protein.

The incidence of lung adenocarcinoma is increasing in the U.S.A. The progenitor cells for adenocarcinoma include type II pneumocytes and Clara cells which are the metabolically active progenitor cells of peripheral airways. The pre-malignant changes of this are not understood. The characterization of genes specific for peripheral airway cell differentiation SP-A(the major surfactant associated protein) and Clara specific protein prompted us to investigate the expression of PAC differentiation genes and oncogenes in the progenitor cells for lung cancer in non-neoplastic lung.

Methods Employed:

1.Tissues. A frozen tissue bank has been established composed of resection specimens of lung carcinomas and corresponding non-neoplastic lung that reveals pre-neoplastic changes. The study will initially concentrate on NSCLC of all types, with specific interest in adenocarcinomas since tissue samples for SCLC are more difficult to collect(patients are not undergoing resections of their SCLC tumors). A great emphasis was placed on appropriate collection of specimens to preserve tissue RNA and morphology. The collection and cataloging is an ongoing project to obtain a series of progressive premalignant changes occurring randomly in resected non-neoplastic lungs. In addition to frozen tissue blocks, RNA and DNA will be prepared from all specimens.

2.Methods. RNA-RNA in situ tissue hybridization with S 35 labelled probes of c-myc(2nd exon 420bp),L-myc(3rd exon, 580 bp) and N-myc (3rd exon 660bp) were used. Immunohistochemical staining using p53 antibodies and Ki67 proliferation cell antibody was performed using the avidin biotin peroxidase technique.

Major Findings:

c-myc oncogene was overexpressed in 8 out of 17 tumors, 2 of which also expressed L-myc, while 15 out of 17 lungs showed low levels of c-myc both in airway epithelium and alveoli. N-myc levels both in tumors and lung tissue remained undetectable. In situ hybridization results were confirmed by Northern blot analysis. In contrast to c-myc, Ki67 was intensely positive only in basal cells of bronchial epithelium. In c-myc positive tumors a subset of tumor cells were positive for the proliferation antigen Ki67. The expression of PAC differentiation genes SP-A and Clara cell protein was focal in 4 tumors and restricted to type II cells in alveoli nad bronchiolar cells of the lung respectively. By Immunohistochemistry 5 tumors were positive for p53 staining signifying the possible presence of mutated form of this tumor suppressor gene. Furthermore, positive staining was also detected in the basal cells of bronchial epithelium. We conclude that 1) overexpression of the myc-protooncogenes is common in NSCLC; 2) c-myc expression is not restricted to a single cell type in non-neoplastic lung; 3) c-myc and Ki67 expression may characterize different aspects of proliferation and 4) PAC differentiation is restricted to subpopulations of bronchopulmonary cells.

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the identification of the expression of multiple oncogenes, tumor suppressor genes, growth factors and receptors simultaneously at the cellular level in the same surgically removed specimens. This enables to establish the order of genetic events and their correlation to premalignant changes and tumor histology. Similar techniques can be used to identify oncogene expression in clinical cytology specimens and as possible adjunct in the early detection of lung cancer. The results will help to define the early versus late genetic events in the development of lung cancer.

Proposed Course:

1. The expression of growth factors and receptors, oncogenes and tumor suppressor genes will be correlated with individual cell types of lung in malignant and non-neoplastic lung obtained through routine surgical removal of NSCLCs and the surrounding lung tissue.

2. Specimens from patients at risk for getting a lung cancer will be collected and analyzed for the expression of growth factors and receptors, oncogenes and tumor suppressor genes and results will be correlated with the clinical outcome.

3. Cell culture and animal models will be used to define the relative growth /transformation potential of the bronchopulmonary cells which express selected oncogene/differentiation profiles.

Publications:

Gazdar, A.F., Linnoila, R.I., Kurita, Y., Oie, H.K., Mulshine, J.L., and Whitsett, J.A.: Peripheral airway cell differentiation in human lung cancer cell lines. Cancer Res. 50:5481-5487, 1990.

Brennan, J., O'Conner, T., Makuch, R.W., Simmons, A.M., Russel, E., Linnoila, R.I., Phelps, R.M., Gazdar, A.F., Ihde, D.C., and Johnson, B.E.: myc family DNA amplification in 107 tumors and tumor cell lines from patients with small cell lung cancer treated with different combination chemotherapy regimens. Cancer REs. 51:1708-1712, 1991.

Mitsudomi, T., Viallet, J., Linnoila, R.I., Minna, J.L., and Gazdar, A.F.: Mutations of ras gene distinguish a subset of non-small cell lung cancer cell lines from small cell lung cancer lines. Oncogene. In press.

Linnoila, R.I., Jensen, S.M., Steinberg, S.M., Mulshine, J.L., Eggleston, J.C., and Gazdar, A.F.: Peripheral airway cell diferentiation in non-small cell lung carcinoma: Association with distinct clinicopathologic features. Am. J. Clin. Pathol. In press.

Linnoila, R.I., Mulshine, J.L., Steinberg, S.M., and Gazdar, A.F.: Expression of surfactant-associated protein in non-small cell lung cancer: A discriminant between biological subsets. JNCI Monographs. In Press.

Linnoila, R.I.: Pathology of non-small cell lung cancer: New diagnostic approaches. Hematology Oncology Clinics of North American. Vol. 4, No 6, pp 1027-1051, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06813-09 PB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Carol J. Thiele	Senior Investigator	PB, NCI
Other:	Kazue Matsumoto	Chemist	PB, NCI
	Carlo Gaetano	Fogarty Fellow	PB, NCI
	Leonard Wexler	Clinical Associate	PB, NCI
	Sandra Doren	Biologist	PB, NCI
	Fabrizio Ensoli	Visiting Fellow	PB, NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Pediatric Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, Maryland 20891

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4	4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Summary:

We use human pediatric tumors as a model system to study the molecular events associated with the development of malignant tumors during childhood. Since neuroblastoma (NB) can be induced to differentiate in vitro, it is a particularly useful system in which to study the molecular mechanisms regulating growth and differentiation. Our current focus is to identify chemicals and biologic response modifiers that control cell growth and/or induce differentiation and use recombinant DNA technology to identify the molecular mechanisms and clone the genes important for the regulation of these processes in pediatric peripheral neuroectodermal tumors. During the past year a clinical protocol utilizing trans-retinoic acid to treat children with neuroblastoma evolved in part from in vitro studies demonstrating its efficacy. Furthermore, the inclusion of interferon-gamma pre-treatment of children with neuroblastoma in a TIL(tumor-infiltrating lymphocytes) therapy clinical protocol developed from in vitro studies analyzing the expression of Class I Major Histocompatibility Complex antigens on neuroblastoma tumors. Ultimately our goal is to develop new strategies and novel therapeutics based on understanding the specific alterations in these pediatric malignancies.

Accomplishments and Results:

1. Evaluation of neuroblastoma cell differentiation:

Recent evidence indicates that cell cycle genes respond to the actions of oncogenes and suppressor genes. We have recognized the importance of studying cell cycle genes and feel that an understanding of the mechanisms controling cell proliferation is critical to delineating mechanisms that regulated cell growth in cancer. Previously, we have characterized NB cell lines that are growth arrested by retinoic acid (RA) and using molecular genetic analysis we have determined a pattern of proto-oncogene, growth associated gene and differentiation gene expression. Using this model we have compared the phenotypic changes associated with other growth controlling or differentiation inducing agents in neuroblastoma. While RA induces a neuronal phenotype and decreases expression of genes associated with a neuroendocrine phenotype, cAMP induces rapid increases in the expression of genes associated with a neuroendocrine phenotype. When the combination of RA and cAMP are used a neuronal phenotype predominates and the expression of an adrenal-specific anonymous gene is extinguished. This provides a model to investigate lineage-specific issues in the development of chromaffin and neuronal cells. Studies carried out this year indicate that vasointestinal peptides (VIP) whose signal transception pathways is presumed to be mediated by increases in intracellular cAMP do not inhibit the growth when induced differentiation in our model neuroblastoma cell line. In fact, VIP simulates IGF-II expression and concommittment with this increase in IGF-II expression is an increase in mRNA4 cell cycle related gene $p34^{cdc2}$ and cyclin. In collaboration with Drs. Mario Maggi and Mario Serio, endocrinologists at the University of Florence, we have analyzed NB cell lines for expression of somatostatin receptors as well as biologic response to somatostatin and analogs. We have identified a transient decrease in cell proliferation. Current studies are aimed at elucidating the somatostatin signal transduction pathway in NB cells. Interferon gamma treatment of neuroblastoma cells indicates that it is capable of arresting growth and inducing differentiation. We have expanded our evaluation of these biological response modifiers to include other pediatric peripheral neuroectodermal tumors such as Ewings Sarcoma and primative peripheral nueroepithelioma. Our results indicate that agents such as dibutyrl cAMP inhibit but do not arrest the growth of Ewings or neuroepithelioma cell lines. This is seen in approximately six of eight cell lines tested. We have found that interferon gamma is able to arrest the growth of a neuroepithelioma cell line, TC32, and inhibit the growth of six out of eight others. In addition to these studies, we have investigated the anti-proliferative effects of ICRF-187, a heavy metal chelator currently undergoing clincial trial as

an adriamycin cardioprotectant. Preliminary studies reveal that continuous exposure to clinically achievable levels of this drug for 48 hours results in >75% cytotoxicity in 4/6 NB, and 3/3 ES/NE cell lines. Additional studies are planned to explore whether enhanced in vivo cytotoxicity might be achieved by more optimal dosing and/or scheduling of ICRF-187 in combination with cytotoxic chemotherapy. This is particularly promising since current protocols to treat neuroepithelioma and Ewings sarcoma have only a 12% survival after 40 months. We are continuing to explore additional biological response modifiers such as cytosine arabinoside, TGF-β and these agents in combination with retinoic acid.

2. Evaluation of neuroblastoma cell growth:
An understanding of the mechanisms controlling cell proliferation is critical to delineating mechanisms of dysregulation in cancer. Furthermore control of cell growth is, usually, a prerequisite for terminal differentiation. We have initiated studies to examine the regulation of the human homologs of the recently described yeast cell-cycle genes (cdc2 and cyclin A and B) in human tumor cells. We have found that the expression of $p34^{cdc2}$ is not down-regulated when tumor cells are growth arrested by nutrient deprivation in contrast to normal cell lines. However, when retinoic acid is used to control cell growth and induce differentiation in NB cells a 25-fold decrease in $p34^{cdc2}$ levels is detected, suggesting that such treatment restores normal growth regulation to these tumor cells. Similar results have been obtained in the promyelocytic tumor cell line HL60. We have observed the decreased expression of $p34^{cdc2}$ only in cell lines that are growth arrested and differentiated suggesting that regulation of cdc2 may be an important link between the ability of a cell to continue to proliferate and its ability to differentiate. $p34^{cdc2}$ expression is regulated post-transcriptionally since protein levels are undetected despite cdc2 mRNA levels being comparable to untreated cells. Furthermore our studies have linked decreased expression of $p34^{cdc}$ with activation.

3. Evaluation of resistance to retinoic acid:
Since retinoic acid (RA) has been approved for clinical trials we have instituted a study to evaluate the inability of some NB cell lines to be growth arrested and differentiated by RA as well as the in vitro development of resistance to RA. We have found that many cell lines that are resistant to RA express IGF-II mRNA. IGF-II has been shown to be constitutively expressed in some NB cell lines and function as an autocrine growth factor. We have developed an in vitro model in which a NB cell line that initially is growth arrested by RA rapidly develops resistance and RA-resistant cell lines have been isolated. In this model, RA causes a transcriptional increase in IGF-II mRNA which is reversible

upon removal of RA. We have developed 4 RA resistant cell lines and characterizing them in order to understand the nature of the development of resistance to RA.

4. Evaluation of suppressor genes in pediatric peripheral neuroectodermal tumors (PNET):
Numerous genetic alterations have been described in PNET including chromosome 1p deletions, loss of heterozygosity on chromosomes 11, 14, and amplification of MYCN in NB and t(11:22) and amplification of MYC in Ewing's sarcoma (ES) and peripheral neuroepithelioma (NE). Although ES and NE are distinct clinical entities, cytogenetic, molecular genetic and biochemical analyses suggest that these tumors may have a common origin. We have initiated a study to determine if PNETs have complementing genetic alterations by making somatic cell hybrids between these NB, NE and ES tumor cell lines and evaluating tumorigenicity. As a prerequisite for such an analysis drug resistant cell lines are required. We have established the following drug resistant cell lines which will serve as hybridization partners in subsequent analysis: $G418^r$ KCNR (NB-MYCNamplified), AS (NB), SY5Y (NB), TC32 (NE) and $Hygromycin^r$ TC106 (ES) and TC32 (NE). Studies are planned to evaluate NB x NE, NE x ES and NB x ES hybrids for suppression of tumorigenicity.

Despite the numerous cytogenetic alterations in NB, RA is capable of restoring growth control in many cell lines. We have initiated studies using subtractive cDNA cloning to isolate genes expressed when RA induces growth control in NB cells and to analyze if these genes are capable of suppressing growth and tumorigenicity when transfected into other NB cell lines. Currently we are evaluating expression vector cloning strategies and optimizing transfection techniques.

5. Class I Major Histocompatibility Antigen Expression:
NB tumors and cell lines express low levels of Class I MHC antigens and our studies indicate that this may be related to their developmental stage since Class I MHC is regulated during adrenal medullary development. Although some studies indicate amplification of MYCN may specifically down regulate Class I expression, our studies indicated that transfection of MYCN into PNET expressing high levels of Class I failed to alter its expression. We are studying the ability of IFNγ to alter Class I expression in low Class I expressing NB as well as alter tumor cell growth and induce differentiation.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CM 06830-21 PB

PERIOD COVERED
October 1, 1990 - September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Infectious Complications of Malignancy and HIV Infection in Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip A. Pizzo Chief, Pediatric Branch	Head, Infectious Disease Section,	PB, NCI
Others:	Thomas J. Walsh	Medical Officer	PB, NCI
	Karina Butler	Senior Staff Fellow	PB, NCI
	Emile (Pim) Brouwers	Visiting Scientist	PB, NCI
	Robert Husson	Medical Officer	PB, NCI

Continued on next page

COOPERATING UNITS *(if any)*
Medicine Branch, Surgery Branch, NCI; Diagnostic Microbiology, Department of Transfusion Medicine, CC; Medical Illness Counseling Center

LAB/BRANCH
Pediatric Branch

SECTION
Infectious Disease

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	11.0	11.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies are devoted to developing methods to define cancer patients who are at risk for developing serious infection, to improving the ability to diagnose these infections early, to treat them effectively, and ultimately to prevent them. We are developing new therapeutic approaches based on the ability of new antibiotics particularly the beta-lactams and the quinolones. We have shown that certain beta-lactams used as single agents can replace the need for combination antibiotic therapy. Our studies are also defining the appropriate antibiotic therapy for documented infections, particularly the role of oral antibiotic therapy; the necessary duration of empiric therapy for patients with unexplained fevers and the choice of empiric antifungal therapy.

We have developed a unique model for studying the pathophysiology, natural history, treatment and prevention of invasive candidiasis in the neutropenic host. This model permits the testing of new antifungal agents as well as immunoregulatory agents. To prevent infections we are evaluating the role of passive immunization with a pooled immunoglobulin preparation that contains activity against the enterobacteriaceae as well as the pseudomonads. We are also studying other immunoregulatory agents that may serve as adjuncts to the treatment of infection, including interleukin 1 and 2, GM-CSF and M-CSF.

We have developed a program to evaluate the benefits of antiretroviral agents in children with HIV infection. To date, these have focused on studies with dideoxynucleosides. Studies with immunoregulatory agents and with biologicals (e.g., rCD4) are also underway.

Professional Personnel (Continued):

Alison Freifeld	Medical Officer	PB, NCI
Linda Lewis	Medical Officer	PB, NCI
Emmanuel Roilides	Visiting Scientist	PB, NCI
Brigitta Mueller	Visiting Associate	PB, NCI
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Miriam Weinberger	Visiting Fellow	PB, NCI
Tore Abrahamsen	Special Volunteer	
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Susan Mertins	Biologist	PB, NCI
Robert Schaufele	Biologist	
Janet Gress	Nurse Specialist (Research)	PB, NCI
Freda Jacobsen	Nurse Specialist (Research)	PB, NCI
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Gayle Lovato	Nurse Specialist (Research)	PB, NCI
Doris Marshall	Nurse Specialist (Research)	PB, NCI
Karen Montrella	Nurse Practitioner	PB, NCI
Susan Sandelli	Nurse Specialist (Research)	PB, NCI
Patricia Whitcomb	Nurse Specialist (Research)	PB, NCI

Accomplishments and Results:

A. Diagnosis, Management and Prevention of Infectious Complications in Cancer Patients

1. To determine the role of new beta-lactam antibiotics in providing simpler, safer and effective therapy for neutropenic cancer patients who become febrile, we have conducted a randomized trial comparing a third-generation cephalosporin (ceftazidime) to a carbapenem (imipenem-cilastatin) for initial empirical therapy. The goal of this study is to both evaluate the role of these agents in providing safe initial therapy as well as determining whether the numbers of modifications of the primary antibiotic varies in patients with defined infection or prolonged granulocytopenia. From March, 1986 through June, 1991, we enrolled 600 evaluation episodes of fever and neutropenia, randomizing these to initial ceftazidime (251 episodes) or imipenem (249 episodes). Both regimens provided comparable primary therapy. More modifications of the initial regimen were necessary for patients with documented infection who were randomized to ceftazidime, and there were more second infections in this group. These were primarily with gram-positive bacteria. However, there were no differences in infection related morbidity or mortality. On the other hand, there were more complications with imipenem, including higher incidence of C.*difficile* diarrhea and a higher degree of intolerance due to nausea and vomiting. Overall, both antibiotics appear useful, have different strengths and weaknesses and confirm that various alternatives can be employed to provide safe monotherapy for the majority of febrile neutropenic cancer patients.

2. In a randomized trial, we have demonstrated that it is appropriate to continue empiric antibiotic therapy for a limited (i.e., 2 week) course for patients who have defervesced following the initiation of antibiotics but who remain febrile.

In a follow-up study, we are comparing the use of a new class of oral antibiotics, the quinolines ,for patients who have defervesced on parenteral therapy, have no defined site of infection, and remain persistently granulocytopenic. This study has considerable importance, since it can serve to re-define the role of inpatient versus outpatient therapy 65 patients have been randomized.

3. To decrease the frequency of infectious complications associated with indwelling intravenous catheters of the Hickman-Broviac type, we have performed a randomized trial to compare the Hickman-type catheter to a subcutaneously implanted catheter (Port-a-Cath). Since the subcutaneous catheter requires less manipulation, our hypothesis is that it will have a low incidence of infection. However, if infected, it is possible that these infections will not be capable of eradication unless the catheter is removed. One hundred patients were randomized, 48 to a Hickman catheter and 52 to a Port-a-Cath. Overall, 10,592 days of catheter placement were evaluated in the Hickman group and 14,634 catheter days for the Port-a-Cath group. Analysis to date shows no difference in the frequency of infectious or non-infectious complications.

4. In preparation for *in vivo* administration of elutriated monocytes to patients with progressive infection, a variety of studies are underway. We have demonstrated that these cells have good phagocytic, chemotactic and microbicidal activity. We are studying their production of cytokines such as IL1, TNF and GM-CSF, in order to obviate potential toxicities and maximize efficacy. Optimal culture and storage conditions are being determined, and potential for *in vitro* activation of these cells explored.

   A novel method for elutriation and transfusion of rabbit peripheral blood monocytes is being developed to further investigate the therapeutic potential of this modality for experimental fungal infections.

   Preliminary data has been obtained from two patients with aplastic anemia and a rapidly progressive aspergillus infection. One patient was given nine transfusions with elutriated monocytes. Preliminary results indicate little toxicity, trafficking of the cells to the site of infection, and stabilization of the infectious process. A protocol to evaluate this modality further has been submitted to the Institutional Review Board.

5. In an attempt to reduce the duration of neutropenia associated with cytotoxic chemotherapy, we have initiated a prospective randomized trial in children with sarcomas whereby, following chemotherapy, they are randomized to receive or not receive rGM-CSF. The goal of this study is to determine whether the cytokine will reduce the incidence and severity of the fever of infection usually associated with neutropenia. Should this prove effective, it may permit altering chemotherapy schedules in a manner that might better optimize their antitumor efficacy.

6. We have evaluated the course of infectious complications during the last decade in over 150 patients with aplastic anemia and have determined that fungal infections, especially Aspergillus, are the major cause of mortality.

B. Invasive Mycoses: Preclinical and Clinical Studies

Invasive fungal infections are significant and increasing problems of morbidity and mortality in cancer patients and those with AIDS. Accordingly, we investigated the antifungal activity, pharmacokinetics, and immunomodulatory properties of several of these most promising agents for potential use in our high risk patient populations.

1. We demonstrated that three potent antifungal triazole compounds (itraconazole, fluconazole, and SCH-39304) were most effective when administered as preventive or early antifungal chemotherapy and have the clinical potential for use in early empirical antifungal therapy.

2. We demonstrated that the new antifungal triazoles (itraconazole, fluconazole, and SCH-39304) were as effective as amphotericin B plus flucytosine in early treatment of experimental disseminated candidiasis but that amphotericin B plus flucytosine was more effective against chronic (hepatosplenic) candidiasis.

3. These experimental antifungal studies provided the scientific rationale for design of a multicenter clinical trial to test the concept of early empirical antifungal therapy with fluconazole and for the first phase I trial of a systemic antifungal agent (fluconazole) in children. This phase-I study has been completed and the randomized double-blind multicenter trial investigating fluconazole vs placebo has been initiated.

4. We have completed the first phase I-II pharmacokinetic study of fluconazole in children with cancer. This study found that fluconazole was safe and well-tolerated. Moreover, fluconazole had a shorter mean plasma half-life than that of adults.

5. We have demonstrated the potent efficacy of cilofungin (LY-121019) when administered by continuous infusion against disseminated candidiasis in persistently granulocytopenic rabbits, representing for the first time an experimental rationale for continuous infusion of a systemic antifungal compound.

6. We have demonstrated that cilofungin, the model compound of the class 1,3-β-glucan synthase inhibitors, known as echinocandins, is highly fungicidal *in vitro*. We also demonstrated that cilofungin has non-linear saturable plasma pharmacokinetics. We have further shown that, when the non-linear saturable plasma pharmacokinetics of cilofungin are implemented, the antifungal effect is strikingly augmented. These findings have important implications for the delivery and pharmacodynamics of several classes of potent cell wall active antifungal agents. As echinocandins are also lethal to *Pneumocystis carinii*, these properties may also impact upon treatment of this organism.

7. We demonstrated that cilofungin (LY-121019) is excreted via the biliary tract, has a short plasma half-life following first order kinetics with single dose administration but with continuous or frequent intermittent infusion, we demonstrated the non-linear saturable pharmacokinetics of cilofungin (LY-121019), thus accounting for the heretofore unexplained basis of accumulation of this promising compound in human volunteers.

8. We found that the novel combination of amphotericin B (AMB) plus fluconazole was more active than the combination of AMB plus flucytosine or the agents used alone in the rabbit model of chronic disseminated (hepatosplenic) candidiasis. Such a novel combination offers hope for more effective antifungal therapy for this often refractory infection.

9. We demonstrated that a new antifungal triazole (BAYR-3783) is converted into active metabolites, one of which has an exceedingly long plasma half-life with penetration into the central nervous system.

10. We developed a novel method of continuous intravenous infusion and simultaneous monitoring of plasma levels of investigational compounds in ambulatory non-tethered rabbits. Continuous infusion was administered by means of a portable programmable micropump, which permitted adjustable dosing. Simultaneous plasma pharmacokinetic monitoring during infusion was accomplished by dual central silastic venous catheters. This method provided a safe, reliable, and well-tolerated method of studying the experimental pharmacokinetics of antimicrobial compounds, immunomodulators, and other compounds with short plasma half-lives in rabbits.

11. Corticosteroids cause impaired cell-mediated immunity which may encourage development of gastrointestinal and respiratory infections, especially those due to invasive fungi. In order to better understand the effects of corticosteroids on gastrointestinal immunity, we examined the immunological and histological changes in gut-associated lymphoid tissues after intravenous administration of dexamethasone to rabbits. In treated animals, lymphoid domes and follicles were considerably reduced in size, and the dome epithelial layer was markedly depleted of M cells and lymphocytes. There were numerous open lesions at the luminal surface of dome epithelium, consistent with necrosis of M cells, and a striking depletion of follicular B cells in treated animals. These immunologic and histologic effects of corticosteroids could have found profound, deleterious effects on mucosal immune responses and host resistance to invasive fungal, bacterial, and protozoal infections.

12. We demonstrated that the depth, duration and recovery from granulocytopenia are important determinants in the clearance of experimental disseminated candidiasis; we further showed that recovery from granulocytopenia is not a sufficient condition for clearance of tissue candidiasis if profound granulocytopenia was present during the time of infection. These studies served as the foundations for developing a rational approach to the use of G-CSF for the prevention and treatment of disseminated candidiasis in granulocytopenic hosts.

13. Recombinant human G-CSF was found to be most effective in the prevention rather than the treatment of disseminated candidiasis in granulocytopenic rabbits. G-CSF was able to shorten duration but not depth of neutropenia. When the *ex vivo* effects of the administration of G-CSF on PMN function were assessed in rabbits in this study, G-CSF was found to enhance superoxide production in response to FMLP and opsonized *C. albicans* blastoconidia as well as the phagocytic and microbicidal activity of PMN against *S. aureus* but not against *C. albicans* blastoconidia.

14. *Trichosporon beigelii* is an emerging fungal pathogen in patients with cancer. In order to further understand the pathogenesis, immunodiagnosis, and treatment of disseminated *Trichosporon beigelii* infection, we developed models of disseminated and gastrointestinal infection in persistently granulocytopenic rabbits. Antigenemia cross-reactive with cryptococcal polysaccharide (described in cases of disseminated trichosporon infection) were reproduced. We further demonstrated the immunohistological origin of cryptococcal antigenemia in invasive trichosporonosis as arising from cell wall and matrix of *Trichosporon beigelii*. Infection developed in rabbits with *T. beigelii* gastrointestinal colonization following cytotoxic chemotherapy.

15. We demonstrated that antifungal triazoles (fluconazole and SCH39304) were significantly more effective in clearing tissues and improving survival than miconazole amphotericin B or liposomal amphotericin B in experimental disseminated. Antigenemia declined during the course of antifungal therapy. These studies have afforded new understanding of this and other emerging resistant pathogens.

16. We further identified and characterized the biochemical and physiological factors that may regulate germination, an important virulence factor of *T. beigelii*. We further identified key morphological, microscopic, isoenzyme, metabolic, and biochemical markers, as well as a PCR-amplified 5.2kB fragment of ribosomal DNA from clinical isolates of Trichosporon that distinguished invasive versus non-invasive strains.

17. Following extensive pre-clinical investigation, we completed a multi-center trial demonstrating the expression of antigenemia due to Candida cytoplasmic enolase (a 48 kD Ag) as a new marker of invasive candidiasis in cancer patients.

18. We demonstrated that anti-Candida enolase antibody (Ab) [titer>1:100] but not enolase antigen (Ag) was present in serum of non-neutropenic surgical patients with invasive candidiasis. Patients with invasive candidiasis who were recovering from neutropenia also had rise of anti-enolase Ab and decline of Ag. Anti-Candida enolase Ab also was associated with negative serum antigen detection tests and was indicative of favorable outcome in invasive candidiasis. These data indicate that both serum Ag and Ab should be measured in order to optimally utilize *Candida* enolase as an immunodominant marker of invasive candidiasis.

19. Hepatosplenic candidiasis (HSC) is an increasingly recognized infectious complication of patients with neoplastic diseases. Whether patients with HSC should continue to receive antineoplastic therapy at the risk of progressive HSC or breakthrough fungemia is an important therapeutic dilemma. We found that when patients with HSC at the NCI were treated with ongoing cytotoxic chemotherapy with little or no modification of their antineoplastic regimen, that there was no breakthrough fungemia or significant progression of HSC.

20. Little is known about the daily dosage, total dose, duration, and dose intensity of amphotericin B, the mainstay of systemic antifungal therapy. We are therefore investigating these pharmacodynamic properties both amphotericin B and amphotericin B lipid complex (ABLC) in our model of chronic disseminated (hepatosplenic) candidiasis. Our initial findings indicate that duration and daily dosage, rather than total dose, are more important determinants of antifungal response.

21. We developed a novel model of primary pulmonary aspergillosis in persistently granulocytopenic rabbits. This model histologically, pathophysiologically, and immunologically closely resembles the human infection of primary pulmonary aspergillosis and permits the study of antifungal chemotherapeutic agents, recombinant cytokines, and markers of invasive disease.

22. We characterized the plasma pharmacokinetics and demonstrated the efficacy of a unilamellar formulation of liposomal amphotericin B (LAMB) in our model of primary pulmonary aspergillosis. This system demonstrated that LAMB administered at 5 and 10 mg/kg/d was significantly more effective than conventional desoxycholate amphotericin B (AMB) in improving survival and in preventing pulmonary infarction and hemorrhage due to Aspergillus. The LAMB compound was also less nephrotoxic than AMB.

23. These *in vivo* findings were rapidly translated to patient care when a persistently granulocytopenic patient at the NCI with progressive pulmonary and paranasal sinus aspergillosis responded to high dose (5 mg/kg/d) LAMB. The positive outcome of this patient, who received the first compassionate release of this compound in the United States, has encouraged a nationwide program for compassionate release of this agent in selected mycoses.

24. We found that the Aspergillus metabolite, d-mannitol, as measured by mass spectroscopy and gas-liquid chromatography is present in serum and bronchoalveolar lavage specimens obtained from persistently granulocytopenic rabbits with primary pulmonary aspergillosis.

25. We have developed a new method for measuring phagocytosis of fungi. Whereas conventional methods do not reliably distinguish between intracellular and extracellular but attached fungi, our fluorescent quenching method distinguishes between ingested and attached organisms.

26. We found that empirical amphotericin B was not effective in preventing the development of invasive pulmonary aspergillosis (IPA), that the onset of IPA was earlier than previously reported, that corticosteroids contributed to increased risk of IPA, and that concomitant infections obscured an early diagnosis.

27. During a study of catheter-associated fungemia, we found that the onset of fungemia occurred earlier than previously reported and was associated with high mortality if amphotericin B was not initiated within 48 hours of onset of fungemia.

28. We assessed the effect of G-CSF and IFN-γ on the oxidative metabolic burst (superoxide production) of normal PMNs in response to opsonized or nonopsonized hyphae of *C. albicans* and we compared it with that in response to FMLP and to blastoconidia of the same organism. Both G-CSF and IFN-γ enhanced the responses to blastoconidia as well as to hyphae of *C. albicans*, although G-CSF showed some effect at higher only concentrations. Studies of the effects of these cytokines on the PMN- or elutriated monocyte-induced killing of *C. albicans* hyphae are underway.

29. In experiments using PMNs from healthy adult donors and hyphae of *Aspergillus fumigatus*, we found that both G-CSF and IFN-γ enhance the superoxide production in response to hyphae and the degree of damage caused by the PMNs to the hyphae. In other experiments, we found that both hydrocortisone and dexamethasone in higher concentrations inhibit the antihyphal capacity of normal PMNs but G-CSF and IFN-γ appear to correct this steroid-induced defect. The combination of the two cytokines together shows greater effect than each of them separately. Similar studies with elutriated monocytes as effector cells are underway.

30. Studies investigating the effects of IFN-γ in the prevention and treatment of invasive candidiasis and primary pulmonary aspergillosis are currently being conducted to further establish an understanding of these agents as a guide to potential clinical trials.

C. Pediatric AIDS

1. We have continued our Phase I-II studies of children with symptomatic HIV infection. Since beginning this project in December, 1986, we have evaluated over 200 children, enrolling the majority into clinical trials.

2. Our initial study of AZT, administered either by continuous intravenous infusion or on an intermittent schedule, are completed. Both routes of therapy appeared to offer benefit, particularly for children with neurodevelopmental deficits. However, the extent of this benefit, appear to be greater for children treated by the continuous intravenous schedule. To validate this, we have begun a randomized study comparing AZT administered on a schedule that maintains steady-state kinetics in the plasma and CSF to one in which the drug is delivered on an intermittent schedule to children with evidence of encephalopathy or to children who have developed dementia while receiving antiretroviral therapy. This protocol focuses on the impact of these therapies on neurodevelopmental function and should provide insights that will be of benefit to both children and adults. To date, 18 patients have been enrolled.

3. We initiated a Phase I/II protocol to assess the efficacy of subcutaneously administered G-CSF in increasing and maintaining the neutrophil count in HIV infected children who have developed neutropenia as a consequence of AZT. We are also studying the effect of human erythropoietin on overcoming AZT-induced anemia. To date 12 patients have been enrolled with promising early results.

4. In a search for effective, less toxic regimen, we initiated a Phase I-II trial of dideoxyinosine (ddI) in children in January, 1989. To date, 95 children have been enrolled at several dosage levels (20, 40, 60, 90, 120 mg/m^2/every 8 hours. This protocol enrolled both children who have received no prior anti-retroviral therapy as well as children who have become refractory or intolerant to AZT. We have completed the 6 month follow-up on the first 43 class P2 symptomatic HIV-infected children, (27 previously untreated children and 16 prior AZT recipients) and have evaluated doses of 60, 120, 180, 360, and 540 mg/m2/day. ddI was rapidly absorbed after oral administration, however, there was significant variability in its bioavailability. Pancreatitis occurred in two patients, one at each of the two highest dose levels. Median CD4 cell count increased from 218/mm^3 at baseline to 327/mm3 at 24 weeks (P=-0.001). Patients with baseline CD4 cell counts greater than 100/mm3 were significantly more likely to show an increase in this parameter. Median p24 antigen declined from baseline to 24 weeks (p=0.005), and there was a significant correlation between ddI plasma concentration and decline in p24 antigen level. A significant correlation was also found between ddI plasma concentration and improvement in cognitive function. Improvements in clinical and immunological parameters occurred in previously untreated patients and in prior AZT recipients. Dideoxyinosine was well tolerated and shows promising antiretroviral activity in HIV-infected children. The correlation between response and plasma ddI concentration indicates that bioavailability is an essential consideration for optimizing ddI activity in the treatment of HIV infection. We have also completed the long team follow-up of the entire group, the data which was used to support the NDA for ddI.

5. We completed a phase I study of recombinant soluble CD4 (rCD4) administered by continuous infusion to children with HIV infection. The initial treatment period of rCD4 alone was followed by the addition of oral ddI at a dose of 270 mg/m^2/day. rCD4 at doses as high as 1000 µg/kg/day was well-tolerated alone and in combination with ddI, however no marked changes in p24 antigen or CD4 counts were observed in patients receiving CD4. The CD4 infusion part of this protocol was ended in May, 1991 and the patients remaining on this protocol continue to receive ddI.

6. We initiated a Phase I-II dose escalation trial of combination antiretroviral therapy with AZT and ddI in September, 1990. This study is being conducted in collaboration with the Children's Hospital of Los Angeles and Los Angeles County/USC Medical Center. To date 29 children have been enrolled at doses ranging from 60 to 180 mg/m2 every 6 hours of AZT, and 60 to 135 mg/m2 every 12 hours of ddI. This protocol enrolls children who have not received prior antiretroviral therapy (Arm A), or those who have experienced hematologic intolerance on AZT (Arm B). An interim analysis of this study indicates that this combination is well-tolerated over a wide range of doses, without evidence of new short-term toxicities or of enhancement of known toxicities. Significant increases in CD4 counts, decreases in serum p24 antigen, decreases in viral load in plasma and PBMC's and increases in cognitive function have been observed in 14 patients who reached the initial 12 week major evaluation point. Particularly striking improvements in CD4 cell count were observed in patients at the dose level incorporating the highest dose of ddI. These data , suggest that this combination is active in vivo, however the longer-term tolerance and the optimal doses remain to be determined in this study.

7. All cases of mycobacterium avium-intracellulare infection in our HIV patient population were reviewed and clinical and laboratory characteristics of infected children were determined. 19 cases of disseminated MAI and 1 case of localized adenitis were identified in 196 patients attending the POB Clinic. All patients were receiving antiretroviral therapy. Recurrent fever, weight loss, and neutropenia were the most commonly found symptoms. MAI-infected children had mead CDF % of 2% and all had absolute CD4 counts less than 50 cells/mm^3. MAI affected 10.1% of our HIV patients but the incidence increased to 22% in those with CD4 counts <100.

8. We initiated a Phase I-II dose escalation trial of oral clarithromycin for pediatric patients with disseminated Mycobacterium avium complex infection. This study is being conducted in collaboration with the Children's Hospital of Los Angeles. To date 11 patients have been enrolled at doses of 7.5 and 15 mg/kg/day. A borderline decrease in hearing has been the only significant possible toxicity observed to date.

Improvements in energy levels, appetite and decreased fever have been observed, however recurrence of symptoms has occurred after several weeks in most patients at the first dose level. Tolerance and toxicities of this agent, as well as the durability of clinical response, remain to be determined at the higher doses incorporated into this protocol.

9. We have been evaluating the development of viral resistance to antiretroviral therapy in virus isolates from patients on Pediatric Branch treatment protocols who have received long-term therapy with AZT/ddC or with ddI. While the development of AZT resistance appears to be a common occurrence in this setting, we have not observed high level resistance to either ddC or ddI in HIV isolates obtained from these patients. Verification of a trend toward a small increase in IC_{50} in post-therapy isolates remains to be determined.

10. We have also been evaluating the activity of antiretroviral therapy in reducing viral load in blood as determined by quantitative viral culture of plasma and peripheral blood mononuclear cells (PBMC's) from patients receiving combination antiretroviral therapy with AZT and ddI in the Pediatric Branch protocol 90-C-09. Preliminary results indicate significant decreases in viral titer in both plasma and PBMC's relative to baseline after 12 to 20 weeks of therapy.

11. PMN from HIV-infected children were demonstrated to have significant impairment in their bactericidal capacity against S. aureus. In vitro incubation of defective PMN with GM-CSF corrected the bactericidal impairment. These findings may help explain the increased incidence of bacterial infections in this population, and suggest a potential therapeutic role for GM-CSF.

12. We have shown that G-CSF enhances the phagocytic and microbicidal activity of PMN against *S. aureus* but not against C. albicans blastoconidia. G-CSF also corrected the bactericidal defect of PMN from HIV+ patients.

14. To better understand the humoral deficiency of HIV+ children, we measured serum levels of IgG subclasses in a number of patients and correlated them with the frequency of bacterial infections. No association was found between low levels of specific IgG subclasses and increased susceptibility to bacterial infections. We concluded that other functional parameters rather than quantities of antibodies are more important in the humoral deficiency found in these patients.

15. Bacterial infections is a frequent problem causing increased morbidity and mortality in HIV+ patients. In a retrospective study we analyzed the bacterial infections that occurred in a cohort of HIV+ pediatric patients. The central venous catheters were shown to contribute to increased number of bacterial infections especially in association with younger age and lower CD4 counts. Antiretroviral therapy may have an effect on reducing non-catheter related infections.

16. Because T helper cells are the critically involved immune cells in HIV infection, we assessed their function in a group of HIV+ children and compared it to that of HIV- adults and healthy control children. Different patterns of unresponsiveness of T helper cells to recall and allogeneic antigens as well as to PHA were found, and there was a significant correlation between T helper cell dysfunction and the susceptibility to opportunistic and bacterial infections.

17. Follow-up of the T helper function of these patients during therapy with ddI showed that asymptomatic patients improved significantly more than symptomatic patients, and the improvement observed in the symptomatic patients was associated with fewer opportunistic and bacterial infections. T helper cell function may serve as a surrogate marker of HIV infection during antiretroviral treatment.

18. In studies using mononuclear leukocyte cells of monozygotic twin pairs one of which was HIV-infected, we found that the cells of the HIV-infected sibling suppress the T helper function of the cells of the healthy sibling and the suppressive factor is released in the supernatant of the cells without being the virus itself.

19. Our interest in the T helper cell function of children with HIV infection led us, in collaboration with the Experimental Immunology Branch of NCI and the Children's National Medical Center, to test the function of healthy children with ages ranging from birth to 14 years. We found that the T cell responses of neonates, infants and children to T cell mitogen PHA and to HLA allogeneic antigens are comparable to those of healthy adults. However, the T cell responses that require the interaction of the antigen presenting cells with the CD4+ cells are defective in infants and children younger than 24 months of age but not in neonates. These findings suggest a unique maturational process of the T helper cell function possibly influenced by the presence of various maternal cytokines in the neonates.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06840-16 PB

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Treatment of Acute Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Poplack	Head, Pharmacology and Experimental Therapeutics Section	PB, NCI
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	P. Adamson	Medical Staff Fellow	PB, NCI
	S. Berg	Medical Staff Fellow	PB, NCI
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COOPERATING UNITS *(if any)*

Medicine Branch, NCI (A. Fojo, K. Cowan, L. Neckers); Navy, NCI (L. Kirsch); Children's Cancer Study Group (G. Reaman).

LAB/BRANCH

Pediatric Branch

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Clinical research into the biology and treatment of acute leukemia is pursued with particular emphasis on acute lymphoblastic leukemia (ALL) of childhood. Major issues being addressed include: 1) development of therapeutic strategies aimed at improving overall prognosis of children with ALL, 2) investigation into the mechanisms of treatment failure with particular emphasis on evaluation of pharmacologic approaches to leukemic therapy, 3) characterization of adverse sequelae of antileukemic therapy and design of treatment regimens which avoid them, and 4) studies of the biology of ALL aimed at improving our basic understanding of the biology of this disease, identifying new diagnostic and prognostic tests and providing insight into the biologic basis for treatment failure.

An earlier ALL treatment protocol demonstrated that high-dose, protracted systemic methotrexate infusions could substitute for cranial radiation as central nervous system (CNS) preventive therapy for the majority of patients with ALL. Analysis of data from this study also identified a patient group at particular risk for CNS relapse. A new, high risk protocol has been devised in an attempt to improve the prognosis for these and other poor risk patients. The results to date indicate that this therapy is highly effective in preventing both systemic and central nervous system relapses while avoiding the use of cranial radiation. In patients in the average risk category, a comparison of two forms of CNS preventive therapy (intrathecal vs high dose methotrexate) is under way. A major, multi-institutional pharmacologic monitoring protocol is in progress which is studying the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL. Detailed analysis of the immunologic and molecular phenotype of acute lymphoblastic leukemia has led to the concept of a hierarchy of differentiation for both T cell and pre-B cell ALL. Studies are in progress to determine the relationship of molecular phenotype to prognosis. Evaluation of the P53 gene, a candidate tumor suppressor gene, suggests this gene may play a role in the pathogenesis of this disease.

Objectives

1. To develop effective treatment strategies which will improve the event-free survival of children with ALL, with particular emphasis on a) the development of alternative CNS preventive therapy and b) improvement of treatment for patients with poor risk features.

2. To characterize the long-term adverse sequelae of antileukemic therapy and design treatment regimens that avoid them.

3. To study the clinical pharmacology of antileukemic agents with the objective of optimizing ALL treatment through: a) exploration of the pharmacologic basis of treatment failure in ALL, b) development of new treatment strategies, with currently available antileukemic agents, which are based on sound pharmacologic rationale, and c) identification of promising new antileukemic agents.

4. To conduct studies of the biology of ALL in an attempt to increase our basic understanding of this disease and to identify biological characteristics which will provide avenues for new therapeutic approaches.

Methods and Major Findings:

A. Treatment Studies of Acute Lymphoblastic Leukemia

1. NCI 77/02/CCG 191 Treatment Protocol

A randomized protocol investigating the efficacy of high dose intravenous methotrexate infusions as CNS preventive therapy. Patients received either cranial radiation plus intrathecal methotrexate or high dose 24-hour intravenous methotrexate infusions. One-hundred-eighty-one (181) average and high risk patients were randomized on this study. The overall remission rate was 98%. The continuous complete remission rate is approximately 67% at three years for the entire study group. With a median duration on study of 9.4 years, there is no significant difference in the CNS relapse rate for either treatment group. Longitudinal evaluation of neuropsychological function has demonstrated a striking decrease in IQ test scores and impaired academic achievement in children treated with cranial radiation and intrathecal chemotherapy. No such changes have been observed in children treated with high dose methotrexate. The results of this study not only demonstrated that alternative CNS preventive therapy is feasible and as efficacious as cranial radiation and IT MTX, but also served to focus attention on the importance of avoiding neurotoxic regimens using cranial radiation. This study led to the development our two current clinical trials discussed below.

2. NCI 83-P/CCG 134P

The major aim of this pilot protocol is to demonstrate that high risk patients can be effectively treated on a regimen that uses CNS preventive therapy devoid of cranial radiation. To date, 119 patients have been entered on study; 96% achieved complete remission. With a median potential followup of 4.3 years, the event

free survival (at 3 years) is between 55 and 60%. The occurrence of isolated CNS relapse in only three patients, to date, suggests that effective CNS preventive therapy can be achieved without the use of cranial radiation in high risk patients.

3. NCI 84-A/CCG 144

This protocol randomizes average risk patients in one of two forms of CNS preventive therapy - either high dose methotrexate infusions or intrathecal methotrexate alone. One hundred sixty-six patients have been randomized on study. With a median potential followup of 4.3 years, there is no significant difference in the CNS or bone marrow relapse rate in either treatment arm. Although these results suggest that intrathecal MTX is as effective for CNS preventive therapy as HDMTX infusions for average risk patients, further follow-up is necessary before this statement can be made definitively.

4. Treatment of Newly Diagnosed Children with High Risk Acute Lymphoblastic Leukemia on a Dose Intensified Schedule: A Trial Evaluating the Efficacy of Adjunctive Therapy with Granulocyte Colony Stimulating Factor (G-CSF).

A new treatment protocol has been developed which will begin entering patients in September of 1990. Based on clinical and laboratory features evident at diagnosis, it is possible to delineate those groups of patients with ALL who are at highest risk for treatment failure . These children, usually with a high initial white blood count and an unfavorable age at diagnosis (e.g., <1 or >10 yrs), represent the major current challenge in the therapy of childhood ALL. In recent years, the use of more intensive ALL treatment regimens has improved the outlook for high risk patients. However, myelosuppression has limited further attempts to intensify treatment. Therapy-induced neutropenia leads to frequent delays in treatment and places patients at a significant risk of infection. In this protocol, we plan to evaluate whether the event-free survival of children with high risk ALL can be improved using a dose intensified regimen. The study is divided into two stages. In the initial stage we seek to determine the maximal dose intensity at which the drug combinations in this protocol can be safely administered to patients who are receiving concomitant G-CSF during the most critical and intensive periods of their therapy - induction and intensification. In the second stage, this dose intensified regimen will be evaluated, in the context of a non-randomized pilot study, to ascertain whether: 1) it produces a significant improvement in event-free survival; and 2) this improvement is sufficiently promising to warrant further evaluation in a subsequent, randomized, multi-institutional study. This Pediatric Branch study will be run collaboratively with selected institutions of the Childrens Cancer Study Group.

B. Pharmacologic Approaches to Leukemic Therapy: Relationship to Treatment Failure

A detailed study of the bioavailability of the major orally administered antileukemic agents is being undertaken in an attempt to examine the reasons for treatment failure in children with ALL.

1. Prospective Evaluation of Oral 6-MP and MTX Bioavailability.

This study is attempting to correlate the results of prospective periodic pharmacokinetic bioavailability studies of 6-MP and methotrexate with relapse rate and remission duration in a multi-institutional setting. Approximately 100 patients have been entered to date.The bioavailability and pharmacokinetics of oral 6-MP and MTX are studied on four separate occasions during the course of maintenance therapy in children with average and good risk ALL. Erythrocytes are periodically examined for MTX and 6-MP nucleotide content. To date, clinical information regarding disease status and toxicity in this group is still too incomplete for meaningful analysis. However, we have begun to analyze the "population" pharmacokinetics of these two agents. We have confirmed the wide inter-patient variability in plasma MTX and 6-MP concentrations following oral administration under standardized conditions, and have defined the "normal" range of the area under the plasma concentration-time curve (AUC) for both drugs. We are also able to evaluate the intra-patient variability in drug bioavailability; preliminary analysis reveals much greater variability with 6-MP than with MTX. This variability within the same patient may limit the application of therapeutic drug monitoring of 6-MP therapy. The absorption of these two agents does not appear to decline over the course of maintenance therapy, and the degree of absorption of one agent does not correlate with how well or poorly the other drug is absorbed. When patient accrual is complete and sufficient follow-up is available, the final pharmacokinetic analysis and clinical correlations will be made.

2. Laboratory Studies of 6-Mercaptopurine

6-Mercaptopurine (6-MP) has been the mainstay of maintenance therapy for children with leukemia for several decades. The optimum dose and schedule for 6-MP administration, however, has not yet been accurately defined. We therefore investigated the dose and schedule dependency of 6-MP using a human leukemia cell line, MOLT-4. Cell viability was determined with the MTT assay. In a series of experiments, the minimal cytotoxic concentration of 6-MP was found to be approximately 1 μM. Cytotoxicity was correlated to duration of exposure to concentrations > 1μM, with a minimum of 12 hours being required to achieve 90% growth inhibition. No cytotoxicity was observed with durations of exposure < 4 hours.

Although numerous loci of action for 6-MP have been described, the site of action that is ultimately responsible for cytotoxicity is not known precisely. Recently, a paradoxical cytotoxic effect of 6-MP was described, where decreasing *in vitro* cytotoxicity was noted as the concentration of 6-MP increased. We are presently investigating this *in vitro* phenomenon using human leukemia cell lines. Paradoxical cytotoxicity has been observed only at concentrations exceeding 100μM of drug. These concentrations are never achieved with 6-MP administered orally or intravenously to patients. The only clinical setting in which high (>100μM) concentrations are achieved is when 6-MP is administered intrathecally in doses of 10 mg. We therefore are investigating the basis for this *in vitro* phenomenon.

Other investigators have suggested that the ATP depletion that results from 6-MP is responsible for the lack of drug activation by ATP dependent guanylate synthetase.

We have, however, determined that this is not the basis for the paradoxical effect. Using a human B cell line (Wilson), a paradoxical effect was noted not only with 6-MP but also, to a lesser extent, with 6-TG, a drug not dependent on ATP for activation. Furthermore, there was no dose dependent decrease in intracellular ATP at doses of 6-MP > 10 μM. We postulate that at high concentrations of 6-MP, the initial enzymatic step in activation (HGPRT) becomes saturated, leading to an increase in other catabolic pathways. One pathway results in hypoxanthine formation, which could via IMP formation directly interfere with 6-MP metabolism. Hypoxanthine production was found to increase linearly with the dose of 6-MP. Additional *in vitro* studies are being performed to confirm this mechanism of action.

3. Alternative Maintenance Agents.

We are actively studying nonclassical antifolates which may be alternatives to MTX, such as piritrexim. A phase I trial of piritrexim has recently been completed. (see *Clinical Pharmacology Project Report*).

C. *In vitro* Chemosensitivity

Protocols designed for the treatment of childhood ALL include at least 6 drugs. It is known from single agent studies, however, that individual patients can have potential *de novo* resistance to any of the agents employed. It would therefore be advantageous to exclude agents that the individual is known to be resistant to, allowing addition or intensification of other active agents. The development of an *in vitro* chemosensitivity assay that is rapid, has the potential for automation, and yields results in a high percentage of samples is therefore being pursued.

We and others have studied *in vitro* chemosensitivity using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). MTT is reduced to formazan by the mitochondria of viable cells. The amount of formazan generated can be determined spectrophotometrically. In initial studies using a human leukemia cell line, MOLT-4, we demonstrated that the MTT assay was highly correlated with the clonogenic assay, suggesting that the assay may be predictive of *in vivo* response. When studying patient samples, however, the great majority of leukemic cells do not metabolize MTT efficiently. The results from cytotoxicity testing would lead to acceptable results only in a minority of samples.

We currently are investigating another non-clonogenic assay, the fluoroscein diacetate (FDA) assay. FDA is cleaved by non-specific cellular esterases in viable cells. The liberated fluoroscein can be quantitated by a microtiter plate fluorometer. Initial studies have demonstrated that fluorescence is linearly related to cell number over a greater than 2 log range, allowing for accurate determination of IC_{50} and IC_{90} concentrations. The FDA assay, similar to the MTT assay, was strongly correlated to the clonogenic assay when studied with MOLT-4 cells. More importantly, the signal produced by patient leukemic samples was comparable to the signal produced by cell lines. When comparing relative signal strength of the FDA *vs* the MTT assay using patient samples, the FDA signal was significantly stronger ($p=0.001$). The FDA assay appears to be a suitable assay for *in vitro* chemosensitivity testing of patient samples, and these studies are in progress.

D. Molecular Biology of Acute Lymphoblastic Leukemia

Molecular Phenotyping of Leukemic Lymphoblasts

Collaborative studies are investigating the status of immunoglobulin gene rearrangement and T-cell receptor gene status in acute leukemic lymphoblasts. Studies to date have enabled us to construct a hierarchy of differentiation for both pre-B cell precursor ALL (by immunoglobulin gene rearrangement) and for T-cell rearrangement (using T-cell receptor gene rearrangement). Recent studies, have been aimed at determining whether there is a correlation between molecular genotype in ALL and a variety of biologic and clinical features known to have a prognostic import (e.g. cytogenetics, initial white blood cell count, FAB morphologic classification, etc.) as well as with treatment outcome. Lymphoblasts obtained at diagnosis from patients treated on our "front line" ALL treatment protocols have been prospectively studied with cytogenetics, immunophenotyping (using FACS analysis and a panel of monoclonal antibodies), and molecular characterization. An analysis of this data suggests that genotypically less mature leukemias may manifest a more difficult course, and that genotype heterogeneity may be of clinical relevance. In an attempt to determine a possible biological basis for the aggressive clinical behavior of leukemic cells presumably transformed at the earliest stages of B-lymphoid development, lymphoblast DNA was studied by more sensitive PCR methodology. Leukemia-specific CDRIII sequences of VDJ rearrangements were PCR amplified using consensus V and J primers, and the products studied by agarose gel electrophoresis and hybridization with consensus J probes Each of 24 cases showing distinct rearrangements by Southern analysis, showed only 1 or 2 PCR detectable rearrangements. In contrast, each of 4 cases manifesting a germline pattern of Ig H genes on Southern blotting, showed a smear of bands when PCR products were electrophoresed and hybridized. This pattern suggests the presence of many PCR detectable Ig H rearrangements in some cases where a germline pattern is found on Southern blotting. Sequencing studies are now underway to determine more precisely the number of different clones present, and any clonal relationships between the multiple rearrangements detected by PCR. These preliminary investigations suggest that the refractory nature of "germline" cases if childhood B-cell precursor ALL may be related to difficulties in eradication of multiple subclones, present below the threshold of detection of the Southern method. These early data might represent a possible biological basis for differences in clinical behavior.

Several questions will be addressed using similar methodology in a new high risk treatment protocol which employs front end loading induction therapy and cyclic intensification. These include:

1. Within a high risk population of children , are specific Ig and TCR genotypes of prognostic importance?

2. Within a high risk population of children, is leukemic transformation at earlier stages of lymphoid development more common?

3. What is the PCR detectable tumor burden at defined time points during and at the completion of "front end-loading" induction therapy?

4. Is the degree of tumor burden as quantitated by PCR methodology predictive of outcome? Are PCR detectable Ig gene rearrangements related to therapeutic efficacy?

Studies of the p53 Gene in Acute Lymphoblastic Leukemia

The p53 gene is a candidate tumor suppressor gene located on chromosome 17 at band p13. Base upon experiments in transgenic mice where a mutated p53 gene under its own promoter resulted in lymphoid tumors, as well as anticipated tumors of lung and bone, the potential role of alterations in this gene in the pathogenesis of childhood acute lymphoblastic leukemia (ALL) is currently being explored. Bone marrow peripheral blood lymphoblasts of 25 pediatric patients with B-cell precursor ALL, have been examined for point mutations by the method of RNase protection using probes spanning the entire p53 coding region, and abnormalities were identified in 4 cases. The nature of these abnormalities was fully characterized by both cDNA synthesis, PCR amplification and sequencing of subclones, as well as by direct sequencing of genomic PCR products. These studies have revealed that p53 mutations, expression of these mutations at the RNA level, and loss of heterozygosity may occur in childhood ALL, but at a low frequency. An exploration of family histories revealed two pedigrees suggestive of cancer susceptibility. One was consistent with the Li-Fraumeni syndrome and a hereditary G to T transversion at p53 codon 272 (valine to leucine) was present. Children and adults in several generations of another family were affected with leukemia, but a 2 bp deletion with frame shift and premature termination in exon 6 was nonhereditary. These data suggest that a p53 mutation may not always be inherited in certain cancer-prone individuals and involvement of a different mutant gene is possible in families with multiple members affected by leukemia. The other two nonhereditary p53 mutations included: 1) codon 270 T to G transversion (phenylalanine to cysteine); and 2) a codon 248 G to C transversion (arginine to proline). These data support the role of both hereditary and acquired p53 mutations in the pathogenesis and/or progression of some cases of childhood ALL. Other studies of familial ALL and the status of the p53 gene in children who develop second tumors are also underway.

E. New Agent Studies in Relapsed Patients

1. Phase I and Phase II Trials.

The major focus of our studies for relapsed patients with ALL is on phase I and phase II trials of investigational agents. Emphasis is placed on those new drugs, examined in our laboratory, for which there exists a significant pharmacologic rationale for their use in the treatment of leukemia. Within the past year we have carried out and completed two phase I trials, including piritrexim and Interleukin-2. For a detailed listing and discussion of these new agent studies the reader is referred to the *Clinical Pharmacology Project Report.*

2. New Intrathecal Agents.

In recent years, we have focused attention on the development of new pharmacologic approaches to the treatment of CNS leukemia. Although numerous drugs are available for systemic administration to treat ALL, the number of agents suitable for intrathecal use is limited; no new intrathecal agents have been identified in over 25 years. In contrast to the successful treatment of systemic leukemia which is predicated on the use of combination chemotherapy, the extremely limited number of intrathecal agents restricts clinicians to the use of only one or two agents (e.g. MTX and Ara-C) which belong to the same drug class (antimetabolites). It is conceivable that if effective new intrathecal agents could be identified the development of combination intrathecal chemotherapy regimens could have the same impact on the control of CNS leukemia as

combination chemotherapy has had on control of bone marrow disease. In addition, since CNS preventive therapy with cranial radiation is associated with adverse CNS sequelae, new intrathecal agents are also needed for CNS preventive therapy. Thus, the identification of effective new intrathecal agents has become an appropriate and important priority. Four new intrathecal approaches developed in our nonhuman primate model are currently undergoing clinical study including intrathecal diaziquone (AZQ), intrathecal 6-mercaptopurine, intrathecal mafosfamide and continuous intraventricular methotrexate infusions. These studies are detailed in the *Clinical Pharmacology Project Report.*

F. Studies of the Late Effects of Childhood Leukemia Therapy

1. Memory and Learning Sequelae in Long-Term Survivors of Acute Lymphoblastic Leukemia

A systematic study of verbal and nonverbal memory and learning was undertaken in long-term survivors of acute lymphoblastic leukemia to assess the incidence and pattern of impairments and to determine the relationship between these deficits and computed tomography (CT) brain scan abnormalities. Twenty-three children who had received cranial irradiation (2,400 cGy) and intrathecal chemotherapy as central nervous system (CNS) preventive therapy and who were off all therapy for at least 4 years were evaluated. On the basis of their CT brain scan findings, patients were divided into three groups: those with intracerebral calcifications ($n = 5$), those with cortical atrophy ($n = 8$), and those with normal CT findings ($n = 10$). Significant deficits in verbal memory ($p < 0.025$) and verbal learning (p 0.05) were observed that were associated with the presence and type of CT brain scan abnormalities; the greatest impairments were observed in patients with calcifications. No significant differences between CT scan groups were found for nonverbal memory and learning. Previous evaluation of attentional processing in these patients using reaction time tests had revealed the presence of deficits primarily in the ability to sustain attention. Combining those data with findings from the present study showed that memory impairments, particularly those in short-term memory, were primarily attributable to an underlying attentional defect that affect the encoding stage of memory processing.

2. *In vitro* studies of the effect of folates on radiation sensitivity

Adverse central nervous system sequelae have been well documented in patients with acute lymphoblastic leukemia treated with cranial radiation and methotrexate (MTX). We have also demonstrated that treatment with chronic low dose MTX substantially reduces folate levels in the brain. We are currently examining the *in vitro* effects of folates on the radiation survival (RS) of tissue cultured chinese hamster ovary cells (CHO-K1). RS curves have been generated for asynchronous and confluent CHO-K1 cells grown in either complete (2.3mMfolic acid) or folate-free medium and subsequently cloned in folate-defined medium following irradiation. For both asynchronous and confluent cultures, CHO-K1 grown in folate-free medium and cloned in minimum-required folate medium (.023mM folic acid) have shown increased radiosensitivity when compared to CHO-K1 grown in complete medium (2.3 mM folic acid), demonstrating that RT effects are probably not due solely to alterations in cell cycle kinetics. Other mechanisms associated with

increased radiosensitivity in tissue cultured cells will be studied and folate levels in the cerebrospinal fluid of children who have been treated for ALL will be measured in order to better understand the observed clinical side effects of combination radiotherapy and MTX.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06880-14 PB

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Clinical Pharmacology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

Medicine Branch, NCI (C. Allegra, J. Grem); Childrens Cancer Study Group (J. Holcenberg, J. Sato, V. Avramis); St. Jude Children's Cancer Research Hospital (R. Heideman).

LAB/BRANCH

Pediatric Branch

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies is studied with emphasis on the role of pharmacologic monitoring and on both pre-clinical and clinical pharmacologic studies of Phase I agents. The clinical pharmacology of orally administered antileukemic agents has been evaluated and the limited bioavailability and variable drug levels of 6-MP achieved following oral administration has been documented. Studies are underway to determine the extent to which this phenomenon is the cause of treatment failure. Preclinical and clinical pharmacokinetic studies of a variety of new agents including Piritrexim, All-trans retinoic acid and Thiotepa plus GM-CSF are being completed. A major effort of this project is to investigate experimental approaches to the treatment of CNS malignancy. A unique primate model is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities of various CNS treatments; and to evaluate and screen newer CNS treatment modalities and drug schedules. Information gained from the studies with this model is then applied to the design of clinical treatment protocols. Protocols evaluating strategies such as prolonged intravenous 6-MP infusions and intravenous Thiotepa for brain tumors are under way. Clinical studies of intrathecal AZQ, intrathecal 6-MP, and intrathecal mafosfamide, all approaches developed in this model, are in progress. A clinical study evaluating continuous intra-CSF drug infusion via a unique indwelling drug delivery device also is under way. As part of the Pediatric Branch AIDS research effort, the Leukemia Biology Section is studying the clinical pharmacology of antiretroviral agents in children. The study of these agents is a natural extension of our work on the clinical pharmacology of anticancer drugs, since most of the antiretroviral agents are nucleoside analogs, similar to the antimetabolites used in the treatment of ALL. The CNS pharmacology of antiretroviral therapies is being systematically evaluated in our non-human primate model, to determine which agents may be most effective against CNS HIV infection. We have also participated in the design of clinical trials of antiretroviral agents in children and performed detailed pharmacokinetic studies in the patients treated on these trials.

Objectives:

1. To perform pre-clinical and clinical pharmacologic studies on new agents with particular emphasis on those being used to treat pediatric malignancies and those with potential activity against CNS malignancies.

2. To study the CNS pharmacokinetics of drugs either currently employed or potentially useful to treat CNS malignancy.

3. To study the pre-clinical and clinical pharmacology of new anti-retroviral agents undergoing Phase I testing in children.

Methods Employed and Major Findings:

A. Clinical Pharmacology of Antineoplastic Agents

1. Clinical Studies on Thiotepa

We have evaluated the clinical pharmacology of Thiotepa in children with malignancy. Thiotepa is an active alkylating agent with a steeper dose-response curve than cyclophosphamide. Our studies have demonstrated that substantial amounts of both thiotepa and its metabolite Tepa are present in the CNS following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent than intrathecal injection. As a result of these studies, a Phase I study of intravenous thiotepa in pediatric patients was undertaken and completed. The results suggested that systemically administered thiotepa may be a valuable agent for the treatment of CNS malignancies. In addition, this study demonstrated that thiotepa can be safely administered to pediatric patients at significantly higher doses (the MTD was 65mg/m^2) than those used conventionally in adults. *In vitro* studies of the activity of thiotepa and tepa against human CNS tumors have been performed using medulloblastoma and glioma cell lines. Both thiotepa and tepa show significant *in vitro* activity against these CNS tumor cell lines at drug concentrations achievable in patients at the dose recommended in our phase I trial.

Based on these findings the following studies have been pursued:

Phase II Trial of Intravenous Thiotepa in Pediatric Brain Tumors

A collaborative study of intravenous thiotepa at a dose of 65 mg/m^2 for pediatric patients with brain tumors is in progress.

Phase II Trial of Thiotepa in Pediatric Solid Tumors

A Phase II study of thiotepa in pediatric solid tumors is being pursued together with the Children's Cancer Study Group.

Phase I trial of the Combination of Thiotepa and GM-CSF

High dose thiotepa, in combination with other agents, is being used as preparative therapy for autologous bone marrow transplantation for the treatment of brain tumors. Although promising clinical responses have been observed, the inability to repeatedly perform autologous bone marrow transplantation limits this therapeutic approach. Granulocyte-macrophage colony stimulating factor (GM-CSF) is one of several cloned hematopoietic growth factors which have been demonstrated to significantly modify the degree and duration of chemotherapy induced neutropenia. We recently completed a phase I protocol designed to evaluate the feasibility of administering escalating intravenous doses of thiotepa in conjunction with GM-CSF. In this study, patients received intravenous thiotepa on an every three week schedule starting at the MTD defined in our previous phase I study. GM-CSF was administered subcutaneously during the post-chemotherapy period; the thiotepa dose was escalated in 30% increments The aim of this study was to determinie the highest dose of thiotepa which could be safely administered with adjunctive GM-CSF therapy. A total of 11 patients were enrolled in this study. Dose limiting thrombocytopenia (observed at the 85 mg/m^2 dose) prevented safe escalation of the Thiotepa dose above the 65 mg/m^2 dose.

Although dose escalation was not feasible using GM-CSF, subsequent clinical trials using other cytokines (e.g., Il-3) which may prevent the dose-limiting thrombocytopenia observed with Thiotepa are planned in an effort to explore whether significant dose escalation with this agent will be possible.

2. Preclinical Studies of Cyclopentenyl Cytosine (CPE-C)

Cyclopentenylcytosine (CPE-C), a synthetic cytidine analogue, is currently undergoing extensive preclinical testing and has been demonstrated to have significant antitumor activity. It is active in vivo against the P388 and L1210 murine leukemias and against human lung, melanoma and breast cancer xenografts grown in athymic mice. In addition, cytarabine resistant murine leukemia lines are collaterally sensitive to CPE-C. The plasma and cerebrospinal fluid (CSF) pharmacokinetics of cyclopentenyl cytosine (CPE-C) have been studied following i.v. bolus and continuous i.v. infusion in rhesus monkeys. Following an i.v. bolus dose of 100 mg/m^2 plasma elimination of CPE-C was biexponential with a mean $t_{1/2}\alpha$ of 8.8 min, a mean t1/2β of 36 min and a total clearance CL_{TB} of 662 ml/min/m^2, which is 5- to 10-fold higher than clearance rates in rodents and dogs. Less than 20% of the total dose of CPE-C was excreted unchanged in the urine. The remainder was excreted as the inactive deamination product cyclopentenyl uridine (CPE-U). The ratio of the areas under the plasma concentration versus time curves of CPE-U to CPE-C was 7.0 ± 2.4 following i.v. bolus CPE-C. The CSF:plasma ratios of CPE-C and CPE-U were 0.08 and 0.30 ,respectively. Continuous i.v. infusion of CPE-C was compared to continuous infusion of ara-C

in two monkeys. Steady state plasma concentrations, normalized to a dose of 12.5 mg/m^2/h of CPE-C and an equimolar dose of ara-C, were 2.1 μM and 0.53 μM, respectively. The steady state concentrations of their corresponding uridine metabolites (CPE-C and ara-U) were 8.2 μM and 15.5 μM. The rapid elimination of CPE-C by deamination in the primate resulted in a much higher CL_{TB} and considerably lower rate by renal excretion. The significant interspecies differences in the disposition of CPE-C discovered in this study are important and have been incorporated into the selection of the starting dose for phase I trials. Based on these findings a Pediatric phase I study which incorporates a pharmacologically directed dose escalation strategy is planned.

In-vitro Studies of Cyclopentenyl Cytosine

CPE-C is a prodrug which requires phosphorylation intracellularly by uridine/cytidine kinase to CPEC-CTP which depletes endogenous CTP pools. The mechanism of resistance to CPE-C is being studied in a Molt-4 T-cell leukemia line made resistant to CPE-C (Molt-4^R) by culturing it in the continuous presence of increasing concentrations of CPE-C. Using a tetrazolium based colorimetric assay, the IC_{90} for the Molt-4^{WT} cells is 0.5 μM after a 24 hr exposure. In contrast, cytotoxicity is not observed at concentrations as high as 100 μM in the Molt-4^R cells. After a 10 min exposure to [^{3}H]CPE-C, an equivalent amount of parent drug is detected intracellularly in both Molt-4^{WT} and Molt-4^R cells; however, CPEC-CTP is detected only in Molt-4^{WT} cells. Following a 4 hr exposure to CPE-C, endogenous CTP pools in the Molt-4^{WT} cells decreased from 1.5 ± 0.3 to 0.5 ± 0.03 pmol/10^6 cells; whereas, CTP pools in the Molt-4^R cells increased from a baseline of 2.6 ± 0.1 to 3.6 ± 0.9 pmol/10^6 cells. In addition, uridine/cytidine kinase activity is 2.2 ± 0.5 versus 0.7 ± 0.2 pmol/mg/min for Molt-4^{WT} and Molt-4^R cells, respectively ($p = 0.009$). No differences in alkaline or acid phosphatase levels have been detected between the Molt-4^{WT} and Molt-4^R cells. Thus, decreased uridine/cytidine kinase, resulting in lack of formation of CPEC-CTP, appears to be the primary mechanism of resistance of these Molt-4^R cells to CPE-C. Additional studies to evaluate the role of CTP synthetase in these two cell lines are in progress.

Intrathecal CPE-C

CPE-C, has been shown to have significant preclinical activity against ara-C resistant murine leukemia cell lines as well as solid tumors. The feasibility of administering CPE-C by the intrathecal route is currently being evaluated in our nonhuman primate model. Preliminary experiments have shown that CSF CPE-C levels decline biexponentially following bolus administration with a terminal $t_{1/2}$ of approximately 1 1/2 hours. Additional studies to evaluate the long term toxicity of weekly intralumbar injections of CPE-C are in progress.

3. Preclinical studies of Pyrazoloacridine

Pharmacokinetics of Pyrazoloacridine In Primates

Pyrazoloacridine (PZA) is a rationally synthesized acridine derivative with *in vitro* activity against solid tumor cell lines, noncycling and hypoxic cells, and tumor cell lines that exhibit the multidrug resistance phenotype. The pharmacokinetic behavior of PZA after a 1 or 24 hour intravenous infusion was studied in 6 rhesus monkeys that received a total of 10 courses of PZA at 15 or 30 mg/kg. For 1 hr infusions, the plasma disappearance was biexponential with a $t_{1/2}$ alpha of 31 min and $t_{1/2}$ beta of 11 hr. The mean volume of distribution at steady state was 69 L/kg. The clearance was 83 ml/min/kg. For the 15 mg/kg dose, the mean area under the concentration-time curve was 708 μM•min and the mean peak concentration was 1.3 μM. For the 30 mg/kg dose, the AUC was 1244 μM•min and the Cp was 2.5 μM. The steady-state plasma concentrations during the 24 hr continuous infusions were 0.27μM for the 15 mg/kg dose and 0.45μM for the 30mg/kg dose. The mean CL_{TB} calculated from these Css was 121 ml/min/kg. CSF levels were <0.1μM for all doses and schedules. There was no evidence of toxicity at any dose or schedule.

These results contrast strikingly with those obtained in mice and dogs (Stoltz, PAACR 1990;31:442; Liao, PAACR 1990;31:443) in which, despite a more rapid clearance of PZA, significant toxicities were observed at doses that were nontoxic in the monkey. Our findings may have significant bearing on the dose-schedule ultimately chosen for Phase I studies. The extent to which the non-human primate data will accurately predict the behavior of pyrazoloacridine in humans will be closely examined in our planned Phase I trial of this drug.

Cellular Pharmacology of Pyrazoloacridine

In collaboration with the Medicine Branch, studies of pyrazoloacridine cellular pharmacology are underway. Preliminary studies of the cytotoxicity and uptake of this drug in wild type and multidrug resistant MCF-7 cells have been performed. The rate of drug uptake and efflux as well as the localization of drug in various cellular compartments will be examined and correlated with the results of *in vitro* cytotoxicity experiments.

4. Piroxantrone

Clinical Pharmacology

Piroxantrone is an anthracycline derivative synthesized to have decreased cardiac toxicity while retaining antitumor activity. Decreased cardiac toxicity results from a decrease in the potential for semiquinone free radical formation compared with other anthracycline derivatives.

Pharmacokinetically guided Phase I trials in adults have demonstrated a maximum tolerated dose of 150 to 190 mg/m^2.[Ames, 1990; Hantel, 1990] This Medicine Branch trial will investigate the maximum tolerated dose of piroxantrone alone and in combination with G-CSF. Piroxantrone pharmacokinetics will be analyzed in this laboratory utilizing an HPLC assay. In addition, the possibility of non-linear

pharmacokinetics at higher doses and the identification of piroxantrone metabolites will be explored.

CSF Penetration

Virtually nothing is known about the CSF penetration of piroxantrone. The CSF penetration of other anthracyclines and their metabolites is variable; thus it is difficult to predict the behavior of piroxantrone. The nonhuman primate model will be used to determine the plasma and CSF pharmacokinetics of this agent following intravenous administration. In addition, the plasma pharmacokinetics will be correlated with those obtained in the murine and dog models and in the human phase I trials (see #9 below).

5 PEG-L-Aparaginase

L-asparaginase forms an important part of the chemotherapy of acute lymphoblastic leukemia in children. The antitumor effect of L-asaparaginase is due to the depletion of L-asparagine, an amino acid which is essential for malignant lymphoblasts but not for normal cells, from the plasma of patients receiving this drug. A major obstacle to L-asaparaginase therapy is the development in a significant number of patients of allergic reactions to this foreign protein. In an attempt to circumvent these immune-mediated complications, L-asparaginase has been conjugated with polyethylene glycol. The PEG-modified enzyme is less immunogenic and has a longer plasma half-life than the native enzyme.

Studies in the non-human primate model are comparing the pharmacokinetics of two different preparations of PEG-L-asparaginase in plasma and cerebrospinal fluid in an effort to determine the duration of asparagine depletion achieved with each product.

6. Phase I Studies

A variety of Phase I trials are being pursued in an effort to develop active, new componds for the treatment of pediatric malignancies

Phase I Trial of Piritrexim

Piritrexim, an orally administered, lipid soluble antifolate, was evaluated in a multi-institutional phase I trial in children. The starting dose was 10 mg/m^2/dose administered every 8 hours daily for five days for three consecutive weeks, with dose escalations in increments of 5 mg/m^2/dose. Eighteen patients (16 with metastatic sarcoma, 1 with acute lymphoblastic leukemia and 1 with a brain stem glioma), 3.5 to 20 years of age, with malignancy refractory to therapy, were entered onto the study. The dose limiting toxicities (DLTs), which were myelosuppression and mucositis, occurred in 4/4 patients treated at the 25 mg/m^2/dose level, but in none of the patients treated at the 15 and 20 mg/m^2/dose levels. The maximum tolerated dose was 25 mg/m^2/dose, and the recommended dose for phase II trials is 20 mg/m^2/dose. Pharmacokinetic monitoring was performed in 15 of the 18 children entered on study. The area under the concentration time curve (AUC) was linearly related to the dose administered. Piritrexim was rapidly absorbed, with the

median time to peak level occurring 1.5 hours after an oral dose. The terminal half-life of piritrexim ranged from 1.5 to 4.5 hours. A limited sampling strategy (developed in a prior phase I trial of piritrexim) which predicted the AUC based on the plasma concentrations at 3 and 6 hours after an oral dose, was prospectively tested in this trial and proved to be highly predictive of the AUC (r=0.98, p=0.0001). Pharmacodynamic-pharmacokinetic correlations were performed after combining data from this and the prior phase I pediatric trial. Trough plasma piritrexim concentration was strongly correlated to DLT (p=.0.0016). A trough plasma piritrexim concentration greater than 0.5 μM appeared to be predictive of toxicity. Eleven of fifteen patients with trough concentrations exceeding this threshold experienced DLT. Therapeutic drug monitoring may thus play an important role in adjusting the dose and schedule of piritrexim in future trials.

Phase I Trial of All-*trans* Retinoic Acid (*t*-RA)

t-RA is an agent which has demonstrated activity *in vitro* as a tumor differentiating agent. *In vivo t*-RA has demonstrated significant activity in patients with acute promyelocytic leukemia. Whereas there have been several studies of the pharmacokinetics of *cis*-RA, there is little information regarding the pharmacokinetics of *t*-RA in humans and none in children. We recently initiated and subsequently completed a Phase 1 trial of *t*-RA in pediatric patients with refractory malignancies. *t*-RA was given orally on a q 12 hour schedule for 28 days. The starting dose was 45mg/m^2/day. Twenty-one patients were entered into this trial. Eighteen were evaluable for response; 17 were evaluable for toxicity. The maximum tolerated dose (MTD) was 60 mg/M^2. Increased intracranial pressure was the dose limiting toxicity. Complete responses were observed in two patients with multiply relapsed acute promyelocytic leukemia. Preliminary analysis of *t*-RA pharmacokinetics reveal that peak plasma concentrations are relatively low (1 μM) and that the drug is rapidly cleared from plasma. The plasma half-life appears to be significantly shorter for *t*-RA than for *cis*-RA. In order to better define the pharmacokinetic differences between *cis*-RA and *t*-RA, experiments in Rhesus monkeys are being carried out. Drug will be administered intravenously to accurately define the clearance and terminal half-life of both stereoisomers.

Phase I Trial of Amifostine/Melphalan

Melphalan, similar to other alkylating agents, has a steep dose response curve, but its use is limited by myelosuppression. For melphalan to be effectively used in the treatment of pediatric malignancies, strategies to circumvent its dose limiting myelosuppression are needed. One potential strategy is to administer growth factors following alkylating agent therapy. For agents that are stem cell poisons, such as melphalan, however, this strategy has not proven to be effective in preventing severe myelosuppression following repetitive doses of drug. Another approach is to administer a chemoprotective agent prior to melphalan administration. Amifostine has been shown in preclinical trials to protect the bone marrow from the myelotoxicity of melphalan, and in clinical trials to protect from the myelotoxicity of other alkylating agents. Treatment of patients with amifostine prior to melphalan administration may allow for the escalation of melphalan doses beyond those currently tolerable.A phase I pediatric trial is currently being performed to:

(1) Determine the acute toxicity of amifostine (WR2721) in pediatric patients with refractory malignancies at doses escalated to the adult recommended dose.
(2) Determine the maximum tolerated dose of melphalan when administered in conjunction with amifostine.
(3) Study the pharmacokinetics of amifostine and its active metabolite WR1065 in pediatric patients.

Phase I Study of Topotecan

Topotecan, a new antineoplastic agent with a novel mechanism of action (inhibition of topoisomerase I), has demonstrated a high degree of antitumor activity in a broad spectrum of murine tumors including B16 melanoma, B16 melanoma/F10 subline, ADJ-PC6 plasmacytoma, Lewis lung carcinoma, HT 29 colon carcinoma, reticulum cell sarcoma, P388 leukemia and L1210 leukemia cell lines. In addition, Topotecan has been shown to have significant activity against multiple multi-drug resistant P388 leukemia cell lines. Thus, this compound is of significant interest for the potential treatment of solid tumors and refractory hematologic malignancies in the pediatric population.

Interest in synthesis of camptothecin analogues such as Topotecan stems from the recent elucidation of the unique mechanism of action for this class of compounds as well as the results of initial clinical trials with sodium camptothecin. Phase I trials with sodium camptothecin in the late 1960's demonstrated clinical activity with objective responses reported in 13 of 25 patients evaluable for response, including patients with gastrointestinal adenocarcinoma, melanoma, non small lung cell lung cancer and acute myelogenous leukemia. However, in subsequent phase II trials only two partial responses were noted in 61 patients with colorectal carcinoma and no responses were observed in 15 patients with malignant melanoma. Further studies of camptothecin were limited due to unpredictable and severe myelo-suppression, gastrointestinal toxicity and hemorrhagic cystitis. Clinical trials with camptothecin were subsequently never performed in the pediatric population.

Topotecan is a semisynthetic analog of camptothecin with a basic side chain in the 9-position and a hydroxy group in the 10 position of the A-ring (see structure below). These structural modifications result in significantly increased water solubility for this analog versus the parent compound, camptothecin. The E-ring lactone of Topotecan spontaneously hydrolyzes in a pH dependent fashion with a predominance of the lactone ring below pH 7.0 and greater than 80% lactone at pH 6.0. The lactone form of Topotecan stabilizes a covalent complex between topoisomerase I and DNA leading to enzyme-linked DNA cleavage (DNA single-strand breaks) with resultant cytotoxicity. It has been postulated that since hypoxic tumor cells (prevalent in solid neoplasms) have reduced intracellular pH, Topotecan will be more effective in inhibiting topoisomerase I in these cells.

The antitumor effect of Topotecan appears to be phase specific with enhanced activity after prolonged *in-vitro* drug exposure. The schedule dependency of this compound has also been observed in animal studies. Following a single i.v. bolus dose significant antitumor activity was observed only at the MTD, whereas, with an intermittent q 3 hr IV bolus dosing schedule, antitumor activity was observed not only at the MTD but also at several dose levels lower than the MTD. These studies

support the premise that a continuous intravenous infusion of Topotecan will provide the optimal antitumor effect.

A Phase I trial and pharmacokinetic study of Topotecan administered as a 24 hour continuous i.v. infusion in pediatric patients with advanced neoplastic disease is in progress.

Planned Phase I Trials

Protocols have prepared for conducting Phase I trials of CPE-C, pyrazoloacridine, and IL-3 in children as discussed above. In addition, a protocol is being developed for taxotere, a semisynthetic analogue of taxol.

7. Phase II Trials

Once the phase I trial of a new agent has been completed a the optimal dose has been identified, efforts are made to ensure that phase II testing of the drug's antitumor spectrum are conducted. Since these trials require at least 9 to 14 patients with each diagnosis, they can only reasonably conducted within a group setting. The Senior Investigators in the Pharmacology and Experimental Therapeutics Section are members of the Childrens Cancer Study Group (CCSG New Agents Committee which provides an outlet for continued study of many of these agents. Phase II trials are also conducted in collaboration with the Pediatric Oncology Group (POG).

Phase II Trials of Thiotepa

As discussed earlier, 2 phase II trials of thiotepa are underway, including one for brain tumors with a group of Northeastern institutions and one for solid tumors within the CCSG. The brain tumor trial has entered 56 patients with a variety of tumor histologies. Responses have been observed in patients with PNET, and accrual continues for this diagnosis. The drug proved to be inactive in patients with malignant gliomas and brain stem gliomas. No definitive data is available yet from the solid tumor study.

Phase II Trials of 6-Mercaptopurine (6-MP)

The section previously performed a phase I trial of 6-MP administered by continuous intravenous infusion at a dose of 50 $mg/m^2/hr$. This study demonstrated that an infusion duration of up to 48 hours was tolerable and that the drug penetrated well into the CSF. Subsequently, 3 phase II trials of continuous infusion 6-MP were initiated with the POG. Trials in leukemia and solid tumors have been completed and failed to demonstrate appreciable activity. The phase II trial in patients with brain tumors is still ongoing. Twelve evaluable patients have been accrued including 4 with brain glioma and 4 with PNET.

Phase II Trial of Fazarabine

A phase I trial of the new antimetabolite, fazarabine (a.k.a. Ara-AC), was also conducted by the investigators in our section. This agent has a broader spectrum of

solid tumor activity than Ara-C in preclinical models. A phase II trial was recently approved and opened within the CCSG and has started to accrue patients.

8. Other Pharmacologic Studies

Studies with Carboxypeptidase-G_2

High dose methotrexate (HDMTX) can be safely administered when followed by leucovorin (LV) rescue. $CPDG_2$, an enzyme which rapidly hydrolyzes MTX into inactive metabolites, may act as an alternative form of rescue for HDMTX. The gene for bacterial $CPDG_2$ has recently been cloned and the enzyme purified on a large scale. $CPDG_2$ has potential advantages over LV rescue: $CPDG_2$ does not cross the blood brain barrier, raising the possibility that patients could be rescued systemically from HDMTX while selectively excluding CNS tumors from rescue. In contrast to LV, $CPDG_2$ could be used to rescue patients with renal dysfunction and delayed MTX excretion, as it can effectively rescue from systemic plasma MTX concentrations > 10μM. The plasma pharmacokinetics of MTX with and without this new purified preparation of $CPDG_2$ was studied in adult rhesus monkeys. Animals received a 300 mg/m^2 loading dose of MTX followed by a 60 mg/m^2/hour infusion over 18 hours (steady state MTX plasma concentration 12.0 μM). Without $CPDG_2$ rescue, the initial half-life ($t_{1/2a}$) of MTX after discontinuation of the infusion was 4.5 minutes, and plasma MTX concentration remained > 0.1 μM for > 6 hrs. After administration of 50 units/kg $CPDG_2$ at the end of the MTX infusion, the $t_{1/2a}$ of MTX was 0.8 minutes, and plasma MTX concentrations fell to non-toxic levels (< 0.1 μM) within 15 minutes. The post infusion area under the plasma concentration time curve (AUC) of MTX was 260 μM•min without $CPDG_2$, compared to 32 μM•min with $CPDG_2$. Previous experience with the non-recombinant bacterial preparation, $CPDG_1$, demonstrated that allergic reactions occasionally limited its use. Studies are underway to assess the immunogenicity of $CPDG_2$. Animals have received up to 8 bi-weekly doses of enzyme without manifesting allergic symptoms. The recombinant $CPDG_2$ product may be less immunogenic than its non-recombinant predecessor. Administration of $CPDG_2$ appears safe and well tolerated, and may be useful as an alternative to LV rescue of systemic MTX in patients with renal dysfunction or in the treatment of CNS tumors. (The ability of intrathecal $CPDG_2$ to rescue monkeys from an intrathecal methotrexate overdose is discussed below)

Pharmacokinetics of ICRF-187

ICRF-187, an EDTA analog that protects myocardial cells from the toxic effects of doxorubicin-induced free radicals, is currently undergoing evaluation as part of the Pediatric Branch high risk sarcoma protocol. The pharmacokinetic behavior of ICRF-187 after a 1000 mg dose is being analyzed in each patient who is randomized to receive this agent. To date, 11 patients have been studied. The mean clearance of ICRF-187 is 266 ± 54 ml/min with a distribution half-life of 10 ± 7 minutes and an elimination half-life of 114 ± 22 minutes. The mean AUC is 14600 ± 3600 μM•min.

When the cardioprotective effects of ICRF-187 are evaluated, the correlation between pharmacokinetic parameters and clinical efficacy will be examined.

Pharmacokinetics of 5-Fluorouracil

In collaboration with Dr. J. Grem and Dr. C. Allegra of the Medicine Branch we are evaluating the pharmacokinetics of 5-fluorouracil an antimetabolite used in the treatment of a variety of common adult tumors, including colorectal and breast cancer. The primary purpose of these studies has been to establish pharmacokinetic-pharmacodynamic relationships between plasma concentration of the drug (as measured by area under the plasma concentration time curve) and toxicity and response, and to evaluate possible drug interactions between 5-FU and biologic agents given in combination with 5-FU such as α-interferon and GM-CSF. Preliminary analysis has suggested a relationship between plasma concentration and toxicity following a bolus dose schedule and we demonstrated that α-interferon does appear to delay the elimination of 5-FU in a dose dependent fashion leading to a 50% increase in the total 5-FU exposure when the drugs are given in combination.

9. Pharmacology of Intrathecal Drug Administration

Based on our work in the non-human primate model three new intrathecally administered agents are being investigated in clinical trials, AZQ, 6-MP, and mafosfamide. An additional, novel approach being studied involves the continuous intraventricular administration of methotrexate using a portable, computerized delivery pump. We are also evaluating additional strategies in our pre-clinical models which have potential application relevant to the treatment of patients with CNS malignancy including the use of carboxypeptidase as a rescue from intrathecal methotrexate overdose.

Intrathecal AZQ (Diaziquone).

AZQ is a lipophilic alkylating agent designed for enhanced penetration of the blood-brain barrier. In preclinical studies, we demonstrated that following intravenous infusion, significant levels of AZQ were achieved in CSF . However, in subsequent clinical phase II studies evaluating parenteral AZQ for treatment of brain tumors, the systemic administration of this compound was found to be associated with severe, cumulative and dose-limiting hematologic toxicity. Because of the considerable preclinical data indicating that AZQ is active against a variety of CNS tumors as well as leukemias, we evaluated the possibility of administering AZQ intrathecally. Initially we studied the CSF pharmacokinetics of AZQ following intraventricular injection in our sub-human primates and found that ventricular and lumbar CSF drug exposure (AUC) were 20- and four- fold higher, respectively, than the CSF AUC achieved with intravenous administration of 80 times the intraventricular dose . The feasibility and safety of intraventricular AZQ was also confirmed in the model. As the result of these studies, we developed a phase I/II trial of intrathecal AZQ which is currently in progress. Two dose schedules of AZQ are being evaluated in patients with refractory meningeal neoplasia, including standard bolus intrathecal administration of 1 mg twice weekly or a CxT schedule (0.5 mg intraventricularly every 6 hours x 3 doses). The CxT approach is designed to take advantage of the greater antitumor activity that we noted with this agent *in vitro* following prolonged drug exposure. To date, a total of 38 patients with refractory meningeal malignancy have been entered onto this protocol. Complete

responses have been achieved in 15 patients, ranging from one to nine months in duration. No significant neurologic or systemic toxicity has been observed. These promising results in a group of heavily pretreated patients suggests a future role for intrathecal AZQ in the treatment of CNS leukemia and other meningeal malignancies.

Intrathecal 6-Mercaptopurine.

We have examined the feasibility of administering 6-MP by the intrathecal route. In initial studies in the nonhuman primate model we demonstrated that 6-MP could be safely administered by the intraventricular route. CSF 6-MP concentrations were found to decline biexponentially with t1/2's of 40 minutes and 2.8 hours. In addition, our results indicated that concentrations of 6-MP found to be cytocidal *in vitro* against a variety of human tumor cell lines could be readily achieved in CSF at doses that are well tolerated. As an extension of these studies we recently initiated a clinical phase I trial of intrathecal 6-MP in patients with refractory meningeal malignancy. Both bolus administration (at a dose of 10 mg) and a CxT schedule (10 mg administered every 12 hours for 6 doses) are being studied. Complete remissions have been achieved in four of the nine patients treated on the bolus schedule. The remission durations range from two to five months. Entry onto the CxT arm of the study has only recently begun. Although preliminary, these data indicate that intrathecal administration of 6-MP is tolerable and suggest that this approach may eventually prove useful, not only for the treatment of overt meningeal leukemia, but also as CNS preventive therapy in childhood ALL.

Intrathecal Mafosfamide.

The highly active alkylating agent, cyclophosphamide, is a prodrug, which must be converted by hepatic microsomal enzymes into 4-hydroxycyclophosphamide before expressing its antitumor effects. Because of this requirement for hepatic activation, cyclophosphamide is inactive *in vitro* and would not be an appropriate agent for regional administration. In contrast, 4-hydroperoxycyclophosphamide and mafosfamide, preactivated derivatives of cyclophosphamide, exhibit activity *in vitro* equal to that of 4-hydroxycyclophosphamide. 4-hydroperoxycyclophosphamide has demonstrated activity against a variety of malignant cells lines including L1210 leukemia, Burkitt's lymphoma, and breast cancer, and it is used for purging leukemic cells from human bone marrow prior to autologous bone marrow transplantation. We are currently investigating the feasibility of administering mafosfamide intrathecally. In our nonhuman primate model intrathecal injection of this compound was not associated with either acute or chronic neurotoxicity or with systemic toxicity. The demonstration that cytocidal levels of these agents can be achieved in CSF following intraventricular administration of a non-toxic dose suggests that further study in the clinical setting is warranted. A clinical phase I trial of mafosfamide in patients with refractory meningeal malignancy has recently been initiated, and has rapidly accrued patients. The drug has been well tolerated on both an acute and chronic basis and we have been able to escalate the dose to the 5 mg biweek dose level.

Continuous Intrathecal Infusion

Intrathecal agents are currently administered by bolus injection, despite the fact that the most commonly used agents, MTX and cytarabine, are antimetabolites which have been shown to be more cytotoxic with prolonged exposure. In addition, because other intrathecal agents (AZQ, thiotepa) are cleared rapidly from the CSF following bolus injection, they must be given in higher doses to maintain a minimal cytotoxic concentration for any significant length of time. In some instances a CxT approach has been used to circumvent these problems. The ultimate extension of the CxT approach is to administer the drug by continuous infusion, an approach we have studied in a new Rhesus monkey model. In previous studies in our laboratory, pharmacokinetic modeling with cytarabine demonstrated the potential pharmacokinetic advantages of continuous intrathecal administration in maintaining a minimal cytotoxic concentration in the CSF for a prolonged period with a much lower total dose. In addition, the chemical arachnoiditis frequently associated with intrathecal therapy has been linked with the high peak CSF concentrations following bolus injection. This can be avoided when the drug is given by low-dose continuous infusion. The Rhesus monkey model was adapted to enable us to perform these studies. A new technique was developed in which a cannula is inserted into the lateral ventricle and then attached to a subcutaneously implanted catheter with a reservoir which is attached to a portable infusion pump containing the drug to be studied. In preliminary studies we have found that with continuous infusion of MTX, ventricular CSF MTX concentrations are maintained at 1 μmol/L for two- to three-fold longer than with the bolus dose, despite the fact that only one tenth of the total bolus dose was administered by infusion. Thus, these studies directly demonstrate the clear pharmacokinetic advantage for continuous intrathecal infusion. A clinical protocol evaluating this approach has recently been initiated, and pharmacokinetic studies in the first patient have confirmed the results of the animal studies. In addition, this new model promises to provide new insights into the mechanisms of drug distribution and disposition within the CSF which could also lead to more effective use of intrathecal agents.

Rescue of Intrathecal Methotrexate Overdose with Carboxypeptidase - G_2

MTX is the most commonly used intrathecal (IT) agent for the treatment and prevention of meningeal malignancy. The frequency of overdose is low (probably 6 to 12 cases per year), but the prognosis of patients administered an overdose is grave. The carboxypeptidase G class of enzymes rapidly hydrolyze methotrexate (MTX) into the inactive metabolites 4-deoxy-4-amino-N^{10}-methylpteroic acid and glutamate. We evaluated the use of carboxypeptidase-G_2 ($CPDG_2$) as a potential intrathecal (IT) rescue agent for massive IT MTX overdose. The cerebrospinal fluid (CSF) pharmacokinetics of MTX with and without $CPDG_2$ rescue was studied in adult rhesus monkeys (*Macaca mulatta*) using a nontoxic IT 5 mg dose (equivalent to 50 mg in humans). Without $CPDG_2$ rescue, peak CSF MTX concentration was 2904±340 μM. Within 5 minutes of administration of 30 U IT $CPDG_2$, CSF MTX concentrations decreased greater than 400-fold to 6.55±6.7 μM. Subsequently, groups of three monkeys received either 25 mg IT MTX (equivalent to 250 mg in humans) followed by 150 U IT $CPDG_2$ or 50 mg IT MTX (equivalent to 500 mg in humans) followed by 300 U IT $CPDG_2$. All animals survived without neurotox-

icity. These studies suggest that $CPDG_2$ may prove to be an important addition to currently recommended approaches for the management of IT MTX overdose.

C. Clinical Pharmacology of Maintenance Therapy in ALL

Traditional maintenance therapy for ALL has consisted primarily of orally administered 6-MP and MTX. Although these drugs have been in use for over three decades, the clinical pharmacology of orally administered maintenance therapy has only recently been studied in detail. We have been studying the clinical pharmacology of drugs used in maintenance therapy. These studies are detailed in the *Leukemia Project Report.*

D. Clinical Pharmacology of Antiretroviral Agents

As part of the Pediatric Branch AIDS research effort, the Pharmacology and Experimental Therapeutics Section is studying the clinical pharmacology of antiretroviral agents in children. The purpose of this project is to investigate the clinical pharmacology of antiretroviral drugs in children as they become available. Specifically we are studying 1) the pharmacokinetics of antiretroviral agents in order to determine the optimal route and schedule of administration and to establish correlations between pharmacokinetic parameters and both treatment response and toxicity; and 2) the central nervous system pharmacology of existing and proposed AIDS therapies in order to predict the potential clinical efficacy against AIDS dementia complex. In addition, preliminary efforts are underway to develop new cell and virus-free drug screening techniques to identify inhibitors of the viral enzyme, integrase.

1. Pharmacokinetics of AZT in Children

We previously characterized the pharmacokinetics of AZT in 37 children with symptomatic HIV infection treated in the Pediatric Branch. These children were being treated on one of two phase 1 protocols utilizing either an intermittent (every 6 hour) or continuous infusion schedule of AZT. With intravenous bolus dosing the elimination of AZT in children was rapid and biexponential with half-lives of 14 and 90 minutes and a total clearance of 680 ml/min/m^2. There was also considerable interpatient variation in the rate of drug elimination. Oral bioavailability of AZT was also determined to be 68%. A simulation of the dose and schedule of AZT (180 mg/m^2 every 6 hour) proposed for children revealed that, with intermittent dosing of AZT, plasma concentrations of the drug remain above the target level of 1 μM for less than half of the dosing interval, and that the steady state trough concentrations are less than 0.2 μM suggesting that this dose and schedule may be inadequate In contrast, plasma concentrations of AZT with continuous infusion were maintained above 1 μM even at the lowest dose level, demonstrating a clear pharmacokinetic advantage for this schedule over intermittent administration. Neutropenia was the dose-limiting toxicity with continuous infusion AZT, and the degree of neutropenia appeared to be related to the plasma concentration of AZT. Patients who dropped below an ANC of 500/mm^3 during the first six weeks of therapy had significantly higher plasma AZT concentrations (mean 3.6 μM) than those who remained above 500/mm^3 (mean 2.6 μM).We have suggested that 3.0 μM may be considered a toxic

level on the continuous infusion schedule, pending more extensive studies. The identification of this toxic level along with the significant interpatient variability noted indicates a potentially important role for therapeutic drug monitoring in AZT therapy.

Current pharmacokinetic studies of AZT are focused on patients being treated on an ongoing randomized trial comparing the 2 schedules studied in prior phase I trials (intermittent vs. continuous infusion). We hope to confirm pharmacokinetic-pharmacodynamic relationships noted in the phase I trial of continuous infusion AZT and develop a limited sampling for the intermittent study which would allow estimate of the average steady state concentration from 1 to 3 measurements (an approach that we demonstrated to be feasible for ddI - see below). We are also evaluating potential pharmacokinetic drug interactions on a trial of combination AZT/ddI. In addition, an attempt is be made to evaluate the pharmacokinetics of AZT and other antiretroviral agents in patients with excretory organ dysfunction to formulate recommendations for dose modifications in these patients, and a patient in renal failure who was on dialysis was recently studied in detail while on AZT and then after being switched to ddI. Renal failure had essentially no effect on the clearance of either of these drugs.

Preclinical studies of AZT

Previous reports have demonstrated the effect of probenicid on prolonging the elimination of AZT. However, small animal models have also suggested that probenicid may enhance the CNS penetration of AZT also. We are currently investigating this interesting interaction in our Rhesus model. If the interaction proves to be true, these studies could result in a new approach to the treatment of dementia in AZT sensitive patients.

Another approach to the treatment of HIV dementia in patients who are intolerant of AZT may be the intrathecal administration of the drug. This approach is being evaluated in the monkey model as a continuous intrathecal infusion, and members of the section are collaborating with investigators in the Neurology Institute to develop a protocol to study this approach clinically.

2. Pharmacokinetics of Dideoxyinosine in Children

As a part of a Phase I/II trial of ddI undertaken by the Pediatric Branch in symptomatic HIV infected pediatric patients we evaluated the pharmacokinetics of this new agent in 47 children treated at 5 dose levels of ddI (60, 120, 180, 360, and 540 mg/m^2/day). The drug was administered orally in three divided doses for a minimum of 24 weeks. The pharmacokinetics of ddI were determined following a one hour intravenous infusion and also after delivery of the same dose administered orally. Thirty-three children had plasma samples drawn following iv dosing of ddI. The peak concentration and the area under the plasma concentration time curve (AUC) increased proportionally with dose. Mean peak ddI concentrations ranged from 3.1 μM at the 20 mg/m^2 dose to 22 μM at 180 mg/m^2; the mean AUCs ranged from 3.4 μM•h to 30 μM•h. The half life of ddI following the intravenous dose was 0.8 hours and the total body clearance was 510 ml/m^2. Forty-five children were evaluated following an oral dose of ddI. Oral ddI was rapidly absorbed with peak

levels occurring at 0.5 hours in most patients. However, the plasma concentrations achieved with oral administration were considerably lower than with the equivalent intravenous dose. Overall, the fraction of the oral dose absorbed was 19% and in two patients (one who received 40 mg/m^2 dose and one 60 mg/m^2) ddI was not detected in plasma at any time following oral administration. Although, as with the intravenous dose, peak ddI concentrations and AUC's increased proportionally with the dose of oral ddI, there was more variability in these parameters within each dose level. There was a correlation between the AUC and response to p24 antigen. Patients who responded with declines of p24 antigen had a higher median AUC than non-responders. In addition, a significant correlation was noted between ddI plasma concentration (AUC) after oral administration and improvement in IQ score. The significance of the relationships between ddI plasma concentration (AUC), ddI dose and both p24 response and cognitive improvement underscores the importance of considering the pharmacokinetics and bioavailability of antiretroviral agents in assessing their activity. The data from this study indicate the need for developing convenient methods of monitoring plasma drug concentrations and that dose modulation should be determined not only by the development of toxicity or clinical response, but also by target plasma concentration.

A limited sampling model was developed for ddI for the data of the patients treated at the 120 mg/m^2 dose and validated in the patients treated at the 180 mg/m^2 dose level. Using stepwise forward regression analysis, plasma concentration at three time points (0.5, 1.5 and 3 hr) was shown to accurately estimate the AUC. We have subsequently monitored 66 patients who have continued on the ddI phase I trial at adjusted doses (either 90 or 120 mg/m^2). These plasma concentrations will be used to make pharmacokinetic/pharmacodynamic correlations and assess intrapatient changes in drug absorption.

3. Central Nervous System Pharmacology of Antiretroviral Agents

Using our Rhesus monkey model we have systematically studied the CSF penetration of the new antiretroviral agents and define those physicochemical properties that influence the degree of CNS penetration. Initially, the pyrimidine dideoxynucleosides AZT and ddC were studied in collaboration with the Clinical Pharmacology Branch. CSF penetration, as measured by the ratio of CSF to plasma drug concentration, was 21% for AZT and only 3% for dideoxycytidine. To determine the portion of the molecule that was responsible for this marked difference in penetration, we subsequently evaluated the penetration of dideoxythymidine, which had a CSF to plasma ratio of 30%, and azidodideoxycytidine which had a CSF to plasma ratio of 1%. These studies clearly indicate that the base (cytosine vs. thymine), rather than the 3'-substitution (azido group vs. none) on the sugar, determines the extent of CNS penetration. Of interest, the plasma protein binding and octanol/buffer partition coefficients of each of these compounds was also determined. None of the compounds was significantly protein bound. The azido group on the sugar resulted in a significant increase in the lipid solubility, but there was no apparent relationship between CSF/plasma ratios and lipid solubility. It appears, therefore, that a carrier-mediated process is primarily responsible for CNS entry of this class of drugs. As part of the phase I trial of continuous infusion AZT in children we measured simultaneous CSF and plasma steady state AZT concentrations in 21 children and found a CSF to plasma ratio of 24% - confirming

the predictive ability of the Rhesus monkey model in studying antiretroviral agents. This degree of penetration correlates with improvements in neurologic status of the patients treated on this trial.

We have also evaluated the CSF penetration of a series of dideoxypurines, ddA,ddI and ddG. The penetration of ddG, a less water-soluble drug pentrated somewhat better than ddI. We are currently studying a series of halogenated dideoxynucleosides to determine the degree to which these analogs, which were developed to optimize CNS penetration, enter the cerebrospinal fluid. Newer agents such as protease inhibitors and "Uniroyal Jr" which was highly active in the NCI drug screen will also be studied.

4. Preclinical Studies of GR109714 and GR103665X

2'-Deoxy-3'-thiocytidine (GR103665X) is a nucleoside analogue that has shown antiviral activity against all HIV-1 lines tested, including a number of AZT resistant HIV-1 isolates. This dideoxy analogue of cytidine contains a sulphur atom at the 3' position of the sugar ring and is a racemic mixture of GR109712X (+)-enatiomer, and GR109714X (-)-enantiomer. The latter compound will soon undergo further study in phase I clinical trials. We are evaluating the CNS penetration of both GR103665X and GR109714X in our nonhuman primate model in an attempt to predict the potential effectiveness of this agent against the CNS effects of HIV infection. Evaluation of both compounds will give us valuable information about the CNS penetration of the enantiomers. Furthermore, since significant interspecies differences in the metabolism and pharmacokinetics of other cytidine analogues such as ara-C and CPE-C have been well documented, we will evaluate the metabolism and clearance between species for this compound as well. Such correlations may allow selection of a more rational starting dose for phase I trials of this compound.

5. Studies of Absorption of Anti-retroviral Compounds in an Animal Model

A sustained release oral preparation of the currently available dideoxynucleosides would have the advantage of providing more prolonged exposure to drug over the dosing interval than current oral formulations. However, sustained-release formulations are only of use with drugs that can be absorbed along the entire length of small intestine. Drugs absorbed at a specific site via a carrier are absorbed poorly from a sustained-release preparation. Therefore, prior to developing a sustained-release formulation, we evaluated the mechanism of absorption of AZT and ddI in an ex vivo rat intestinal loop model. These studies reveal that both drugs are passively absorbed, equally in all segments of the small intestine and would, therefore, be amenable to formulation in a sustained-release form.

6. CSF Neurochemistry Studies in Children with HIV Encephalopathy

These studies are designed to explore the possible biochemical basis for the mental impairment observed in pediatric AIDS patients with HIV encephalopathy, and to identify objective markers of CNS involvement that could be used to monitor the response to therapy. Since the systems potentially involved in this impairment are unknown, the concentrations of three categories of neurotransmitters and their metabolites and related enzymes will be studied. These assays, which have been used to study adult dementia, include "classical" neurotransmitters (acetylcholine,

dopamine, noradrenaline, serotonin), neuropeptides (somatostatin, neuropeptide Y, CRF), and amino acids (GABA, aspartate, glutamate, glycine). Levels will be measured in the CSF of children with AIDS before and after treatment with AZT or ddI in order to determine if there is a neurochemical marker of dementia and a correlate for the previously described improvement in mental performance observed in AZT-treated children. Additionally, these values will be compared to those measured in the CSF of children participating in ongoing clinical trials for the treatment of acute lymphoblastic leukemia.

7. CNS Penetration of Antifungal Agents

The need for effective agents to treat central nervous system fungal disease has become especially evident with the advent of HIV disease. The incidence of CNS fungal infections is significant in the HIV infected population. Although amphotericin B is effective in treating fungal meningitis in other patient populations, it has not been as effective in patients with AIDS. We have used our nonhuman primate model to evaluate new antifungal compounds that have promising properties for treatment of CNS fungal infections. The CSF penetration of SCH 39304, (SCH) a new antifungal triazole compound, was characterized in our rhesus monkeys model with Ommaya reservoirs. The mean CSF:plasma area under the curve (AUC) ratio was 0.71; the maximum concentration of CSF was 1.34 mg/ml; the maximum concentration of plasma was 1.96 mg/ml . Thus, this study demonstrated that SCH 39304 effectively penetrates into the cerebrospinal fluid. Moreover, the concentrations in the cerebrospinal fluid met or exceeded the MIC's of SCH 39304 against most strains of cryptococcus neoformans and candida throughout a 48 hour period following a single dose. In addition to penetrating well into the central nervous system, the long plasma and CSF half lives (>40 hr) may permit alternate day or even once weekly dosing for maintenance or remission of CNS cryptococcus in AIDS. The above drug is a racemic mixture of two stereoisomers. We are currently evaluting one of the purified stereoisomers to see if the CSF penetration of this agent shows any stereospecificity.

8. Integrase Inhibitors

The section is in the preliminary phase of developing a cell- and virus-free assay of the crucial viral enzyme integrase, which is responsible for inserting the proviral DNA into the host genome. This step is crucial because it establishes the latent infection which makes HIV disease a life-long infection. Once an acceptable assay of integrase is developed it could be used to specifically screen compounds for their ability to block this important step in the viral life cycle.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06890-12 PB

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology and Treatment of Non-Hodgkin's Lymphoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ian Magrath	Head, Lymphoma Biology Section	PB,NCI
	Ligita Novikovs	Technician	PB,NCI
	Melissa Adde	Nurse Specialist (Research)	PB,NCI
	Wanda Harris	Secretary	PB,NCI
	Walter Goldschmidts	Biotechnology Fellow	PB,NCI
	Vinay Jain	Visiting Scientist	PB,NCI
	Kishor Bhatia	Visiting Associate	PB,NCI
	Aziza Shad	Clinical Associate	PB,NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Pediatric Branch

SECTION: Lymphoma Biology Section

INSTITUTE AND LOCATION: National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
11.0	5.0	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has clinical and basic components. We continue in our endeavors to understand the determinants of prognosis in the pediatric non-Hodgkin's lymphomas and to develop improved combination chemotherapy. At the present time our clinical studies are directed towards the utility of colony stimulating factors as a means of increasing dose intensity and ameliorating toxicity in small non-cleaved cell (SNCL) and large cell lymphomas. Our basic studies are directed towards the elucidation of the molecular biology and molecular epidemiology of the SNCL. Major areas of investigation include a) detailed characterization of the major goal of the non-random chromosomal translocations (particularly 8;14 translocations) associated with the SNCL, with a view to understanding the immediate causes of neoplastic behavior in these tumors and the determinants of geographic and clinical heterogeneity; b) the elucidation of the nature of the association of Epstein-Barr virus (EBV) with the SNCL; c) examination of selected biological and clinical aspects of lymphomas associated with HIV infection; and d) exploration of other molecular abnormalities of potential interest in the SNCL, in particular mutations in p53, examination of the 3' breakpoint region of c-myc with a view to determining the significance of the "pvt" region and exploration of the possible pathogenetic role of genes that prevent apoptosis stemming from these studies, we are also investigation the possibility that the molecular abnormalities can be used as a target for tumor-specific treatment approaches.

OBJECTIVES AND FIELDS OF RESEARCH

Our goals include 1) to improve the management and treatment results of children with non-Hodgkin's lymphomas and 2) to develop a more detailed understanding of the molecular pathogenesis of the SNCL, and, ultimately, to develop novel therapeutic strategies based on the molecular genetic abnormalities.

A. Clinical Studies

Protocol 89-C-41 is a short duration, high dose intensity protocol which incorporates alternating regimens of known efficacy. In addition to examining the efficacy of this general strategy, the protocol seeks to determine the role of colony stimulating factors in the treatment of SNCL and large cell lymphomas. There are two specific questions which are being addressed in a randomized study: 1) will GM-CSF ameliorate toxicity and permit an increased dose intensity and 2) if the latter occurs, will this translate into a survival advantage. An ancillary question being asked is the value of MRI in determining the presence and extent of bone marrow involvement.

The large series of patients with childhood lymphomas continues to provide a resource for the initial evaluation of potential prognostic factors.

B. Molecular Studies

Our ongoing areas of research fall into several overlapping categories:

1) *Molecular analysis of chromosomal breakpoint locations*

a)in different world regions

b)With respect to regions known to be relevant to the regulation of c-myc expression

2) *Exploration of the functional consequences of different breakpoint locations with respect to the regulation of myc transcription by immunoglobulin enhancer regions.*

3) *Examination of anti-sense transcription from various myc gene fragments designed to represent structural changes that actually occur in SNCL.*

4) *Direct examination of the possibility that EBV may collaborate with the translocations in causing deregulation of c-myc*

5) *Development of PCR techniques for the identification of chromosomal translocations and detection of minimal residual disease*

6) *Examination of the prevalence and functional significance of p53 mutations p53 in high grade NHL*

7) *Exploration of the use of antisense molecules as a means of altering the expression of relevant genes in SNCL, specifically, c-myc and EBV genes*

8) *Exploration of the possible role of genes which inhibit apoptosis in SNCL*

SIGNIFICANCE

Therapeutic Studies

Protocol 89-C-41, should result in an improved outcome with a shorter duration

of treatment (4 cycles for high risk patients, 3 cycles for low risk patients). We hope that the prognosis of patients with very extensive disease - e.g involving the bone marrow - a category with a particularly low expectancy of survival will be markedly improved.

Biological Studies

The SNCL, although relatively rare tumors, have provided a crucially important model which has influenced all branches of oncology. It is probably safe to say that the cause and nature of neoplastic growth is better understood in the SNCL than in any other tumor. Our establishment of a library of cell lines derived from SNCL has been crucial to our ability to study the biology of these tumors and has benefited not only ourselves, but many other investigators.

Our studies are comprehensive, in that they encompass molecular epidemiology, molecular pathogenesis, clinical correlates of molecular findings and therapeutic trials. The molecular categorization, in terms of the chromosomal breakpoint locations of these tumors, provides not only a new and considerably more precise epidemiological and diagnostic tool, but is also generating important leads to the understanding of the genesis of the chromosomal translocations, the mechanisms whereby the c-*myc* gene is deregulated, and insights into the possible role of EBV in pathogenesis. The latter has been considerably expanded by direct examination of the possibility that EBV genes may transactivate c-myc and thus collaborate with the chromosomal translocations in the deregulation of this gene. Further, this new knowledge could lead to completely novel, tumor-specific treatment approaches as exemplified by our work with anti-intron and anti-EBV antisense oligomers. While it is obviously too early to estimate their impact, such approaches, if successful, could confer a totally new perspective on cancer therapy. Because of the paucity of pathogenetic information in the vast majority of tumors it is probably only in the SNCL that such approaches can be seriously contemplated at present.

We have recently demonstrated the frequent involvement of p53 mutations in SNCL. In characterizing these mutations, we have been able to make novel observations with respect to the mechanisms whereby this gene - abnormalities of which constitute the most frequent genetic abnormality in cancer - contributes to neoplasia, not only in SNCL, but also in inherited cancer predispositions, such as the Li-Fraumeni syndrome.

Finally, we are beginning to formulate general principles regarding the pathogenetic lesions required for the formation of the SNCL lymphomas. In this respect, our studies of the functional significance of the chromosomal translocations, the influence of EBV, and the significance of p53 mutations have been supplemented by a new endeavor - examination of the potential role of genes that are relevant to cell survival - namely genes such as bcl-2, which appear to inhibit programmed cell death, i.e apoptosis.

PROGRESS REPORT AND FUTURE DIRECTIONS

Clinical Results

Protocol 89-C-41

Low risk patients.

Four patients with low risk SNCL have been entered onto protocol 89-C-41, and all remain free of disease. None of these patients remain at significant risk to relapse.

High risk patients

To date, we have entered 15 high risk patients onto protocol 89-C-41, and although only about half of them are out of the high risk period for relapse, we have not so far observed any relapses in these patients, in spite of the short duration of therapy. If results remain as good, it will not prove possible to examine the second objective of this protocol - to determine whether increased dose intensity produced by a colony stimulating factor will result in improved survival.

Evaluation of the effectiveness of GM-CSF in ameliorating toxicity and permitting increased dose intensity.

Initial examination of the effects of GM-CSF on toxicity and dose intensity shows no differences between the two arms of the study, although it remains possible that selected patients may benefit.

Analysis of CNS involvement in the SNCL

We have completed analyses of the associated disease patterns and relevance to prognosis of CNS involvement. Our results suggest that CNS disease nearly always accompanies extensive systemic disease, and is not necessarily a high risk factor per se.

Molecular and Biological Studies

1. Molecular Analysis of Chromosomal Breakpoint Locations

Molecular Epidemiology of the Small Non-Cleaved Cell Lymphomas.

Non-random chromosomal translocations, the most important being an 8;14 translocation, provide a critical element in the pathogenesis of the small non-cleaved lymphomas. We have previously shown differences between the endemic and sporadic forms of SNCL in that the former is nearly always associated with EBV, and has breakpoints on chromosome 8 some distance outside the c-myc gene. The sporadic variety is much less often associated with EBV (15%-20%) and nearly always has breakpoints close to or within c-myc. We have now shown that tumors in South America have a pattern which differs from both North American and Equatorial African tumors. The breakpoint is predominantly in the immediate 5' region of c-myc. There even appear to be differences between Argentinean and Brazilian tumors, the latter more closely resembling African tumors. Interestingly, the EBV association of South American tumors is also intermediate between African and US tumors, and is higher in Brazil (75%) than in Argentina (55%). It appears probable that there are at least three molecular types of Burkitt's lymphoma, and that in different geographical regions, these are represented to different extents. These observations are likely to have important pathogenetic, clinical and therapeutic implications and we plan to expand them and to extend them to other world regions.

Delineation of Breakpoints in Relationship to Recognized Regulatory Regions in c-myc

One of the consequences of breakpoints is the removal of upstream regulatory sequences from their normal juxtaposition with c-myc. This applies only to those breakpoints that are close to or within the gene. The remaining sequences must permit transcription from c-myc, and therefore require a potential promoter and possibly sequences required for synergism with the heterologous enhancer regions which have been juxtaposed by the translocation. We have completed an analysis of immediate 5' breakpoints, and find them to cluster in a very small (<130bp) region, strongly suggesting the deletion of an upstream regulatory element and retention of a downstream regulatory element necessary to drive transcription from P1 and P2. We have also commenced a more detailed localisation of breakpoint regions in the first

intron and anticipate that they will cluster between the MIF region and P3. We plan to further analysis exon breakpoints in this way.

Recognition of Germline status of Ig genes in SNCL

We have shown that a high proportion (approximately 45%) of SNCL, one IgH allele is unrearranged. This strongly suggests that the translocation occurs in very early B cells (i.e at the time of D-J joining0, such that, frequently, the cell has not initiated rearrangement of its immunoglobulin genes. The translocation presumably prevents rearrangement of the translocated allele, while if the other allele does not rearrange, the cell is likely to undergo apoptosis. Hence all SNCL have a functionally rearranged Ig gene.

Exploration of the Role of Immunoglobulin Enhancers in the Deregulation of c-myc

We have developed plasmid constructs containing a reporter gene (luciferase or beta-galactosidase) in which we can examine the influence of immunoglobulin enhancer regions on the transcription of myc from fragments which reproduce, as far as possible, some of the structural configurations of myc/Ig genes that result from the chromosomal translocations. We have been able to demonstrate, by transfection assays, that the intronic enhancer from the immunoglobulin region significantly increases transcription from c-myc fragments that lack the normal c-myc promoters (P1 and P2) but contain the c-myc intronic promoter region, P3. Moreover, this enhancer effect is cell line specific, suggesting that some cell lines may lack some or all of the protein factors necessary for enhancer function, or may contain negative regulatory proteins. This is surprising, since these cells are all of B lineage, but the roles of the 5' and 3' enhancers in the heavy chain locus are not defined, and it is possible that in cell lines that do not support the intronic enhancer, the 3' enhancer is operative. The in vitro system that we have developed should enable us to explore these issues in detail. These findings are highly relevant to the development of an understanding of the functional consequences of the chromosomal translocations that occur in the SNCL.

2. Pathogenetic Role of EBV in Small Non-Cleaved Lymphomas

Direct Examination of Influence of EBV on c-myc Expression

We have hypothesized that structural changes in the c-*myc* gene may sometimes be sufficient in themselves to effect deregulation and neoplasia, but in other cases an effect of EBV on one or more of the c-*myc* regulatory elements may be essential. To explore this issue, we have transfected the above constructs into cell lines that have been transfected with EBNA-1 expression vectors (demonsrated to be functional by use of a second EBV-luciferase reporter gene which is transactivated by EBNA-1) or control vectors. We have shown that EBNA-1 increases the expression of myc, and that this effect appears to be mediated via the juxtaposed immunoglobulin enhancer. We have also shown, in collaborarion with L. Frappier, that EBNA-1 does not directly bind to myc sequences. These observations provide a potential mechanistic explanation of the pathogenetic signicance of EBV in Burkitt's lymphoma. It is relevant to note that EBNA-1 appears to be invariably expressed in Burkitt's lymphoma, whereas other EBNAs are usually down regulated. We plan to explore the effect of other EBNAs on myc transcription, and to examine the importance of the particular immunoglobulin enhancer (heavy and light chain, 5' and 3') for the effect.

Examination of EBV Subtype in Burkitt's Lymphoma from Different World Regions and in HIV Positive Patients.

Two major subtypes of EBV have been identified which differ in their latently

expressed nuclear antigens. Early observations suggested that type 2 was more prevalent in normal Africans and African Burkitt's lymphomas. More recently, it was shown that type 2 EBV is actually quite prevalent in the nasopharynx in USA subjects. We have now shown that type 2 EBV is more often associated with HIV positive EBV associated lymphomas (40%) than with non-HIV associated lymphomas in the USA (10%) and Argentina (10%). The prevalence of type 2 EBV in HIV associated lymphomas is similar to that in African Burkitt's lymphoma (40%). Type 2 EBV is known to be quite frequently present in epithelial cells, and we surmise that it is less likely to be established in lymphoid cells (perhaps because of increased immunogenicity) in patients with intermittent (African) or continuous (HIV infected) immunosuppression.

3. Antisense Regulation of c-*myc* Translation in Small Non-cleaved Lymphomas

Antisense Inhibition of c-myc Expression

We have continued to pursue our objective of attempting to demonstrate that knowledge of the molecular abnormalities of a tumor may lead to novel treatment approaches by exploring the possibility of developing a means of specifically inhibiting the translocated c-*myc* gene in SNCL, while not affecting the c-*myc* gene of normal cells. We have developed inducible vectors containing antisense sequences derived from the intron of c-myc and transfected them into Burkitt's lymphoma cell lines. This should enable us to explore the target genes for myc transactivation as well as to investigate the feasiblity of antisense-based therapy in a nude mouse system.

Endogenous Production of Antisense Transcripts from c-myc

We have shown that antisense transcripts are readily detectable from myc gene fragments in transient and stable transfection assays, and that the ratio of sense to antisense transcription is increased in fragments which are structurally similar to a subset of rearranged c-myc genes in Burkitt's lymphoma - namely those with breakpoints in the first intron. This observation may be important to an understanding of the functional consequences of different types of chromosomal translocation and suggests that antisense transcripts may normally have a role in the regulation of the expression of c-myc. We plan to examine the precise start sites of antisense transcripts via primer extension assays, and further explore their functional significance.

4. p53 Mutations in SNCL

Presence of p53 Mutations in SNCL

We have demonstrated that mutations of the p53 gene are present in some 70% of our cell lines derived from small non-cleaved cell lymphomas (SNCL). Since the mutation prevalence appears to be higher in cell lines, derived predominantly from relapse tumors than in primary tumors (30%), these findings are consistent with the possibility that p53 mutations are important to tumor progression in SNCL in the USA.

Molecular Epidemiology of p53 Mutations

We have shown that in Argentinean SNCL, some 50% have p53 mutations, and that the pattern of mutations in SNCL appears to differ from that seen in other tumors (e.g colon cancer). These findings are consistent with the notion that p53 mutations differ with respect to the functional end-result, that there may be differences in the pattern, not only in different tumors but in different geographical regions, and that in some cases, p53 mutations may be necessary to the pathogenesis of the SNCL. We plan to extend these studies to other world regions. The implications for response to treatment clearly need to be examined.

Functional Significance of Heterozygosity of Mutations at Codon 248

We have further demonstrated that only one of the mutations we have observed in SNCLs - a mutation at codon 248 - is not associated with stabilization of the p53 protein in the heterozygous state. This mutation, however, results in high levels of p53 when homozygous. This observation suggests that heterozygous mutations at 248 are innocent, providing an explanation for the clustering of cancer predisposing, inherited p53 mutations (e.g in families with the Li-Fraumeni syndrome), around this region of p53. Mutations in the Li-Fraumeni "cluster region" also represent a potential predisposing genetic factor to SNCL - a future research project.

Demonstration of p53 mutations in Anaplastic Large Cell Lymphoma

We have also found a p53 mutation in a relapsed Ki-1 positive large cell lymphoma. We are presently examining the primary tumor to determine if the mutation was associated with relapse, and plan to study a series of large cell lymphomas for p53 mutations.

5. Characterization of the Mouse bcl-3 Gene

We have cloned the mouse bcl-3 gene, the human equivalent of which is involved in a translocation observed in small cell lymphoma/leukemias, and demonstrated that its pattern of expression is remarkably similar to the bcl-2 gene. This raises the possibility that bcl-3, like bcl-2, is involved in apoptotic pathways. The prevention of apoptosis appears to be a potentially important step in the pathogenesis of many neoplasms, perhaps particularly the hemopoietic neoplasms, and further exploration of genes involved in apoptosis is therefore, clearly warranted.

6. Collaboration with Cancer Centers in Less Developed Countries

We have developed collaborations in less developed countries in order to further assist local scientists and clinicians in the characterization and treatment of lymphoid neoplasms occurring in these geographic regions. We are interested in exploring the influence of different environmental circumstances on the frequency of various subtypes of leukemias and lymphomas, and have a particular interest in characterizing the SNCL occurring in these regions at a molecular level (see below). We have provided assistance in the development of therapeutic protocols in India and have provided advice and in some cases reagents for the phenotypic characterization of the lymphoid neoplasms in both India and Egypt. Data regarding socioeconomic status, occupation and rural/-urban residence is being routinely collected. A particularly important aspect of our efforts has been the provision of a detailed data management system and assistance to the centers with respect to the use of electronic storage of clinical protocol results.

In Madras, the disease free survival of patients with ALL appears to have doubled in the study period. In addition, in this center preliminary results of immunophenotyping strongly suggest a marked increase in the proportion of T cell ALL, with a corresponding reduction in common ALL. New projects have recently been approved in Egypt, Bombay and Bangalore.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06891-03 PB

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Solid Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Marc E. Horowitz	Senior Investigator	PB, NCI
Others:			
	Linda Weaver-McClure	Nurse Specialist (Res)	PB, NCI

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, NCI (E. Glatstein); Surgery Branch, NCI (S. Rosenberg); Lab Pathology, NCI (M. Tsokos); Cardiology, NHLBI (R. Bonow); Critical Care Medicine, CC (F. Ognibene)

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Research into new therapeutic strategies for the treatment of pediatric solid tumors is focused on bone and soft-tissue sarcomas including Ewing's sarcoma, peripheral neuroepithelioma, rhabdomyosarcoma and osteosarcoma as well as neuroblastoma and malignant brain tumors. These common pediatric tumors remain diagnostic and therapeutic challenges for which new approaches are needed.

The sarcomas serve as an excellent "model system" for the exploration of strategies and hypotheses that have broad applicability to both pediatric and adult solid tumor oncology. The overall goal of these protocols is to learn how to use drugs that have been determined to be active in the pediatric sarcomas with sufficient intensity to maximize their therapeutic potential.

Previous Pediatric Branch protocols have demonstrated a very high response rate for intensive vincristine, adriamycin and cyclophosphamide in newly diagnosed sarcoma patients (83-C-73) and a high level of activity for ifosfamide, mesna and etoposide in those with recurrent tumors (85-C-154). The current front-line sarcoma protocol (86-C-169) is studying the integration of the ifosfamide, mesna, etoposide combination with intensive vincristine, adriamycin, cyclophosphamide, and local irradiation. In an effort to circumvent the major toxicity associated with this protocol, myelosuppression, we are studying the hematopoietic growth factor rh-GM-CSF in a randomized trial to determine whether its use will decrease the myelosuppression, related delays and toxicity (88-C-165). We are also studying the iron chelator ICRF-187 in a randomized trial (89-C-07) in patients on the sarcoma protocol to learn whether it will protect the heart from adriamycin induced myocardial damage. These studies of ICRF-187 and rh-GM-CSF are unique in that they are the only ongoing front-line trials of these promising new approaches in pediatric solid tumor patients.

CLINICAL STUDIES

Protocol 83-C-73 - Treatment of Patients With Ewing's Sarcoma With Central Axis Primaries and/or Metastatic Disease, Rhabdomyosarcoma, and Other High Risk Soft Tissue Sarcomas

In 1983 Pediatric Branch study 83-C-73 was initiated to test the response to intensive VAdrC and local irradiation with consolidation by total body irradiation (TBI) and autologous bone marrow reconstitution. Seventy-five patients were entered and treated at the NCI over a three year period. The diagnoses were: Ewing's (n=32), PN (n=14), rhabdomyosarcoma (n=24), and undifferentiated sarcoma (n=5). Thirty-six patients had metastatic disease at diagnosis and the majority central axis primary lesions. Over 90% of the patients responded completely to irradiation and chemotherapy. Despite the excellent initial responses the survival and event-free survival for the entire group at approximately four years is 49% and 29% respectively. A major difference was seen for those with or without metastatic disease at presentation. Event free survival at approximately three years is 25% versus 49% respectively. Event free survivals for those with Ewing's, PN and rhabdomyosarcoma are not significantly different. The method used to obtain local control was, in 80%, a surgical biopsy and local irradiation. The actuarial local control rate at approximately three years was 70%. In 10 patients local and distant failure was noted simultaneously. Three failed with local disease only. Of 13 patients with local failure, three had metastatic disease at diagnosis and nine had tumors of the trunk for which complete resection was not an option.

Protocol 86-C-169 - A Pilot Study for the Treatment of Patients With Metastatic and High Risk Sarcomas and Primitive Neuroectodermal Tumors

This protocol is designed to define the initial response rate, overall effectiveness and toxicities of a combination of intensive vincristine, adriamycin and cyclophosphamide with the new combination ifosfamide and etoposide for patients with sarcomas. Eligible patients are those less than 25 years of age with Ewing's sarcoma, peripheral neuroepithelioma and primitive sarcoma of bone, metastatic unresectable rhabdomyosarcoma or spindle cell sarcoma. Treatment commences after a surgical biopsy. A complete surgical resection is not attempted unless this can be easily accomplished without mutilating surgery and a major delay in the initiation of chemotherapy. Induction chemotherapy is delivered over twelve weeks prior to the initiation of radiotherapy. This "neo-adjuvant" design is supported by the results of study 83-C-73. Radiotherapy is delivered after week 12 chemotherapy. The primary site is treated to a field encompassing the original tumor volume with approximately 45 Gy. An additional 15 Gy is delivered to a coned down field.

To date there have been 65 protocol entries with the following diagnoses: Ewing's sarcoma (n=17), PN (n=15), primitive sarcoma of bone (n=6), rhabdomyosarcoma (n=12), other soft tissue sarcomas (n=6), other (n=5), and 4 diagnoses pending. Forty-four patients had central axis lesions and 34 metastatic disease at diagnosis. The numbers are too small and the duration of follow-up too short to judge the efficacy of this treatment. Response to the four pre-irradiation induction chemotherapy cycles (VAdrC-IE-VAdrC-IE) have, with the exception of two patients, been excellent (> 50% tumor reduction). There have been 27 protocol failures with progressive tumor in 24 and 3 deaths from toxicity (sepsis 1, cardiomyopathy 1, bleeding 1). The toxicity of this protocol has been significant. 93% of treatments have been associated with grade IV neutropenia (AGC nadir < 500) and in 41%, infection. Although the majority of infections have been fever, without a source, the incidence of sepsis is 3% with one toxic death from septic shock. The myelosuppression has resulted in delays in treatment.

Instead of the scheduled treatments every 21 days the average interval between treatments is 25 days. During or after radiation therapy the average interval between treatments is 28 days. Cardiac toxicity has also been significant. The patients are prospectively evaluated by radionuclide angiography (MUGA). There have been two episodes of clinically apparent cardiomyopathy; one resulting in death. Many have a drop in MUGA scan ejection fraction to the lower levels of normal as they approach the cumulative 410 mg/m2 called for in the protocol. In some patients adriamycin was discontinued early because of the ejection fraction changes.

Although it is premature to judge the efficacy of this treatment as a general statement it is unlikely that significant gains will be realized by the introduction of new drugs if they result in a degree of toxicity that precludes their optimal utilization. We are therefore developing ways to decrease myelosuppression and cardiac toxicity in order to allow maximal benefit from VAdrC-IE by increasing dose intensity over time.

Protocol 88-C-165 - A Randomized Placebo-Controlled Trial of Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor in Pediatric Patients Following Intensive Combination Chemotherapy

This protocol was initiated as a randomized double blind study of rh-GM-CSF in patients on the sarcoma protocol to learn whether it will significantly reduce myelotoxicity and resultant delays in therapy. Patients received rh-GM-CSF at 10 uG/kg subcutaneously daily beginning 24 hours after completion of the chemotherapy regimen and continuing for 10 days. Seven patients have been entered in the study. The results were "unblinded" when it became clear that the effects of the agent precluded a true double blind comparison. From the 6 patients receiving the GM-CSF we have learned that it will not obviate neutropenia. In 20 cycles analyzed, the GM-CSF was discontinued after ten days with an absolute neutrophil count still below 500 in every case. From these initial patients the protocol has been amended in order that the study be randomized but not blinded. The GM-CSF dose was increased to 15 uG/kg daily through day 19 from the initiation of the chemotherapy cycle. It will be continued until the absolute neutrophil count remains above 500 for 48 hours. Studies elsewhere have demonstrated that GM-CSF may decrease the duration of neutropenia if not the nadir. More recently the dose has been decreased to 5 μg/kg based on a recommendation by CTEP. Twenty-three patients have been randomized.

Protocol 89-C-07 - A Phase III Study of ICRF-187 (Bisbiodoxopiperazine, ADR-529), an Adriamycin Cardioprotector, in Pediatric Sarcoma Patients

Patients on the sarcoma protocol are randomized to receive ICRF-187 with adriamycin or adriamycin alone to learn whether this iron chelating agent will decrease the significant incidence of clinical and subclinical adriamycin associated cardiomyopathy. The patient's cardiac function is monitored closely with radionuclide angiography which is the endpoint for the study. Twenty-eight patients have been entered on this study.

Protocol 87-C-68 - A Randomized Trial of Pre-Surgical Chemotherapy Vs. Immediate Surgery and Adjuvant Chemotherapy in the Treatment of Non-Metastatic Osteosarcoma - A Pediatric Oncology Group Phase III Study

The Pediatric and Surgery Branches of the NCI have a long history of studying osteosarcoma. Since 1981 studies have been carried out in collaboration with the Pediatric Oncology Group as the "Multi-Institution Osteosarcoma Study (MIOS)". The Pediatric Branch participation in this effort was essential for the completion of the study published in 1986 by Link et. al. in the New England Journal which demonstrated the value of adjuvant chemotherapy in osteosarcoma. Fully 50% of the randomized patients were treated at the NCI. The current study

is testing the relative merits of immediate surgery versus neo-adjuvant chemotherapy. As the majority of osteosarcoma patients have resectable tumor at diagnosis, important questions are adjuvant in nature and must be addressed with phase III studies. The numbers of patients required for such studies necessitate multi-institution collaborations. Investigators from the NCI have been intimately involved with the design, conduct and analysis of the MIOS studies.

Protocol 90-C-210 - A Phase II Trial of Recombinant Human Interleukin-2 (IL-2) Plus Tumor-Infiltrating Lymphocytes (TIL) with Low-Dose, Recombinant Human Interferon-Gamma (IFN-γ) for the Treatment of Advanced Neuroblastoma in Children

The Pediatric and Surgery Branches of the NCI are collaborating to test the efficacy of IFN-γ, IL-2, and TIL in children with recurrent or progressive neuroblastoma. Eligible patients are treated with IFN-γ prior to surgery for TIL harvest. In the interim postoperatively and prior to the time that sufficient TIL are grown, approximately 6 weeks, patients receive a single dose of carboplatin in order to prevent rapid, progressive disease. Once sufficient TIL are grown, patients are treated with IFN-γ followed by TIL and IL-2 administered in the intensive care unit. Two patients have been treated. The first patient's tumor progressed whereas the second patient has responded.

Protocol 90-C-211 - A Phase II Study of High-Dose Cyclophosphamide with GM-CSF in Malignant Brain Tumors in Children

There is increasing evidence to support a role for chemotherapy in the treatment of brain tumors. Cyclophosphamide is the most active single agent against pediatric brain tumors. The thrust of this study is to develop a regimen of high-dose cyclophosphamide and GM-CSF that will be used in front-line studies for the treatment of children with high-risk brain tumors. Patients with recurrent malignant brain tumors after radiation therapy for at most one prior chemotherapy regimen or newly diagnosed patients with high-risk brain tumors such as brain stem glioma or ependymoma are treated with cyclophosphamide at 4.5 g/m^2 administered every 2 to 3 weeks. In addition, patients receive GM-CSF at 5 mg/kg daily from day 3 until the absolute granulocyte count is greater than 1500. To date 15 patients have been treated. Responses have been seen in medulloblastoma (PNET) and ependymoma. Over 70% of the chemotherapy courses have been complicated with infection. The duration that the absolute granulocyte count is less than 500 is 8 ± 2 days. This study is ongoing to estimate the response rate in the major categories of pediatric brain tumors.

Publications:

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Abrahamsen TG, Horowitz ME, Lie SO. Cytokines. In: Voute PA, Barrett A, Bloom HJG, Lemerle J, Neidhardt MK, eds. Cancer in children - clinical management - third edition. New York: Springer-Verlag, in press.

Feuerstein IM, Jicha DL, Pass HI, Chow CK, Black WC, Chang R, Ling A, Hill SC, Travis WD, Horowitz ME, Steinberg SM, Frank JA, Doppman JL. Magnetic resonance imaging of pulmonary metastases: A prospective study with surgical correlation. Radiology, in press.

Helman LJ, Horowitz ME. Rhabdomyosarcoma. In: Tonini G-P, Thiele CJ, Sansone R, eds. Molecular genetics of solid pediatric tumors. Basic concepts and recent advances. London: Harwood Academic Publishers, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM06892-02 PB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Helman	Senior Investigator	PB, NCI
Others:	G. Crouch	Special Volunteer	PB, NCI
	C. Minniti	Visiting Associate	PB, NCI
	T. Kalebic	Fogarty Scientist	PB, NCI
	C. Kappel	Biologist	PB, NCI

COOPERATING UNITS *(if any)* Standord Univ. Medical Ctr., Dept. of Pediatrics (R. Rosenfeld). Natl. Cancer Inst., LMB, DCDB (I. Paston), St. Jude's Children's Res. Hospital (P. Houghton), Washington Univ. School of Medicine (W. Daughaday), Lab. of Path. (M. Tsokos), Natl. Cancer Inst., CPB (O. Sarter, M. Cooper, C. Myers)

LAB/BRANCH

Pediatric Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER.
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the molecular mechanisms involved in the pathogenesis of rhabdomyosarcoma (RMS). This neoplasm probably arises due to a developmental disturbance during muscle formation. Since much has been learned recently about the molecular mechanisms underlying the commitment to muscle lineage and the mechanisms involved in normal muscle development, the study of rhabdomyosarcoma offers a unique opportunity to evaluate the relationship between differentiation arrest and the development of this pediatric embryonal tumor. For example, the activation of genes such as MyoD and myogenin have been shown to be required for the commitment of stem cells to myogenic differentiation. Additionally, several growth factors including TGF-beta and insulin-like growth factors have been implicated in the normal growth and maturation of muscle tissue. Finally, alterations of the tumor suppressor gene p53 have recently been demonstrated to play a role in a hereditary cancer syndrome (Li - Fraumini) of which rhabdomyosarcoma is a component. Our current focus has therefore been aimed at identifying the roles that such growth factors and the tumor suppressor gene p53 may play in the development of the striated muscle tumor, rhabdomyosarcoma. In particular we have focused on the role of insulin-like growth factor II in the development of this tumor since previous work has shown that this growth factor is expressed at abnormally high levels in these tumors compared to normal muscle.

We have identified IGF II as an autocrine growth factor in this set of tumors and have initiated a Phase II study aimed at disrupting this autocrine loop. We have also characterised p53 mutations in cell lines and tumor samples and found that the majority of tumors have mutations in thos tumor suppressor gene. We are currently attempting to define the precise role that each of these alterations play in the development of childhood RMS. We have also been evaluating various agents in an attempt to in vitro differentiate tumor cell lines. These studies are aimed at identifying particular lesions within the normal differentiation pathway that may occur in the development of rhabdomyosarcoma. One such agent was identified that was able to both differentiate tumor cell lines as well as reverse the transformed phenotype. This work led to the initiation of a second Phase II study. We are also using cDNA cloning approaches to identify potential molecular mechanisms which may distinguish between the unregulated continual growth of the embryonal tumor, rhabdomyosarcoma, compared to the normal regulated growth of normal embryonal human muscle.

Accomplishments and Results:

1. The role of insulin-like growth factor II (IGF-II) in rhabdomyosarcoma: We have identified IGF-II as an autocrine growth factor in rhabdomyosarcomas. This growth factor is over-expressed in 30 of 30 tumors evaluated to date. In situ hybridization analysis on tumor sections has demonstraited that IGF II is expressed directly in the tumor cells and that both alveolar and embryonal histologies express this growth factor at high levels. In addition, several cell lines have been demonstrated to secrete authentic IGF-II into culture media, and these tumors have been demonstrated to contain typical type I IGF receptors which are known to mediate the mitogenic response of IGF-II. We subsequently were able to show that a monoclonal blocking antibody to the type-I receptor could substantially inhibit the in vitro growth of rhabdomyosarcoma cell lines both of the embryonal and the alveolar subtype. Interestingly, we were also able to demonstrate that the IGF-II secreted by the tumors was also capable of stimulating motility in these cell lines suggesting that the same growth factor may play a role not only in the unregulated growth of this tumor but also contribute to the high metastatic potential that these tumors have since motility is a major step in the metastatic pathway. Surprisingly, the blocking monoclonal antibody to the type-I receptor was not able to inhibit IGF-II induced motility in these tumors. Subsequent studies demonstrated that the IGF - II stimulated motility is signaled through the IGF II/Mannose-6-Phosphate receptor. This is the first demonstration of an autocrine peptide factor synthesized by tumor cells having defferent effects depending on the receptor activated.

Since the polysulfated compound suramin has been shown to bind several growth factors including FGF and PDGF, we have evaluated the ability of suramin to interfere with the IGF-II autocrine growth loop. We have demonstrated that suramin inhibits the ability of IGFS to bind to the type-I receptor, and that this compound causes growth inhibition in numerous rhabdomyosarcoma cell lines. The ability of suramin to displace IGF's from the type I receptor exactly parallels its ability to inhibit cell growth. Finally, exogenously administered IGF-II to suramin treated cells can reverse this growth inhibition. These data suggest that suramin inhibits the growth of RMS in vitro by disrupting the IGF-II autocrine growth loop. This has led to the submission of a Phase II study to test the activity of suramin in relapsed RMS. The protocol will be the first study of suramin in a pediatric population.

In collaboration with the Laboratory of Molecular Biology, DCBD, we have created an IGF-I-PE40 oncotoxin. Since IGF-II appears to be an autocrine growth factor that is mediated through the IGF-I receptor, and other embryonal tumors such as neuroblastoma and Wilm's Tumor have also been shown to have such an autocrine growth loop, we reasoned that specifically targeting pseudomonas exotoxin

to this receptor may be of interest. We have demonstrated that this genetically engineered oncotoxin binds specifically to the type-I IGF receptor and is capable of killing tumor cells bearing such receptors on their cell surface, including three individual rhabdomyosarcoma cell lines. We are currently working to improve the binding of this IGF-I-PE40 oncotoxin molecule by structurally modifying the protein. In addition, we have recently fused the PE40 toxin molecule to the monoclonal antibody that is capable of binding to the type-I receptor.

Finally, we have just begun studies of the anti-type I IGF receptor, αIR-3, as an anti-tumor agent in nude mouse xenogrsft models.

2. In Vitro Growth and Differentiation of Rhabdomyosarcoma Cell Lines: We initiated studies on the effects of retinoic acid (RA) since this compound has been reported to be a limb morphogen in the developing chick limb-bud. In vitro treatment of rhabdomyosarcoma cell lines with all trans retinoic acid resulted in a greater than 70% inhibition of cell growth using nanomolar concentrations of RA. Interestingly, treatment of the same cell lines with 13-cis retinoic acid resulted in only a 30% decrease in cell growth. This growth inhibition was not accompanied by any morphological or biochemical evidence of differentiation. It therefore appears that these cells are sensitive to retinoic acid in a stereo-specific way and that growth inhibition is not accompanied by evidence of differentiation. Once again these results have been similar in both alveolar and embryonal rhabdomyosarcoma cell lines.

We have subsequently treated cells with low-dose ara-c, since this has been shown to in vitro differentiate hematopoietic cells. Treatment of rhabdomyosarcoma cell lines for four days with 0.5 uM ara-c resulted in 80-90% growth inhibition. Removal of ara-c from the culture medium resulted in the recovery of normal growth characteristics in an embryonal RMS cell line. However these cells were no longer tumorigenic in nude mice compared to untreated control cells which were tumorigenic in 100% of animals. Further treatment of these once treated cells to a second 4-day exposure of 0.5μM ara-c led to biochemical and morphological evidence of differentiation. These studies have led to the initiation of a Phase II study of low-dose (100mg/m^2 SQ) ara-c in relapsed RMS patients. The study was just opened and 1 patient has been entered.

We have just completed the construction of cDNA libraries from normal human embryonal muscle as well as from a human embryonal rhabdomyosarcoma tumor. Screening studies have identified a cDNA clone that is expressed specifically in RMS tumors but not in normal fetal or adult muscle. This cDNA is currently being sequenced and analysis to date fails to show any significance homology to sequences in the data base.

3. Analysis of p53 Mutations in Rhabdomyosarcomas: Mutations of the p53 tumor suppressor gene have been identified in the Li-

Fraumeni syndrome, of which RMS is a component. We have therefore been screening cell lines and tumor specimens to define the frequency and diversity of p53 mutations in these tumors. Four of five cell lines studied were found to have homozygous mutations that predict significant protein alterations in highly conserved regions of the p53 protein. Two of three tumor samples evaluated to date also showed homozygous alterations. One tumor had deleted both alleles by Southern analysis, and a second tumor had 2 point mutations leading to an alteration of the predicted protein structure. These preliminary data suggest a high frequency of p53 mutations in RMS and we are currently evaluating a larger series of tumor specimens to get a more accurate reflection of frequency of p53 mutations.

4. Involvement of human fetal glial cells in the activation of HIV in chronically infected monocytic cells: We previously demonstrated that LPS-stimulated astrocytes from newborn rats may activate the expression of latent HIV in chronically infected cells. We therefore extended our study to the human system. By using glial cell lines SVG and POJ derived from human fetal brain, we demonstrated the release of soluble factors which have the capacity to stimulate the activation of latent HIV in chronically infected monocytic U1 cells. Conditioned media from fetal glial cells of human origin cause an increase of total HIV proteins and an increase in reverse transcriptase activity. The capacity of conditioned media from fetal glial cells to induce the expression of HIV reduced by 45% in the presence of antibodies against human TNFα, suggesting that one of the HIV-activating factors released by these cells was TNFα. These results suggest that glial cells may induce the activation of HIV expression in chronically infected monocytes and in brain-infiltrating giant monocytic cells during human fetal development, thereby contributing to the progression of encephalopathy in children with neonatally acquired HIV infection.

5. GSH in combination with AZT suppresses the activation of HIV more efficiently than either of these agents alone: We previously demonstrated that in chronically infected U1 cells, reducing agents glutathione (GSH), glutathione ester (GHS-E) and N-acetyl-cysteine (NAC) suppressed the activation of latent HIV, mediated by several viral inducers (PMA, TNFα, IL-6). Due to its suppressive effect on HIV activation, GSH and NAC may represent potential candidates for adjuvant antiretroviral therapy. We demonstrated that AZT in combination with glutathione may completely suppress *in vitro* activation of HIV in chronically infected cells stimulated with viral inducers PMA and TNFα. In contrast, the same doses of GSH (5mM) or AZT (5mM) alone suppressed only partially the activation of HIV. Further studies are ongoing in order to determine at which molecular levels these compounds may act to prevent the activation of latent HIV in this cellular system.

Publications:

Prior, TI, Helman, LJ, Fitzgerald, DJ, and Pastan, I. Cytoxic Activity of a Recombinant Fusion Protein between Insulin-like Growth Factor I and Pseudomonas Extoxin. Cancer Res 1991;51:174-80.

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Kalebic, T, Helman, LJ, and Pizzo, P. Human glial cells from fetal brain activiate the expression of HIV in chronically infected monocytes. American Association for Cancer Research (Houston, Texas) 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 00650-36 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*: Service Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	E. Glatstein	Branch Chief	ROB, NCI
Others:	T. Goffman	Head, Clin. Ther. Sec.	ROB, NCI
	T. DeLaney	Senior Investigator	ROB, NCI
	L. Pierce	Senior Investigator	ROB, NCI
	B. Kelly	On-Site Coordinator	ROB, NCI
	T. Cushing	Chief Technologist	ROB, NCI

COOPERATING UNITS *(if any)*: Cancer Nursing Service, CC

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10	4	6

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The purpose of this project is to provide expert radiotherapy, consultation, and treatment for patients of the Clinical Center, including patients admitted to services other than the ROB. Support is given to the Medicine Branch, Surgery Branch, Pediatric Branch, NCI/Navy Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and others.

Project Description

Professional Personnel Engaged on the Project:

J. Smith	Clinical Nurse	CNS, CC
R. Smith	Cancer Nursing Specialist	CNS, CC
L. Dachowski	Clinical Nurse	CNS, CC
E. Fuetsch	Clinical Nurse	CNS, CC

Methods Employed

Formal and informal consultation with referring physicians and application of radiotherapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice, as well as modified programs when necessitated by concomitant adjuvant therapies.

Major Findings

Just under 700 patients were seen in formal consultation this year. In addition, between 400 and 500 telephone conversations provided ad hoc advice on treatment for a variety of problems and general information, including nursing management and follow-up for radiation therapy related problems. Approximately three visits per month from nursing staff to observe delivery of radiation therapy and simulation process. Approximately 450 patients will be treated this fiscal year with most of these being protocol patients in the Radiation Oncology Branch, or on collaborative studies.

Significance to Biomedical Research and the Program of the Institute

This project represents the ROB's direct contribution to clinical research and patient care. It also represents ROB's efforts to assist physicians and patients with problems which generally defy simple medical solutions.

Proposed Course

To continue.

Publications

1. Raubitschek A, Goffman T, Glatstein E. A staging of lymphomas: practical thoughts on impractical practices, J National Cancer Institute Monographs 1990;10:13-17.

2. Goffman T, Glatstein E. The caucus race: regionalism in the treatment of Hodgkin's disease, Int J Rad Oncol Biol and Phys 1990;19:805-806.

3. Goffman T, Glatstein E. To lap or not to lap. [Letter to the Editor]. J Clin Oncol 1990;8:941.

4. Raubitschek A, Glatstein E. Radiation pneumonitis. In: Shelhamer J, Pizzo PA, Parillo JE, Masur H, eds. Respiratory disease in the immunosuppressed host. Philadelphia: JB Lippincott, 1990; 504-511.

5. Goffman T, Raubitschek A, Mitchell JB, Glatstein E. The emerging biology of modern radiation oncology, Cancer Research 1990;50: 7735-7744.

6. Raubitschek A, Glatstein E. Hodgkin's disease: Radiation therapy. In: Rakel RE, ed. Conn's current therapy 1991. Philadelphia: WB Saunders, 1991; 350-352.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06310-12 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surgery vs. Radiation Therapy in Treatment of Primary Breast Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L. Pierce Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*

Medicine Branch, NCI; Rehabilitation Medicine, CC; Cancer Nursing Service, CC; Biostatistics and Data Management Section, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6	3	3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to determine whether a breast-conserving treatment program of limited surgery and definitive radiation offers equivalent local control and survival to mastectomy in patients with early stage breast cancer. After work-up confirms localized disease, patients are randomly assigned to either primary surgery or primary irradiation. Patients treated with mastectomy are offered breast reconstruction. All patients undergo complete axillary node removal; those patients with pathologically positive lymph nodes, and those with negative lymph nodes who are estrogen receptor negative receive chemotherapy.

Project Description

Professional Personnel Engaged on the Project:

D. Danforth	Senior Investigator	SB, NCI
K. Cowan	Head, Med. Brst. Cancer Sect.	MB, NCI
W. Schain	Clinic Care Consultant	Rehab. Med., CC
N. Gerber	Chief, Rehab. Medicine	Rehab. Med., CC
T. d'Angelo	Cancer Nursing	CNS, CC
M. Merino	Surgical Pathologist	LP, DCBD, NCI
S. Steinberg	Head, Bio & Data. Mgmt. Section	BDMS, NCI

Objectives: If survival and recurrence data obtained with treatment that preserves a cosmetically acceptable breast are comparable to those obtained with radical surgical procedures, such treatment will probably be more acceptable to most women with localized breast cancer. Availability of an effective alternative to mastectomy may encourage women to seek medical attention with earlier, hence more curable, cancers. The psychological, sexual, and sociological impact of mastectomy vs. lumpectomy and radiation will be noted. Ability to combine aggressive chemotherapy with either local treatment in node positive patients and node negative, ER negative will also be assessed.

Methods Employed

Patients with stage T1-T2, N0-N1, M0 primary untreated breast cancer are candidates for the study. They will be randomized to receive either lumpectomy, axillary dissection, and radiation therapy or total mastectomy with axillary node dissection. Patients receiving mastectomy will be offered breast reconstruction. Patients with pathologically positive lymph nodes, and ER negative patients with negative lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 11 years. It is now open for follow-up only. Two hundred and fifty-six patients have been entered, of whom 128 have randomized to mastectomy, and 128 to radiation. With 10 year actuarial results, no differences have been as yet between the surgery arm and radiation arm in terms of overall survival, 80% vs. 83% or disease-free survival, 67% vs. 65%, respectively. There have been 18 local/regional recurrences in the radiation arm. (Fourteen/seventeen in breast-only failures were salvaged by mastectomy. Ten local/regional recurrences have occurred on the mastectomy arm. Locoregional control is 89% in the

mastectomy arm and 80% in the radiation arm (p= .21), excluding those patients ultimately salvaged by mastectomy.

Significance to Biomedical Research and the Program of the Institute

The study is intended to determine whether breast conserving treatment (lumpectomy and radiation therapy) is equivalent to radical surgery as treatment for early stage breast cancer. If this is the case, this treatment option should be much more acceptable to the majority of women. It is conceivable that the availability of such non-mutilizing treatment would encourage women to seek medical attention sooner, and therefore present with more curable disease.

Proposed Course

The study is open for follow-up only. No new patients are being accrued.

Publications

Straus K, Lichter A, Lippman M, Danforth D, Swain S, Cowan K, de Moss E, MacDonald H, Steinberg S, d'Angelo T, Merino M, Bader J, Findlay P, Rosenberg S, Glatstein E. Results of the NCI early breast cancer trial. Accepted for the NCI Monographs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06320-12 RO

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Response of Mammalian Cells to Chemotherapy Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Cook	Staff Fellow	ROB, NCI
	J. Gamson	Biologist	ROB, NCI
	S. Hahn	Clinical Associate	ROB, NCI
	D. Kaufman	Senior Investigator	ROB, NCI
	J. Liebmann	Clinical Associate	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Experimental Phototherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6	4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several chemotherapy agents with proven utility such as anthracyclines, bleomycins, alkylators, neocarcinostatin, nobel metal derivatives, VP-16, and radiosensitizers are being studied. The detoxification mechanisms, modification of cellular response by altered intercellular redox status, and oxygen metabolism in sensitive and resistant cells are of interest to the area of cancer treatment and directly related to our studies. Deleterious species produced by the antineoplastic drugs and cellular response to these species, as well as sulfhydryl containing compounds as they relate to metabolism, activation, and detoxification of antineoplastics are being explored. It has been demonstrated that depletion of glutathione levels either by directly conjugating or inhibition of de novo synthesis results in sensitization of cells by adriamycin, bleomycin, cisplatin, VP-16, alkylators, and radiosensitizers. Alternatively, increasing glutathione levels by providing direct precursors results in protection of cells from the above reagents. Rescue of cells after treatment by supplying glutathione directly by modifying the molecule such that it becomes membrane permeable is being studied. We have completed synthesis of a series of glutathione esters and and are presently evaluating them *in vitro* under a wide variety of conditions. Following these studies we hope to determine whether or not differential elevations in GSH and tumor versus normal tissues in animals is possible and whether such manipulation can modulate chemotherapy drug response to yield a therapeutic gain. We have also exposed human breast cancer cells to 20 weekly adriamycin treatments (each treatment yields ~ 50% survival) and isolated a clone that is approximately 2 fold more resistant to adriamycin that the original parent cell line. This cell line does not express MDR nor does it have elevated GSH or GSH transferase levels. This cell line will be extensively studied to determine other factors important in drug resistance.

Project Description

Objective: The objective of this project is to determine the importance of biochemical modulation of selected cellular redox compounds upon chemotherapeutic drug cytotoxicity.

Methods Employed

In vitro cell culture and *in vivo* murine tumor models will be exposed to the various reagents mentioned above and assayed by conventional clonogenic assay, dye markers, tumor dose response, and survival advantage. In the *in vivo* studies, both thymic and athymic mouse are available to investigate murine and human tumor response. Standard biochemical enzyme assays, synthetic organic chemistry techniques, high performance liquid chromatography, and molecular biology techniques will and are being used.

Major Findings

Glutathione esters are currently being evaluated to determine if modulation of GSH levels in cells and tissues can alter chemotherapy drug response. The esters work in vitro, that is GSH levels can be greatly elevated over a short period of time and such modulation can result in resistance to cisplatin, and adriamycin. A new adriamycin drug resistant cell line has been developed that is ~2 fold resistant to adriamycin, but does not express MDR or have elevated GSH or GSH transferase levels. Since this cell line was developed in a more clinically relevant method than previous drug resistant cell lines we feel much is to learned by seeking to determine the mechanisms underlying the resistance.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of drug-induced resistance and provide potential means of overcoming such resistant clones. Likewise, work is accumulating that may allow for differentiating normal tissue and tumor response to antineoplastic drugs by manipulating, in part, the redox status of cells.

Proposed Course

To continue to explore the best means of modifying chemotherapy response by manipulation of redox cycles or by adding exogenous protective agents.

Publication

1. Goffman TE, Raubitschek A, Mitchell JB, Glatstein E. The emerging biology of modern radiation oncology, Cancer Research 1990;50:7735-7744.

2. Tochner Z, Barnes M, Mitchell JB, Orr K, Glatstein E, Russo A. Protection by indomethacin against acute radiation esophagitis, Digestion 1990;47:81-87.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-12 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Radiosensitization and Chemosensitization of Aerated and Hypoxic Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	J. A. Cook	Staff Fellow	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Studies from our laboratory and others have identified the intracellular thiol, glutathione (GSH) as being important in the cytotoxicity of certain chemotherapy drugs and hypoxic cell radiation sensitizers. This finding prompted the question as to whether GSH levels are elevated in human tumors and if so could this explain resistance so often encountered in clinical cancer treatment. In collaboration with the Surgery Branch, we have measured GSH levels from 27 lung tumor biopsies and compared them to normal lung GSH. Our findings show that 1) precise GSH measurement of tumor cells are complicated by infiltration of leucocytes (infiltration in some tumors exceeded 40% of the total mass) in lung tumors; 2) normal lung GSH values were remarkedly constant amoung the 27 samples evaluated; 3) several squamaous lung cancer samples had populations of tumor cells in the biopsy with 3-5 fold higher GSH levels than found in normal lung. The techniques we have worked out should aid researchers who wish to measure GSH levels in tumors. In particular, we have shown that a GSH specific stain, monochlorobimane, along with HPLC techniques will enable identification of subpopulations within tumor cell digests and establish their GSH levels. We have demonstrated basic differences in GSH transferases exist between human and rodent tumors which greatly modify the effectiveness monochlorobimane staining. These approaches and techniques should prove useful in clinical trials where agents such as buthionine sulfoximine are being used to deplete tumor cell GSH. These techniques should enable accurate assessment of tumor cell populations from patients.

Project Description

Objective: The objective of this project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the inter-relationships of factors which influence radiosensitivity and chemosensitivity, with an emphasis on intracellular molecules that may detoxify damage such as glutathione.

Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to radiation or selected chemotherapy drugs, either under aerated or hypoxic conditions. Cellular GSH will be measured by spectrophotometric methods and cellular levels altered by drugs that specifically modulate the GSH cycle. Particular attention will be placed toward optimizing flow cytometric assays for GSH determination of fresh human tumor biopsy material.

Major Findings

We have developed a sensitive technique to measure GSH levels in subpopulations taken from digests of human tumor biopsies. We have compared the GSH levels from tumor cell populations to those of normal lung cells. Our finding has been GSH levels of cells taken from tumor and normal lung are approximately the same with the exception of squamous carcinoma of the lung. Subpopulations of cells taken from squamous lung tumors exhibited GSH levels 3-5 fold higher than normal lung values. The techniques we have developed can be used in clinical trials where GSH levels from populations of cells taken from tumor might be correlated with the ultimate treatment outcome. The technique will also be useful in testing the extent of GSH depletion afforded by buthionine sulfoximine in clinical trials where this particular drug is being used inconjunction melphalan treatment.

Significance to Biomedical Research and the Program of the Institute

Agents such as buthionine sulfoximine which inhibits GSH synthesis are currently being evaluated in clinical trials. In order to assess the efficacy of such approaches accurate tumor cell measurements from patients is imperative. The techniques developed in our lab over the past two years are appropriate for these studies. These techniques have been now published and should be incorporated into clinical trials exploring whether or not GSH levels in tumors is a factor of the overall treatment outcome.

Proposed Course

More studies will be conducted at the cellular level on a more efficient means of GSH modulation. A major continued effort will be refinement of the measurement of GSH (and related enzymes) in human tumor and normal tissue.

Publications

1. DeGraff WG, Russo A, Friedman N, and Mitchell JB. Misonidazole hypoxic cytotoxicity and chemosensitization in two cell lines with different intracellular glutathione levels, Eur J Cancer 1990;26:17-20.

2. Cook JA, Iype S, and Mitchell JB. Differential specificity of monochlorobimane for isozymes of human and rodent glutathione S-transferases, Cancer Res 1991;51:1601-1612.

3. Cook JA, Pass HI, Iype S, Friedman N, DeGraff W, Russo A, Mitchell JB. Cellular glutathione and thiol measurements from surgically resected human lung tumor and normal lung tissue, Cancer Research 1991 (In press).

4. Garg PK, Garg S, DeGraff WG, Zalutsky MR, and Mitchell JB. 4-fluorobenzylamine and phenylalanine methyl ester conjugates of 2-nitroimidazole: synthesis and evaluation as hypoxic cell radiosensitizers, International Journal of Radiation Oncology, Biology, and Physics 1991 (In Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-11 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Clinical Radiation Physics Service

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	N. Wersto	Radiation Physicist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI
	M. Thompson	Dosimetrist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This section continues to provide expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to special clinical needs. Regular checking of dosimetric and technical set-up aspects of radiation treatment will continue.

1. The improvement of the quality assurance (QA) program for the three Varian accelerators (Clinacs 4, 18, and 20) and the Scanditronix Microtron M22 is an ongoing effort. A new quality assurance detector using five ionization chambers has been integrated into the QA program. This device consolidates output, energy and symmetry checks and will be useful for electrons as well as photons.

2. Adaptation of the radiation equipment and special supporting equipment for patient treatment and its implementation is a continuing effort, continually adjusted also to the needs of the ongoing and new clinical research programs.

3. The Microtron is to be replaced.

4. The computer programs for clinical radiation treatment planning are being rewritten in C-language for implementation on a Macintosh II system. This project is nearing completion and is being field-tested for both photon and electron beam treatment planning. An extensive "Help" manual has been assembled.

5. Supporting patient treatment and evaluation of clinical research.

Project Description

Personnel: See above

Objectives: To ensure highly flexible and quality physics support for radiotherapy.

Methods Employed

The locally developed highly efficient system for daily and periodic quality assurance is continually used for monitoring the performance of three linear accelerators, the Microtron, the simulator, and the CT scanner. Special mechanical supports and measuring devices are used to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning have been simplified and improved.

The Section continues to provide non-routine in vivo patient dosimetry by means of thermoluminescent dosimeters and diodes. Such ad hoc measurements are usually concerned with doses to sensitive organs, and are sometimes crucial to the continuation of a treatment technique.

Major Findings

This is a continuing project, developing in part in line with developing or new clinical research. Beam monitoring locally developed and other quality assurance support jigs enable daily monitoring of output, beam flatness, symmetry, and alignment of light field and x-ray fields for all three linear accelerators. The method allows simple documentation of performance. Our system continues to impress visitors. The dosimetry of photon beam total-body irradiation, as well as that of total-skin electron beam irradiation for mycosis fungoides, has stabilized.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important aspect is the capability to image irregular fields shaped by individualized specially defined shielding blocks. This is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

The use of locally designed and developed equipment and methodology continues to be a major factor in quality control of equipment, methodology and treatment documentation. This is especially important in view of the generally highly complex clinical studies in this Branch. Reliability of treatment delivery is being improved by implementation of a computer controlled hand-held bar code reader system, developed in-house by Robert Miller.

Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies. The CT scanner is now the principal source of patient data for treatment planning.

Proposed Course

1. Continuation of adaptation of the computer programs to the new radiation machines, with emphasis on an updated system based on the Macintosh II.

2. Integration of alternative imaging systems such as MRI and PET into the updated treatment planning system.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06330-11 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radiation Field Modeling and Computerized Treatment Planning

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	H. Xie	Computer Specialist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is ongoing research and development. The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams to the most general characteristics is fundamental to radiotherapy. The radiation field model has been described before. It takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field." This concept is applied to the beam-modifying devices as well. One virtue of this approach is that it requires few experimental data and thus can be implemented very easily.

The implementation of our photon beam treatment planning programs on a Macintosh II system is continuing. The project is nearing completion for photon beams; an extensive manual with several levels of user guidance has been prepared. The general approach, in particular many "tools" will be directly applicable to other applications, including monoclonal antibodies dosimetry and imaging.

Project Description

Objectives:
1. To extend and verify unified calculative models for the description of absorbed dose produced by beams of ionizing radiation, including photon beams as well as electron beams, as a basis for computer-assisted treatment planning, with special attention to high energy x-ray and electrons.

2. To develop a user friendly, inexpensive and open-ended computerized treatment planning system powerful enough to serve routine requirements in any clinical environment

Methods Employed

Current emphasis is on developing and optimizing coding to implement our own photon and electron beam models on thge Macintosh II. This implies a greaty deal of data acquisition and processing activity, and quality assurance work (QA). For this purpose, the Therados RFA-7 radiation field scanner, in combination with data processing and graphic capabilities of the Macintosh II system are proving particularly useful.

Maior Findings

The system is being clinically tested in parallel with the existing VAX-750 based sytem. Continuous interacting with the users (dosimetrists, physicists) have lead to the assembling of a manual with several levels of user guidance. Van de Geijn was invited to the University of Göttingen, Germany, to introduce and implement the system there. This amounted to extremely useful field testing and feed-back for further improvement as to the user end of operational aspects, and input and QA of characteristic data.

Significance to Biomedical Research and the Program of the Institute

The range of validity of the dose field model determines the potential range of applicability of the clinical treatment program. In turn, the latter determines the degree of refinement in radiation treatment that can be scientifically documented. Current development could also become attractive for dissemination into the radiotherapy community, and improve the exchangeability of treatment documentation in clinical trials.

Proposed Course

1. This project is to be continued, with the emphasis of inhomogeneities in photon and electron beams. In regard to electron beams, the influence of inhomogeneities needs further experimental work and algorithmic implementation.

2. Implementation on a Macintosh II portable system is to be continued. (see Z01 CM 06378-03 RO.)

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06351-09 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Response of Mammalian Cells to Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
	J.A. Cook	Staff Fellow	ROB, NCI
	T.E. Goffman	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When certain halogenated pyrimidines such as bromodeoxyuridine (BrdUrd) and iododeoxyuridine (IdUrd) are incorporated into cellular DNA, cells become more sensitive to ionizing radiation and chemotherapy drugs. This observation has led to several clinical studies over the years and recently at the NCI to evaluate whether selective sensitization of tumors could be achieved by IdUrd infusion followed by radiation. Phase I studies have been completed. We were not able, *within the context of our protocol*, to show a role for high dose hyperfractionation and continuous IV infusion of IdUrd in brain tumors. Our laboratory has been able to give us some possible explanations for these results. Thymidine replacement in tumor cell DNA has been evaluated from 4 patients who received IdUrd infusion for 5-7 days prior to surgery. Replacement values ranged from 0-4%. *In vitro* studies would suggest that replacement values ~6-10% are required to observe significant radiosensitization. High local control rates in large unresectable sarcomas treated with high dose radiation/IdUrd has been confirmed in a more extensive study. We are now randomizing patients with unresectable sarcomas without metastatic disease to receive the halogenated pyrimidine IdUrd as a radiosensitizer. IdUrd replacement data from this set of patients has thusfar revealed much higher replacement values (7.3-14.2%) than was seen for gliomas. Other patients such as gliomas and unresectable head and neck cancers are being treated on a non-randomized basis at this time, with clincal-laboratory interaction to obtain labeling and uptake information. In a small group of head/neck patients IdUrd replacement values have ranged from 2.9-9.1 perhaps explaining the drammatic tumor response observed in these patients.

Project Description

Objectives: To quantitate the amount of IdUrd in tumor vs. normal tissue by flow cytometry, HPLC, and image analysis. With these techniques we will be able to determine if there is a relationship between the tumor cell IUdR replacement and the overall radiotherapy treatment response. With these techniques, optimal timing schedules of incorporation for maximum differential radiosensitization will be determined.

Methods Employed

A monoclonal antibody for IdUrd and HPLC assays will be used to quantitate incorporation of IdUrd in tissues. In vitro studies will employ standard cell survival techniques. Image analysis will be preformed using a fluorescent microscope linked to laser excitation and computer image analysis systems.

Major Findings

Our preliminary findings indicate that a possible reason for the ineffectiveness of IdUrd/radiation in the treatment of high grade gliomas was due to low tumor cell incorporation of IdUrd. In contrast, high IdUrd replacement values have been observed in large unresectable sarcomas and head and neck tumors infused with IdUrd and local control rates have been impressive.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding as to quantities and timing of IdUrd required to radiosensitize cells from tumor and normal tissue in a clinical setting. This parameter may be useful in selecting appropriate treatment approaches.

Proposed Course

Continue work on cellular and tumor quantitations of IdUrd. Evaluate cell survival of other mammalian cells to halogenated purines and pyrimidines and work out timing of incorporation for maximum differential sensitization in *in vivo* models. The influence of biological response modifiers on IdUrd incorporation will be studied.

Publications

1. Cook JA, Glass J, Lebovics R, Bobo H, Pass H, Delaney T, Oldfield E, Mitchell JB, Glatstein E, and Goffman TE. Measurement of thymidine replacement in patients with high grade gliomas, head and neck tumors, and high grade sarcomas after continuous intravenous infusions of 5-iododeoxyuridine. Tampa, 7th International Conference on Chemical Modifiers of Cancer Treatment 1991;78-79.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06353-09 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metal Chelate Conjugated Monoclonal Antibodies For Tumor Diagnosis And Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	O. A. Gansow	Senior Investigator	ROB, NCI
Others:	M. Brechbiel	Chemist	ROB, NCI
	T. McMurry	Senior Staff Fellow	ROB, NCI
	G. Pippin	Staff Fellow	ROB, NCI
	K. Garmestani	Visiting Scientist	ROB, NCI

COOPERATING UNITS *(if any)*

Laboratory of Cellular and Molecular Biology, NCI; Metabolism Branch, NCI; Johns Hopkins Medical School, Baltimore, MD (M. Strand); Argonne National Laboratory, Argonne, IL (R. W. Atcher); University of Nebraska Medical School (D. Colcher), Omaha, Nebraska

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in several animal model systems: one is a tumor virus induced leukemia of mice; another is human tumor xenographs in nude athymic mice.

The cytocidal agents being employed are various radionuclides. Their relative efficacy when conjugated to monoclonal antibodies is being assayed and compared to that of monoclonal antibodies alone or conjugated to toxins. The several radionuclides chosen for study span the range of nuclidic properties available, thus Copper-67 represents a weak, short range, low energy beta emitter, Yttrium-90 is a long range, high energy beta emitter, Bismuth-212 is a short-lived, alpha emitter and Lead-212 provides both short and long range beta emissions and the subsequent alpha emission of its Bismuth-212 daughter. The syntheses of the chelating agents required for linkage of these isotopes to antibody is now complete.

A new compound, (Bi-CHX-DTPA), was synthesized and shown to be useful for labeling monoclonal antibodies with Bi-212. A therapy study in leukemic mice has been accomplished.

These studies will provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytocidal agents to tumors, as well as metastases and as well will allow improved diagnostic imaging of malignancies.

Professional Personnel Engaged on the Project

T. Waldmann	Chief	MET, NCI
M. Strand		Johns Hopkins
R. W. Atcher		Argonne
D. Colcher		Univ. of Nebraska

Objective

The specific goal of these studies is to investigate in vitro and in animal tumor models the therapeutic efficacy of radionuclides attached to tumor-associated monoclonal antibodies. These studies encompass the synthesis of new bifunctional chelates designed for therapy employing a variety of radioisotopes and radiation types.

Methods Employed

Methods for covalently conjugated metal isotopes in bifunctional chelates to monoclonal antibodies are being devised and developed. The inorganic chemistry of new complexing agents for metal isotopes thought to be useful in tumor diagnosis or therapy is being explored. The objectives of the research must thereby of necessity include: (a) the synthesis and characterization of new bifunctional chelates and their metal complexes, both before and after protein conjugation; (b) the evaluation of currently available chelates for use as carriers of isotopes familiar in clinical environments (e.g., Tc-99M) and of less common, but potentially serviceable radionuclides (e.g., Ga-68, Pb-203, In-111, Pb-212, Bi-212, Y-90); (c) the development of chemical procedures (protocols) for routine and reproducible preparations of rigorously stable radiometal chelate conjugated monoclonal antibodies which retain their inherent biological specificity and activity; and (d) the use of animal models for investigating the stability in vivo of metal labeled antibodies.

Major Findings

We report this year progress in implementing the use of Bi-212 and Y-90 labeled antibodies in tumor therapy.

1. The antibody 103A was labeled with Bi-206, 212 by use of the new chelator CHX-DTPA and shown to be stable in vivo.

2. A tumor therapy study in nude mice showed that use of specific antibody labeled with Bi-212 prolonged survival of the animals for twice as long as untreated mice or mice treated with Bi-212-irrelevant antibody.

Major Findings With The Radionuclide Y-90

1. The Y-90 chelate of the 1B4M-DTPA ligand was linked to antibody and shown to be stable in vivo.

2. The 1B4M-DTPA ligand was used to label antibody Anti-TAC with Y-90 and shown to be effective in prolonging rejection of a heart transplant in monkeys.

3. Clinical trials of treatment of T-cells leukemia with Y-90 labeled anti-TAC antibody were begun. Initial results are good.

Proposed Course

Studies of the therapeutic efficacy of the several radionuclides now under investigation are in progress employing: 1) a model for leukemia in which normal mice have been infected with Rauscher leukemia virus; and 2) a human xenograph solid tumor model in mice. Based on these studies, we will be able to select the most appropriate radionuclide for radioimmunotherapy of the specified disease to be treated.

Radiobiology studies of relative in vitro therapeutic efficacy and dosimetry will be performed.

Since protocols for production of clinical doses of chelate linked Yttrium-90 labeled antibody are in place, treatment of lymphoma and leukemia with radiolabled antibody are underway and will continue.

Publication

1. Ruegg CL, Anderson-Berg WT, Brechbiel MW, Mirzadeh S, Gansow OA, Strand M. Improved in vivo stability and tumor targeting of Bismuth-labeled antibody, Cancer Res 1990;50:4221-4226.

2. Greager JA, Chao TC, Blend MJ, Atcher RW, Gansow OA, Brechbiel MW, Das Gupta TK. Localization of pulmonary human sarcoma xenographs in athymic nude mice with Indium-111 labeled monoclonal antibodies, J Nucl Med 1990;31:1378-1383.

3. Langmuir VK, Atcher RW, Hines JJ, Brechbiel MW. Iodine-125-NRLU-10 kinectic studies and Bismuth-212-NRLU-10 toxicity in LS174T multicell spheroids, J Nucl Med 1990;31:1527-1533.

4. Sharkey RM, Motta-Hennessy C, Gansow OA, Brechbiel MW, Fand I, Griffiths GL, Jones AL, Goldenberg DM. Selection of a DTPA chelate conjugate for monoclonal antibody targeting to a human colonic tumor in nude mice, Int J Cancer 1990;46:79-85.

5. Cooper MM, Robbins RC, Goldman CK, Mirzadeh S, Brechbiel MW, Stone CS, Gansow OA, Clarke RW, Waldmann TA. Use of Yttrium-90-labeled anti-TAC antibody in primate xenograft transplantation, Transplantation 1990;50:760-765.

6. Washburn LC, Sun TTH, Lee YCC, Byrd BL, Holloway EC, Crook JE, Stubbs JB, Brechbiel MW, Gansow OA, Steplewski A. Comparison of five bifunctional chelate techniques for ^{90} Y-labeled monoclonal antibody C017-1A, Nucl Med Biol 1991;18:313-321.

7. Roselli M, Schlom J, Gansow OA, Brechbiel MW, Mirzadeh S, Pippin CG, Milenic DE, Colcher D. Comparative biodistribution studies of DTPA-derivative bifunctional chelates for radiometal labeled monoclonal antibodies, Nucl Med Biol 1991;18:389-394.

8. Brechbiel MW, Gansow OA. Backbone substituted DTPA ligands for 90 radioimmunotherapy, Bioconjugate Chem 1991;2:187-194.

9. Pippin CG, Kumar K, Mirzadeh S, Gansow OA. Kinetics of isotopic exchange between Cu(II) and Cu(II) 1,4,7-triazacyclononane-N,N',N" triacetate, J Labeled Compds and Radiopharm 1991;30:211.

10. Gansow OA, Kumar K. Process for synthesizing macrocyclic chelates, United States Patent 4,923,985,1990.

11. Dirbas FM, Brown PS, Mirzadeh S, Griffith PK, Goldman CK, Garsia RJ, Junghans R, Gansow OA, Waldmann TA, Clark RE. Anti-TAC-Yttrium-90 prolongs cardiac graft survival in primate homograft transplantation. Transplantation and its related immunology, reprinted from American College of Surgeons 1990 Surgical Forum, Vol. XLI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06357-08 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*: Clinical Studies on Intraoperative Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	E. Glatstein	Chief	ROB, NCI
Others:	W. Sindelar	Senior Investigator	SB, NCI
	H. Pass	Senior Investigator	SB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC

COOPERATING UNITS *(if any)*: Surgery Branch
Cancer Nursing Service, CC

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	10	PROFESSIONAL	100	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The Radiation Oncology Branch and Surgery Branches of the National Cancer Institute have been involved in prospectively randomized studies evaluating the potential role of intraoperative radiation therapy in several disease sites, including resectable and unresectable carcinomas of the pancreas, resectable carcinomas of the stomach, and retroperitoneal sarcomas. One hundred patients have been treated with experimental intraoperative radiation therapy, and randomized to either receive or not receive radiation therapy intraoperatively with large single doses of electrons. There is really no suggestion of improvement in survival, or in disease-free survival. There is some suggestion of an improvement of local control in the retroperitoneum itself; however, this is off-set by a high predilection for seeding of the abdominal cavity, either peritoneal carcinomatosis or sarcomatosis, thus neutralizing the potential benefit of intraoperative radiation. The trials on pancreatic carcinoma and retroperitoneal sarcomas have been closed. The gastric study is still open for patient accrual.

Project Description

Personnel:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
R. Smith	Cancer Nursing Specialist	CNS, CC

Objectives: These are Phase I and II studies assessing the role of intraoperative radiation therapy as an adjunct to surgical resection in various primary tumor sites, including pancreas, stomach, and retroperitoneum, where local failure following surgery alone is extremely high. Additional pilot studies are ongoing to determine the role of intraoperative radiation therapy with tumors with high-risk of local recurrence.

Methods Employed

Patients are considered for entry on the randomized studies with combined surgical resection and intraoperative therapy that have specific malignant lesions with the abdomen and retroperitoneum, and lack evidence of metastatic spread. In general, the control arm of these studies receives resection with post-operative conventional fractionated radiotherapy, and the experimental arm receives in addition, intraoperative radiation therapy, as well as misonidazole, a known radiosensitizer of hypoxic cells, a single injection of 3.5 gm/m^2. Patients are followed closely to assess local toxicity, and patterns of recurrence.

Major Findings

With over 100 patients having been randomized to receive intraoperative radiation therapy at the NCI, there is no trend to suggest an improvement in local control, disease-free survival, or overall survival. Local control can be made to look quite good, if one talks only about the retroperitoneum. However, the marked predilection for carcinomatosis or sarcomatosis of the peritoneal surface itself, negates this potential gain. Until this problem can be
overcome, intraoperative radiation therapy will not be useful on a large scale. Potentially, intraperitoneal chemotherapy, pre-operative radiation therapy, or intraoperative photodynamic therapy might be useful in overcoming this problem.

Significance to Biomedical Research and the Program of the Institute

Intraoperative radiation therapy studies are the first prospective randomized trials looking at this method of delivering radiation therapy.

Proposed Course

With the renovations of the electronics of the Microtron, we hope to continue these pilot trials. However, until we are able to deal realistically with the problem of peritoneal seeding, this modality will probably not prove to be useful. If we can overcome the problem of peritoneal seeding, this may represent a useful advance in a number of abdominal neoplasms. Photodynamic approaches to prevent peritoneal seeding are presently in Phase I studies.

Publications

1. Antoine JE, Glatstein EJ, and Sindelar W. Intra-Operative Radiotherapy. In: House J, ed. Oxford Textbook of Oncology. New York: Oxford University Press, Inc. (to be published).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06358-08 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Radiolysis, Photolysis and Sonolysis of Cells and their Constituents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Riesz	Research Chemist	ROB, NCI
Others:	Heasook Kim	Visiting Fellow	ROB, NCI

COOPERATING UNITS (if any)
LCM, NIMH (C. Chiueh);
Electrophysics Branch, CDRH, FDA (C. Christman);
Hydrodynamics and Acoustics Branch, CDRH, FDA (G. Harris)

LAB/BRANCH: Radiation Oncology Branch

SECTION: Experimental Phototherapy

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The chemical effects of ultrasound have been studied in relation to sonodynamic cancer therapy. Recent *in vivo* and *in vitro* studies by Umemura et al (Jpn. J. Cancer Res. 81, 962, 1990) have indicated antitumor effects of hematoporphyrin activated by ultrasound. A suggested mechanism involves the sonochemical generation of singlet oxygen. One of the proposed methods of testing for singlet oxygen is the conversion of a sterically hindered cyclic secondary amine (2.2.6.6 tetramethyl-4-piperidone. TMPone) to the corresponding nitroxide which can be detected by electron spin resonance. A detailed study of the sonolysis and gammaradiolysis of TMPone showed that TMPone cannot be used for the detection of singlet oxygen in sonochemistry since the same nitroxide is also produced by the reaction of hydroxyl radicals in the presence of oxygen with a similar pH dependence. Our recent studies have shown that transient cavitation (that is, the formation, growth and violent collapse of gas bubbles) occurs in argon-saturated aqueous solutions exposed to typical diagnostic ultrasound pulses from a Ultramark-9 ultrasound system. The 2.25 MHz ultrasound pulses had a pulse width of 0.8 microsec, a pulse repetition frequency of 50 kHz and a spatial peak pulse average intensity of 100 watts/cm^2. Hydroxyl radical formation was detected by means of a highly sensitive assay using high pressure liquid chromatography with electrochemical detection of picomoles of the hydroxylation products of salicylate.

Project Description

Objectives: The effects of ionizing and ultraviolet radiation and of ultrasound on biological macromolecules and their constituents are being investigated. Ionizing radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals, or by the "indirect effect" where radical species are hydrated electrons, hydrogen atoms, and hydroxyl radicals.

In the chain of events that lead to loss of biological activity, free radicals play an important role. Chemical compounds have been discovered which significantly modify radiation effects. These include: (a) electron affinity sensitizers which act on hypoxic tumor cells; (b) halogenated pyrimidines which are incorporated into DNA; and (c) cancer chemotherapy agents of the intercalating or alkylating type which sensitize tumor and normal cells. Studies of the mechanism of action of radiosensitizers and radioprotectors are necessary to design improved combinations of chemotherapy and radiation therapy.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity in macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

In the last few years, it has become apparent that superoxide anion radicals and hydroxyl radicals are found in many biological systems in the absence of either ionizing radiation or UV-photolysis. Recent reports have indicated that radicals are produced in the presence of certain anti-cancer drugs such as Bleomycin and Adriamycin. The significance of radical reactions is therefore not confined to radiation biology. It has also been shown that damage to tissues following ischemia appears to occur during reperfusion with oxygenated blood. This damage is generally considered to be due to the excessive production of superoxide radicals and hydrogen peroxide. In support of this hypothesis, it has been shown that in several model systems superoxide dismutase, catalase or allopurinol (a xanthine oxidase inhibitor) protect ischemic tissue from oxidative damage during reperfusion.

Methods Employed

Nucleic acids, proteins and their constituents were gamma-irradiated either in the solid state or in aqueous solutions in a 800-curie Colbalt gamma-source. Electron spin resonance studies were carried out with a Varian E-9 Spectrometer connected to an IBM-XT computer. For photolysis studies at specific wavelengths, a 1000-watt high pressure Xenon arc source and monochromator were employed. For ultrasound exposures, aqueous solutions were insulated in a non-perturbing cylindrical cell with 1 mil mylar windows in an anachoic ultrasound exposure apparatus at 30 $\pm$ 0.5 degrees. Specimens were exposed to either continuous wave or tone bursts

of 1 MHz ultrasound to simulate both therapeutic and diagnostic exposure conditions. In the spin trapping method, the short-lived free radicals react with a diamagnetic scavenger (the spin trap) to produce longer-lived radicals (the spin adduct) which can be conveniently investigated by e.s.r. In our studies, 2-Methyl-2-Nitrosopropane, 5,5-Dimethyl-1-Pyroline-N-Oxide, and 3,5-dibromo-2,6-dideuterio-4-nitrosobenzenesulfonate were employed as the spin traps.

Major Findings

I. Effect of Ultrasound and Ionizing Radiation on a Sterically Hindered Cyclic Secondary Amine: An ESR Study (with Takashi Kondo, Department of Experimental Radiology and Health Physics, Fukui Medical School, Fukui, Japan).
The possible use of 2,2,6,6,-tetramethyl-4-piperidone (TMPone) for the detection of singlet oxygen was investigated by gammaradiolysis and sonolysis of oxygen-saturated aqueous solutions. Formation of 2,2,6,6,-tetra-methyl-4-piperidone-N-oxyl (TAN) was observed with both gamma radiolysis and sonolysis with a similar dependence on the concentration of TMPone up to 20 mM and a strong dependence on pH. In oxygen-saturated solutions the sonolysis of TMPone leads to the formation of the cyclic hydroxylamine (approx. 30% of the yield of TAN) while radiolysis does not. In the low pH range (5-6.5) and at high concentration of OH radical scavengers (azide or formate), TAN is produced by sonolysis but not by radiolysis. Sonolysis of argon-saturated solutations of TMPone produces methyl radicals due to the high-temperature regions of the collapsing cavitation bubbles. The methyl radicals were detected by ESR (electron spin resonance) and spin trapping with 3,5-dibromo-2,6-dideuterio-4-nitroso-benzene sulfonate. Since the reaction of singlet oxygen with TMPone is also strongly dependent on pH, it does not seem likely that TMPone could be used for the detection of singlet oxygen in sonochemistry.

II. Free Radical Formation in Aqueous Solutions Exposed to 2.25 MHz Ultrasound Diagnostic Pulses (with Heasook Kim, C.C. Chiueh (LCM, National Institute of Mental Health). C.L. Christman (Electrophysics Branch, CDRH, FDA, and Gerald R. Harris (Hydrodynamics and Acoustics Branch, CDRH, FDA).
The potential for the occurrence of transient cavitation by pulsed ultrasound is of increasing concern during the last few years because of the widespread use of diagnostic ultrasound. The generation of free radicals is a common criterion for the detection of cavitation. We are employing a very sensitive method for detecting hydroxyl radicals using high pressure liquid chromatography with electrochemical detection of picomoles of the hydroxylation products of salicylate (2:5 and 2:3 dihydroxybenzoate). Using an Ultramark-9 Ultrasound system and a specially designed anechoic exposure tank, argon-saturated aqueous salicylate solutions were exposed to 2.25 MHz ultrasonic pulses with a pulse width of 0.8 microsec, and a pulse repetition frequency of 50 kHz and a spatial peak pulse average intensity of 100 watts/cm2. 2:5 and 2:3 dihydroxybenzoate formation was a linear function of the time of ultrasound exposure. This result indicates that transient cavitation is induced by standard diagnostic ultrasound pulses in argon-saturated water. The effects of ultrasound intensity, pulse length and pulse repetition frequency on the threshold for transient cavitation are being investigated.

III. Sonochemistry of Acetone and Acetonitrile in Aqueous Solutions (with A.J. Carmichael. Radiation Biochemistry Department. Armed Forces Radiobiology Research Institute, Bethesda, MD).

Sonolysis of aqueous argon-saturated acetone solutions produces methly radicals by C-C bond scission due to pyrolysis of acetone in the imploding argon cavitation bubbles. Methyl radicals are not formed from the reactions of H atoms and OH radicals which diffuse into the bulk of the solution and react with acetone at ambient temperature to yield CH_3-CO-CH_2 and other radicals. Sonolysis of aqueous argon-saturated acetonitrile solutions results in C-H bond scission in the high temperature region of the collapsing cavitation bubbles. This is followed by H atom addition to the triple bond and decomposition of this intermediate radical to form methyl radicals. Sonolysis studies of aqueous solutations of acetone, acetonitrile, methanol and ethanol are consistent with the generalization that the higher the vapor pressure of the pure solute, the lower the concentration of solute at which the maximum of the plot of sonochemical yield vs. concentration occurs. The initial increase in the sonochemical rate is due to the increasing fraction of volatile reactant in the collapsing argon bubbles, while the subsequent decrease is primarily the result of the lower ratio of specific heats of the gas mixture, as the monoatomic argon gas (gamma = 1.67) is mixed with increasing amounts of polyatomic solute (gamma < 1.20). Sonochemistry is a probable pathway for the formation of complex organic compounds in the primordial ocean. The linear velocity of water in collapsing waves in the ocean is approximately 5 m sec^{-1} and above. This is the range of velocities for which M. Anbar (Science, 1968) has shown that sonoluminescence and hence transient cavitation and the associated sonochemistry will occur.

Significance to Biomedical Research and the Program of the Institute

Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy; (2) carcinogenesis; (3) stability of the genetic pool; (4) the suppression of the immune mechanism; and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal cells, and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained.

Proposed Course

To continue studies on the effects of ionizing radiation on mammalian cells and macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents and the interaction of radiation and cancer chemotherapy agents will be investigated. New areas of interest include photosensitized cell killing by porphyrins and phthalocyanines in relation to photodynamic and sonodynamic therapy and chemical and biological effects of ultrasound.

Publications

1. Riesz P, Kondo T, Krishna CM. Free radical formation by ultrasound in aqueous solutions: a spin trapping study, Free Rad Res Comm 1990;10:27-35.

2. Riesz P, Kondo T, Krishna CM. Sonochemistry of volatile and non-volatile solutes in aqueous solutions: e.p.r. and spin trapping studies, Ultrasonics 1990;28:295-304.

3. Riesz P. Free radical generation by ultrasound in aqueous solutions of volatile and non-volatile solutes. In: Mason TJ, ed. Advances in sonochemistry, vol. 2. London: Jai Press, Ltd, 1991 (in press).

4. Riesz P, and Christman CL. Sonochemical Exposure Methods. In: Mason TJ, ed. Practical Sonochemistry. London: Jai Press, Ltd, 1991 (in press).

5. Kondo T, and Riesz P. Effect of ultrasound and ionizing radiation on a sterically hindered cyclic secondary amine: an ESR study, Radiat Res 1991 (in press).

6. Krishna CM, Uppuluri S, Riesz P, Zigler JS, Balusubramanian D. A study of the photodynamic efficiencies of some eye lens constituents, Photochem Photobiol 1991 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06361-07 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phototherapy of Intracavitary Spaces

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	H. Pass	Senior Investigator	SOB, NCI
	P. Smith	Senior Investigator	BEIB
	W. Frauf	Senior Investigator	BEIB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7	7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The use of hematoporphyrin derivative and other photosensitizing agents in combination with light activation is currently being investigated as an anti-tumor modality for the treatment of intraperitoneal and intrathoracic tumors. A major advantage of this modality is the apparent selective retention of the sensitizing dye within tumors. A murine ascites ovarian carcinoma and a human ovarian tumor have been used to study the characteristics of drug distribution in the peritoneal cavity. Initially, murine models were used to study the tolerance of the thoracic cavity structures to the phototherapy techniques being explored. The limitations of the murine model has required the extensions of the investigation to the canine model for evaluation of the toxicity of Phototherapy. Different wavelengths of light, different laser delivery systems, different sensitizers, different doses of energy, different modes of drug administration, and different monitoring devices were studied. We have shown that Phototherapy can be used to effectively treat a murine ascites tumor. We have also shown that in both the murine and the canine model, the peritoneal serosal surface is tolerant of at least 0.5 J/cm^2 and that this work can be extended to human subjects. In the murine system, we have shown that the thoracic cavity, like the peritoneal cavity, is exquisitely sensitive to treatment with red light (630 nm). The dose rate must be controlled to minimize heat buildup (less than 150 mW fiber output from a forward projecting optical fiber is usually tolerated). We have extended a toxicity study to the canine thoracic cavity and shown that stuctures such as the esophagus, parietal and visceral pleura, heart can tolerate 35 J/cm2 red light. Currently, there is an ongoing Phase I trial using DHE/630nm light to treat unresectal mesothelioma. Currently, 32.5J/cm has been used in the clinical treatment of mesothelioma. We are exploring the use of photoimmunotherapy as an additional means of drug delivery. An athymic murine model transplanted with human lung cancer was used to study photoimmunotherapy. Hematoporphrin was covalently bound to the specific monoclonal antibody directed against the xenograft. The results show that tumor can be eradicated and that dermatophotoxicity is eliminated. We are exploring the use of chemiluminescence as a means of light delivery to the cavitary spaces. A new class of water soluble agents are being used. New forms of energy delivery for activation of sensitizer are being explored.

Project Description

Objective: Our objectives are to establish a laboratory model for treatment of intracavitary malignancies that spread by implanting on serosal surfaces such that Hematoporphyrin derivative and other photosensitizing agents can be used in combination with non-ionizing radiation. Likewise the best means of delivering light and sensitizer and to establish means to better quantitate light delivered to the tumor and normal tissue (dosimetry) are high priorities. We are also focusing on means of improving or circumventing phototoxicity to normal tissue. Lastly, we are exploring the utility of ultrasound as a source of sonoluminescence for activation of sensitizer dyes which would dramatically increase the penetrance of tumors.

Methods Employed

Two different murine (thymic and athymic) systems and a canine model are being used to investigate the peritoneum for Phototherapy. For the study of the chest cavity, murine and canine models are being studied. Response, survival, histopathology are used for evaluation. In vitro cell culture techniques are being used to judge the initial effects of different sensitizers. Both pleiotropic drug resistant cell systems as well as more conventional cell models are being used. Fluorescence spectroscopy is being used to study drug administration routes as they impact on tumor localization and normal tissue distribution. Light dosimetry is being studied by photodiode placement and computer modeling and analysis. Monoclonal antibodies are being covalently affixed to either hematoporphrin because the sensitizers can be purified to homogeneity, have desirable absorbance characteristics, and provide different chemical means of attachment. Antibodies being studied are directed against either human lung tumors that have been developed for growth in an athymic murine model system. General searches for sensitizers that absorb light at longer wavelengths (>600 nm) are being sought that also have the characteristics of being lipid membrane permeable and favorably partition to nucleic acid oligomers. Such sensitizers are investigated for viricidal effect.

Major Findings

Preliminary work in a cell culture system that has been pretreated with HPD shows that chemiluminescence agents provide enough light to be effectively used as a light source. We have evaluated a number of chemiluminescent agents and are still in the process of identifying the most efficient agent. The agents in and of themselves do induce cytotoxicity and of course this is a concern. Plastic models of a canine thoracic cavity suggest that intralipid (fat emulsion) can be used for real-time simultaneous equal light distribution to the pleural surface when three or more fiber sources are concurrently used. Studies using these models have led to determination of toxcitiy in animals of treating the plural surface. The animal studies have been completed and the Radiation Oncology Branch in cooperation with the Surgery Branch have begun Phase I studies in treatment of mesothelioma in the pleural cavity. Differences in the response to photodynamic therapy were evaluated in black versus white guinea pig skin. eschar formation in black skin required over twice the light dose necessary to produce eschar and light skin. These studies underscore the difficulty in treating pigmented lesions such as malignant melanoma with PDT.

The finding further suggests that higher light doses might be required to treat superficial lesions and produce skin photosensitivity in dark skin individuals. Photoimmunotherapy can effect cure of human lung xenograft.

Significance to Biomedical Research and the Program of the Institute

The ROB is involved in clinical use of Phototherapy and this work is being applied to guide the choice of tumors to be treated, the dosing of light to be used, and the best means of administering sensitizer and light.

Proposed Course

Continue to explore the models outlined above to improve the use of Phototherapy in the clinic.

Publications

1. Bernstein EF, Thomas GF, Smith PD, Mitchell JB, Glatstein E, Kantor GR, Spielvogel RL and Russo A. Response of black and white guinea pig skin to photodynamic treatment using 514 nm light and photofrin II, Arch Dermatol 1990;126:1303-7.

2. Bernstein EF, Freauf WS, Smith PD, Cole JW, Solomon RE, Fessler FF, and Russo A. Transcutaneous determination of tissue dihematoporphyrin ether content: a device to optimize photodynamic therapy, Dermatolog 1991; (in press).

3. Manyak MJ, Nelson LM, Solomon D, DeGraff W, Stillman RJ, and Russo A. Fluorescent detection of rabbit endometrial implants resulting from monodispersed viable cell suspensions. Fertil Steril 1990;54 (2):356-359.

4. Perry RR, Matthew W, Mitchell JB, Russo A, Evans S, and Pass HI. Sensitivity of different human lung cancer histologies to photodynamic therapy, Cancer Research 1990;50:4272-4276.

5. Mitchell JB and Russo A. Biological basis for phototherapy, In: Morstyn G, Kaye AH, eds. Phototherapy of cancer. New York, Harwood Academic Publishers, 1990;1-22.

6. Tochner ZA, Pass HI, Smith PS, Delaney TF, Sprague, M, DeLuca AM, Terril R, Bacher JD, Russo A. Intrathoracic photodynamic therapy: a canine normal tissue tolerance study and early clinical experience. Lasers in Surg Medicine 1991; (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06378-06 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

QA of Treatment Delivery by Means of Overlayed Digitized Simulator & Port Films

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	B. Chin Arora	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	.25	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The quality assurance of the consistency of radiation treatment delivery with the prescription is a continual concern, locally as well as nationally. The ROB already employs graticules projecting onto all simulator films and all corresponding portfilms. A project has been started to overlay differently processed digitized films to increase the quality of information, as well as to decrease the volume of documentation to be retained.

The system should be of great interest to inter-institutional studies as well. The project has been on hold until recently because of delays in acquisition of essential hardware due to lack of funds. It is nearing completion now.

Project Description

Objectives: 1) To improve the quality of documentation on the proper implementation of beam treatment set-ups.

2) To intergrate this information with the MacII based treatment planning system.

3) To condense the amount of documentation to be kept, and to increase its objectivity and exchangeability.

Methods Employed

1. Take x-ray films at the simulator, in the planned beam positions, including graticules projected onto the films.

2. Follow similar procedure at the treatment machine, producing port films with graticules.

3. Digitize both categories of films taking care to use the same orientation, cetering and magnification, with help of the graticules projected onto all films, and enter the data into a MacII computer system.

4. Apply appropriate computer enhancement of both simulator films and the corresponding port films.

5. Use overlay techniques to bring out salient anatomical features, graticules, block delineation, etc.

6. Using the computer, do measure significant deviations.

7. Store the results, properly labeled.

8. Evaluate the quality of treatment delivery.

Major Findings

The Macintosh based treatment planning sytem MacTPS has reached a stage where the present project could profitably be incorporated into it.

Significance to Biomedical Research and the Program of the Institute

1. Quality assurance and verification will become much more efficient, self-contained and attractive to use.

2. Documentation will be much more compact and easier to use, as one arm of the treatment planning facility.

3. Quality assurance of joint studies will be much easier and more objective.

Proposed Course

To continue incorporation of this technique into the Macintosh II environment and start a pilot project.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06379-05 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phase I Study of Photodynamic Therapy for Surface Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR; Laboratory of Pathology, NCI; Diagnostic Radiology Department, CC

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photodynamic therapy involves the use of a light activated compound which localizes in tumor, followed by the activation of this compound by light for cytotoxic effects for the treatment of cancer. The current protocol uses the intravenous administration of the Photofrin II preparation of the hematoporphyrin derivative, the only currently approved photosensitizer for use in humans. This is followed by the delivery of light to the affected area using optical fibers coupled to an argon/pumped dye laser. Hematoporphyrin derivative selectively localizes in tumor compared to certain normal tissues. Selective retention of the photosensitizer in combination with focal light delivery to the involved area permits selective destruction of tumor with minimal effect on uninvolved normal tissue. Hematoporphyrin derivative photodynamic therapy may be clinically useful in a number of anatomic sites involved by tumor.

Project Description

Professional Personnel Engaged on Project:

H. Pass	Senior Investigator	SB, NCI
W. Sindelar	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR
W. Travis	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	DR, CC

Objectives

This is a Phase I study designed to assess the toxicity and effectiveness of photodynamic therapy with Photofrin II and laser light in treatment of surface malignancies. Physical parameters of light distribution in tissue are being measured, as well as photosensitizer pharmacology.

Methods Employed

Patients with surface malignancies, cutaneous or mucosal, that are not curable by conventional therapy are eligible for this protocol. Patients receive the Photofrin II photosensitizer by intravenous administration 1.5 - 2.5 mg/kg. Laser light is then delivered in single or multiple fractions to the involved tumor area, using optical fibers for surface illumination, endoscopic treatment, or intraoperative treatment, depending on the patient's clinical problem.

Major Findings

Patients with recurrent tumors involving skin and patients with tumors obstructing the bronchus comprise the majority of patients treated (A small number of patients with intraperitoneal tumors and pleural tumors have been treated in a pilot fashion to permit the design of formal, phase I photodynamic therapy in each of these anatomic areas).

Twenty patients with recurrent breast cancer on the chest wall have been treated on this protocol. Four patients (20%) experienced a complete regression of tumor while 9 patients (45%) experienced a partial response, defined as reduction of tumor greater than 50%. Unfortunately the duration of the complete responses was generally less than 6 months, while the duration of the partial response was only 4 months. A major problem in the use of this modality for the treatment of these breast cancers is the limited light penetration of the light wavelength currently employed. Other patients

succcessfully treated include 1 patient with recurrent squamous carcinoma of the head and neck involving the skin, 1 patient with cutaneous lymphoma, and a patient with multiple recurrent Merkel cell carcinoma lesions of the skin of the face. Pigmented melanoma does not respond because of heavy pigmentation which attenuates light. One patient with epidemic cutaneous Kaposi's sarcoma has received 2 courses of treatment without response. Treatment-related morbidity includes sunburn in five patients, full thickness skin necrosis in 2 patients requiring surgical repair or burn treatment, and moderate discomfort in the treatment field requiring medication.

In patients who have been treated on this protocol for tumors obstructing the bronchus, fifteen had metastatic lesions and seven had tumors of the bronchus or the trachea. Fourteen (64%) had the lobe expanded or airway opened. One (5%) had partial reopening after 1 treatment with complete reexpansion with a second treatment. Two patients (9%) had transient airway opening. Two patients had a mixed response with 1 of 2 treated bronchi reopening and 3 patients (14%) had no response. Three complications were seen consisting of 1 pneumothorax, 1 infiltrate on chest x-ray, and 1 hemoptysis from recurrent tumor.

Nine patients with disseminated intraperitoneal tumors received the hematoporphyrin derivative prior to laparotomy. This was a pilot group of patients prior to the initiation of a formal Phase I study of Surgery and Photodynamic with Laser Light and Photofrin II for Intraperitoneal Malignancies. Of these first 9 patients, 6 were able to get tumor debulking and intraperitoneal photodynamic therapy at progressively increasing light doses from 0.2 - 0.6 J/cm^2 without toxicity. On the basis of the findings in these patients, a formal study of photodynamic therapy for intraperitoneal malignancies was initiated.

Seven patients with tumors involving the pleural space received the photosensitizer prior to thoracotomy/median sternotomy, at which time tumor was debulked to 5mm and at increasing light doses from 5.0 to 15.0 J/cm^2 without significant toxicity. On the basis of the findings in these patients, a formal study of Surgery and Photodynamic Therapy for Pleural Malignancies has been initiated.

One patient with carcinoma of the nasopharnyx who had a recurrence in the primary site has been treated with photodynamic therapy and is in remission 3 months after treatment.

Treatment-related morbidity includes sunburn in five patients, full thickness skin necrosis in 2 patients requiring surgical repair or burn treatment, and moderate discomfort in the treatment field requiring medication.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially curative therapy for selective groups of patients with malignant disease. In particular, patients with a tumor that is accessible to light either by superficial, endoscopic or interstitial illumination may benefit from treatment. Intraoperative treatment is both practical and potentially efficacious. Photodynamic therapy is being explored for use in multiple anatomic sites including the superficial tumors in the urinary bladder, tumors involving peritoneal and pleural surfaces, gynecologic malignancies, brain tumors, and selected skin cancers.

Proposed Course

Phase I study of photodynamic therapy are in progress in the peritoneal and pleural cavity, as well as in the urinary bladder. On completion of these trials we would hope to move on to Phase II studies in the following sites, peritoneal cavity, pleural cavity, and bladder. Long range plans also include examination of other photosensitizers which may be activated by light with deeper tissue penetration and which may have less cutaneous photosensitivity.

Publications

1. Sperduto PW, DeLaney TF, Thomas G, Smith P, Dachowski LJ, Russo A, Bonner R, Glatstein E. Photodynamic therapy for chest wall recurrence in breast cancer, Int J Rad Onc Biol Phys 1991;21:441-446.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06381-05 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modeling of Time-Dose Response of Human Tumors and Normal Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	T. Goffman	Radiotherapist	ROB, NCI
	J. Mitchell	Radiobiologist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	E. Glatstein	Radiotherapist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of radiation therapy is tumor control. In view of clonogen proliferation it makes sense to deliver the necessary dose in as short a time as possible. The limiting factor in tumor treatment is normal tissue reaction: normal tissue reactions must not exceed "tolerance" level. The development and application of high technology particularly in computers and computer-based and assisted imaging has stimulated great progress in tumor localization and treatment planning, and even in the technology of delivery and its quality assurance, i.e., the spatial aspects of the issue. Decision-making as to the amount of dose and its distribution over time (by fractionation or protraction) is still essentially empirical, however. The present project continues the development and exploration of a theoretical description of time-dose response of tumors as well as normal tissues. Its basic concepts have been published in 1988[1]. Since then, further developments have concentrated on extension of the Linear-Quadratic model. The extension concerns a unified description of the influence of incomplete repair and comprises a description of the influence of tumor proliferation and stem cell/transition cell repopulation. A study regarding the implications of high dose arate vs. low dose rate brachytherapy is ongoing. This work concentrates on the implications of uncertainties in the relevant parameters, particularly a/b and the time factor. The work includes the development of an interactive computer program and graphical tools to help clinical guidance in the search for high dose rates compatable with constant tumor control as well as no more severe toxicity.

Project Description

Objectives: To develop a mathematical formalism describing:

1. The attrition of functioning normal tissue cells.
2. The survival rate, per single dose, of viable stem cells.
3. The inter-fraction and post-treatment course repopulation including an account of the sublethal damage repair of viable stem cells.
4. The survival rate of clonogenic tumor cells per single-dose.
5. The effective dose for early as well as late reacting normal tissues.
6. The inter-fraction and post-treatment growth pattern of the clonogenic cells as well as the gross tumor.
7. Extension of the model to cover both high-dose rate fractionated (beam) therapy and protracted therapy including brachytherapy.

Methods Employed

1. The alpha/beta (2-parameter) model is applied for the single-dose response of stem cells and clonogenic tumor cells.

2. Radiation damage is assumed to consist of lethal and sublethal damage.

3. Linear attrition over time is assumed for functioning normal tissue cells as well as non-clonogenic tumor cells.

4. Normal tissue cell loss and replacement is under homeostatic control.

5. Clonogenic tumor cells are assumed proliferate exponentially over time.

6. Stem-cell proliferation is triggered only after some distress signal related to functionality cell levels drop below a certain threshold.

7. Normal tissue tolerance in interpreted as the lower limit of normal tissue functionality: the normal tissue functioning cells dropping below some fraction of their normal count.

Major Findings

1. Initially, a "two component" mathematical model was developed and was shown to be promising. A major paper was published in 1988.

2. Since then, further development has concentrated on extension of the "Linear Quadratic" (LQ) model of single dose response; in particular, we have developed a generalized description of the relative importance of "extra lethal damage" resulting from incompletely repaired sublethal damage still existing at the time of delivery of new dose. The mathematical description covers

both high dose rate fractionated (external beam) therapy and high dose rate as well as low dose rate brachytherapy. A paper on the mathematical aspects has been published recently Medical Physics.

3. Interactive computer programs have been developed which enable automatic search for acceptable parameters, based on reasonable estimated ranges of certain key parameters, a and b, cell doubling times, etc.

4. It is possible to simulate time-dose response patterns for conventional and unconventional fractionation schemes, which are reasonably consistent with published findings in some clinical trials.

5. Current work is concentrating on high dose rate (HDR) vs. low dose rate (LDR) brachtherapy, which is of particular interest in view of technological developments in small "hot" sources for after loading equipment. It now seems possible to determine "acceptable" ranges of dose rates and corresponding total doses in regard to early and late reactions of normal tissues, depending on the relative dose levels at these tissues.

Significance to Biomedical Research and the Program of the Institute

1. The present model shows promise as a tool toward understanding of time-dose response to conventional or "standard" treatment schedules, as well as some hyper-fractionation schemes and other non-standard schemes.

2. The model promises to become useful to explore, by simulation, other unconventional schemes, and provide reasoned guidance to at least avoid work results especially as regards to late reactions and tumor.

3. The developments as to high dose rate vs. low dose rate treatment are of great practical significance, as they may help change the logistic, economic and possibly the clinical results of brachytherapy.

Proposed Course

1. Continuation of theoretical studies
2. Study of clinical data.

Publications

1. van de Geijn J. Time-dose response of human tumors and normal tissues during and after fractionated radiation treatment. A new model, Radiotherapy and Oncology 1988;12:57-78.

2. van de Geijn J. Incorporating the time factor into the linear-quadratic model. [Letter to the Editor]. Brit J Radiol 1989;62:296-297.

3. van de Geijn J. Modeling of time-dose response of human tumors and normal tissues to radiation treatment. In: Paliwal BR, Fowler JF, Herbert DE, Kinsella TJ, and Orton CG, (eds.) Prediction of response in radiation therapy, pp. 108 - 130. AAPM Symposium Series #7, Proceedings of the Third Internat. Conf. on Dose, Time and Fractionation, Madison WI: Sept. 1988;14-17.

4. Chen J, van de Geijn J, Goffman T. Extra lethal damage due to residual incompletely repaired sublethal damage, in hyperfractionated and continuous radiation treatment, Med Phys 1991;18:488-496.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI CM 06382-05 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapy with Radiolabelled Antibodies: Technical & Dosimetric Aspects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	T. Goffman	Radiotherapist	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	N. Wersto	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	J. Carrasquillo	Nuclear Medicine Physician	NM, CC

COOPERATING UNITS *(if any)*

Nuclear Medicine Department, CC; Diagnostic Radiology Department, CC.

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10.0	8.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Administration of radiolabelled antibodies is a relatively new treatment modality for certain forms of cancer. Much of this field is developmental in nature. In particular, the dosimetry of tumor masses, especially at the microscopic level, is at yet unknown. The Radiation Physics and Computer Automation Section is actively assisting in the implementation of clinical protocols. Current research is in two major areas.

Imaging of the organ-specific distribution patterns on a temporal basis is fundamental to the understanding of antibody kinetics and for large volume radiation dosimetry (at the total organ level). The ability to accurately localize biodistribution patterns using nuclear medicine imaging techniques and to accurately register these images with respect to other imaging modalities (CT or MRI) is essential for obtaining quantitative results.

Dosimetry of microscopic tumor masses is approached through the use of computer modeling. The results, where possible, will be validated using quantitative autoradiography.

Project Description

Objectives: To localize the sites of retention of radiolabelled antibodies and to determine the deposition-retention kinetics as well as the clearance pathways. To determine normal organ radiation doses and tumor dose, if possible on a microscopic level for alpha, beta and gamma emitting radionuclides. To determine the optimum combination of imaging modalities for localization and to determine the lower limits of detection of tumor masses with external imaging devices.

Methods Employed

This project will use small animal models to determine the metabolic pathways of various antibodies and their deposition-retention-excretion kinetics. Phantom studies will be conducted to determine the optimum imaging modalities and their lower limits of detection. These will be confirmed using large animal models. Computer models for determining dose distributions on a microscopic level and for alpha emitting radionuclides will be developed and tested with animal models. Patients under treatment will be imaged, as appropriate, and will be bioassayed using external counting techniques. Biopsies will be taken and used to validate metabolic and dosimetric models for each radiolabelled antibody.

Major Findings

Studies at other institutions indicate that therapy with radiolabelled antibodies offers little advantage over conventional forms of radiation therapy in the treatment of large tumor masses, due to the inhomogeneous distribution pattern of organ uptake. This results in large dose gradients within the treated site. Antibody therapy shows real promise, however, in the treatment of small tumor masses, especially microscopic disease. The problem with this approach is that the size of these masses makes them difficult to localize using traditional nuclear medicine imaging techniques. It may be possible to image these masses by employing other imaging modalities, either singly or in combination. Also, the dose calculational formalism for distributed radionuclide sources (MIRD), may no longer be valid under these conditions, since the range of the particulate radiations may be greater than the dimensions of the tumor mass and the distribution of radioactivity may be inhomogeneous. A new formalism will need to be developed for alpha emitting radionuclides, as their energy deposition pattern differs significantly from beta-gamma emitters.

Significance to Biomedical Research and the Program of the Institute

Radiolabelled antibodies are a new, exciting potential treatment modality. They offer the promise of selectively irradiating tumor masses, while delivering minimal radiation doses to normal tissues. This represents the ideal form of radiation therapy. It is possible that, for some forms of cancer, radiolabelled antibody therapy will supplant chemotherapy as the treatment of choice for microscopic disease.

Proposed Course

To be continued. The SPECT camera dedicated to this project has been be replaced by a dual

head, opposed crystal camera. This will permit us to simultaneously acquire AP and PA whole-body scans and will simplify correction for self-attenuation. Depending on the model purchased, this unit may be SPECT capible. A laser system to facilitate patient alignment has been installed in this room. A method has been devised for registering patients on various imaging devices such as a radiotherapy simulator, CT scanner, MRI scanner and gamma camera table, using an aperture emulator. Phantom studies will shortly commence, first employing simple geometries.Existing humanoid phantoms which will permit imaging via multiple modalities are inadequate. Effort will be directed towards development of an organ phantom which accurately retains its internal anatomy and which can be adequately scanned by CT and MRI as well as gamma cameras, SPECT and PET. Image processing techniques will be developed to correlate images from different scanning modalities to aid in diagnosis and treatment planning. Two alternative methods for quantitating whole-body clearance of gamma-emitting radioisotopes will be instituted and compared. The first uses a dedicated microcomputer with both multichannel analysis and multichannel scaling capabilities, while the latter is a much simpler, less expensive system utilizing a portable, data-logging radiation detector.

Publications

1. Miller RW, Orr K, Goffman TE, Harrington FS, van de Geijn J and Glatstein E. A simple CT aperture emulator for use with a radiotherapy simulator, Int J Rad Oncol Biol Phys 1991 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06383-05 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Development of an Improved Treatment Chair for Radiation Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH: Radiation Oncology Branch

SECTION: Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is intended to design a treatment chair which overcomes the inherent design limitations of commercially available chairs. This chair will function independently of the treatment couch so as to permit opposed field treatment in any orientation in an extended isocentric fashion (the center of rotation will be at a distance greater than the standard isocenter of the accelerator). It will be capable of accurate, reproducible rotation and translation in the lateral, longitudinal and vertical planes. If possible, the chair will function with a standard radiotherapy simulator to permit proper localization, immobilization and treatment planning.

The chair is being designed on the "tool platform" principle. That is, the chair will function as a platform, allowing the attachment of various additional devices which can be placed in such a manner that they permit proper immobilization of the patient without unduly restricting treatment delivery.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

Project Description

Objectives: To develop an independent treatment chair to permit multiple-field radiation therapy at either standard or extended SSD.

Methods Employed

The Radiation Therapy Machine Shop fabricates any chair components and accessories that are needed. Selected patients are placed in the chair for simulation and for their course of therapy. Any problems associated with immobilization and repositioning are analyzed on a daily basis and the necessary modifications are made.

Major Findings

The initial version of the treatment chair permitted opposed-field treatments and could be used with the simulator as well as with any treatment unit. Treatment of some forms of cancer with the patient seated is advantageous. The first version of the chair has been used clinically on several occasions and is easy to align and use. Patient positioning has been improved by using a back rest, a silastic seat cushion, lateral hip restrictors and a new back rest "Tennis Racket". "Extended isocentric" setup can also be accomplished with the chair. This will provide for complete clearance of all obstacles, which in the past have limited the rotational freedom of the chair. Extended isocentric distances of up to 140 cm. can be accommodated with the new simulator.

Significance to Biomedical Research and the Program of the Institute

Treatment of the mediastinum with the patient seated can minimize the amount of lung in the irradiated field, minimizing complications. A combined Waldeyer's/mantle field treatment is possible in this position. Also, low dose rate mantle fields can be used by placing the chair at an extended SSD.

Proposed Course

Currently, the chair has undergone a major modification which incorporates a base-mounted turntable to provide isocentric positioning of the patient. This greatly simplifies the initial set-up and treatment. The vertical stability has also been greatly improved. These modifications still permit the chair to be used with our current simulator. The next development phase will include motor drive for the lateral, longitudinal and rotational dimensions with digital readouts of all coordinate and angular positions and the ability to read coordinate offsets. The third phase will involve interfacing the chair rotation to hardware which permits the simulator to provide limited CT capability. This will allow us to obtain axial CT slices of the patient in the treatment position for radiotherapy planning. Additional attachments for positioning and immobilizing the patient will also be developed.

Publications

1. Miller RW, Raubitschek AA, Harrington FS, van de Geijn J, Ovadia J and Glatstein E. An isocentric chair for the simulation and treatment of radiation therapy patients, Int J Radiat Oncol Biol Physics 1991;21 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06386-04 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radioimmunotherapy of Peritoneal Cancer with I-131 Labeled B72.3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Goffman	Head, Clinical Therapy Section	ROB, NCI
Others:	J. Carrasquillo	Head, Antibodies Project	NM, CC
	R. Neumann	Chief	NM, CC
	J. Reynolds	Senior Investigator	NM, CC
	J. Schlom	Chief	LTIB, NCI
	D. Colcher	Senior Investigator	LTIB, NCI

COOPERATING UNITS *(if any)*: Nuclear Medicine Department, CC; Laboratory of Tumor Immunology and Biology, NCI

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	.5	PROFESSIONAL	0	OTHER	0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In cooperation with the Department of Nuclear Medicine, and the Laboratory of Tumor Immunology and Biology, we have initiated clinical trials for the treatment of peritoneal carcinomatosis. A classical phase one study has begun using escalating doses of I-131 labeled antibody administered intraperitoneally.

Dose limiting toxicity appears still to be bone marrow, with patients with extensive previous chemotherapy being limited to 125-150 mCi. Patients without extensive pretreatment may be able to receive as high as 175 mCi, although this dose level has not been reached yet.

The clinical protocol has been recently revised by Dr. Carrasquillo to more accurately define the maximum tolerated doses. A follow-up protocol using a higher affinity antibody CC49, with Lu-177 for isotope is being written in conjunction with the Medicine Department.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06387-04 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Superoxide Dismutase Mimics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	C. Krishna	Associate	ROB, NCI
	Stephen Hahn	Associate	ROB, NCI
	Tom Goffman	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Shortly after our laboratory discovered that nitroxides, which have been used as EPR spin labels exhibit superoxide dismutase (SOD) activity, we have shown that they are quite effective agents in protecting cells against oxidative stress. Our lead compound, Tempol, a water soluble nitroxide has been shown to protect mammalian cells against superoxide generated from xanthine/xanthine oxidase, and direct hydrogen peroxide cytotoxicity. More recently we have demonstrated that Tempol protects both cells *in vitro* and mice against ionizing radiation. Screening of an additional eight water soluble nitroxides has revealed at least two nitroxides that are more efficient than Tempol. Thus, the nitroxides represent a *new class* of radiation protectors that may have widespread use in protecting humans against radiation. Studies are underway to determine if selected nitroxides might exert differential protection between normal and tumor tissue. Topically applied Tempol has been shown to protect against radiation-induced alopecia in guinea pigs. Tempol has been shown to protect cells against mutation induction mediated by superoxide, hydrogen peroxide, and radiation. Tempol has also been shown to protect cells exposed to various chemotherapy drugs including mitomycin C and SR-4233. Not only might these agents be useful in protecting against certain chemotherapy agents but should be instructive in determining mechanisms of action. Since these agents readily penetrate cell membranes, they may be of use in other areas of medical research such as ischemia/reperfusion injury studies. Published data already confirm their utility in this setting.

Project Description

Objectives: The role that nitroxides play in modifying the cellular response and response in animals to various forms of oxidative stress will be continued. We hope to demonstrate that nitroxides will be useful agents against toxicity mediated by chemotherapy drugs and ionizing radiation and consider their development for clinical use. A major objective will be to continue to evaluate some 20-30 analogues of nitroxides that we have either made in the laboratory or are available. A major emphasis will be placed on determining mechanism of action and the use of analogues in *in vivo* systems.

Methods Employed

Electron spin resonance (EPR) spectroscopy allows the study of free radical chemistry and biology. The study of short lived oxy-radicals (spin trapping) or the rate of interaction of superoxide with oxazolidine nitroxides (stable spin labels) is well suited to the use of EPR. Organic synthesis of different oxazolidine nitroxides will follow straight forward procedures. UV, NMR, IR, and Mass spectroscopy will be used to characterize the chemical nature of the compounds. Cell culture techniques will be used to evaluate drug and radiation modulation. Murine systems will be used to investigate the pharmacology, biodistribution, and metabolism of the different nitroxides.

Major Findings

Our initial observation that nitroxides protect against oxidative stress has been confirmed by other laboratories. Of particular importance was the finding that nitroxides protect both cells and mice against the lethal effects of ionizing radiation. These results establishes the nitroxides as a completely new class of radiation protectors with possible widespread use. Additionally, Tempol was shown to protect against mutation induction in mammalian cells exposed to superoxide, hydrogen peroxide, and x-rays. Radiation-induced alopecia could be greatly reduced by topical application of Tempol.

Significance to Biomedical Research and the Program of the Institute

The study of nitroxide and how they protect against agents which impose oxidative stress will further our understanding and hopefully offer clinical avenues to explore the protection of normal tissues against radiation or chemotherapy. The SOD mimics may have applications in the area of coronary reperfusion, arthritis treatment, inflammation resolution, and decreasing harmful effects of the anthracyclines and bleomycin antineoplastic agents (respective cardiac and lung toxicities), as well as changing the dose response to radiation-induced damage.

Proposed Course

To continue to explore the chemical/biochemical reactions of a series of nitroxide analogues as they apply to protecting cells and animals against a variety of forms oxidative stress. Particular

attention will be directed toward determining if differential radiation protection between normal verus tumor tissues exists for nitroxides. Since nitroxides protect against selected chemotherapy drug cytotoxicity they will be evaluated *in vivo*.

Publications

1. Samuni A, Mitchell JB, DeGraff W, Krishna CM, Samuni U and Russo A. Nitroxide SOD-mimics: modes of action, Free Radical Biology and Medicine 1991;12-13:187-194.

2. Samuni A, Krishna CM, Mitchell JB, Collins CR and Russo A. Superoxide reaction with nitroxides, Free Radical Res Commun 1990;9:241-249.

3. Samuni A, Ahn M, Krishna CM, and Mitchell JB. SOD-like activity of 5-membered nitroxide spin labels. In: I Emerit, L Packer, Auclair C, eds. Antioxidants in therapy and preventive medicine. New York, Plenum Press, 1990;85-92.

4. Samuni A, Godinger D, Aronovitch J, Russo A, and Mitchell JB. Nitroxides block DNA scission and protect cells from oxidative damage, Biochemistry 1991;30:555-561.

5. Samuni A, Winkelsberg D, Pinson A, Hahn SM, Mitchell JB and Russo A. Nitroxide stable radicals protect beating cardiomyocytes against oxidative damage, The Journal of Clinical Investigation, Inc. 1991;87:1526-1530.

6. Pogrebniak H, Matthews BS, Mitchell JB, Russo A, Samuni A, Pass H. Spin trap protection from tumor necrosis factor cytotoxicity, Journal of Surgical Research 1991;50:469-474.

7. Mitchell JB, DeGraff W, Kaufman D, Krishna MC, Samuni A, Finkelstein E, Hahn SM, Gamson J and Russo A. Inhibition of oxygen dependent radiation-induced damage by the nitroxide superoxide dismutase mimic TEMPOL. Archives of Biochemistry and Biophysics 1991 (in press).

8. Goffman T, Cuscela D, Glass J, Hahn S, Krishna MC, Lupton G and Mitchell JB. Topical application of nitroxide protects radiation-induced alopecia in guinea pigs. International Journal of Radiation Oncology, Biology, and Physics 1991 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06388-04 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Superficial Carcinoma of the Bladder with Photoradiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bladder cancer can be subdivided into (1) superficial disease confined to the mucosa or submucosa and (2) muscle-invading disease. Standard therapy for superficial disease confined to the mucosa or submucosa consists of transurethral resection and intravesical chemotherapy (thiotepa, mitomycin C, BCG). Recurrence rates may range from 30%-85% depending upon the grade of tumor and multiplicity of lesions. The concept of a full field defect in patients with carcinoma in-situ in association with a solitary papillary tumor is supported by the high incidence of invasive disease developing within two years following resection alone. Five-year survival rates for patients developing muscle invasive disease (T2/T3A) range from 31%-52%. Early control of superficial disease offers a potential advantage towards reduction of the overall death rate in bladder malignancy. Carcinoma in-situ refractory to intravesical chemotherapy is a particularly troublesome clinical entity, as patients are at high risk for the development of invasive disease and may require removal of the urinary bladder (cystectomy). Recent work with hematoporphyrin derivative ($^{H}p^{D}$) sensitized photodynamic therapy of the bladder mucosa suggests high cytotoxic effect, but low systemic toxicity. This modality may permit treatment of superficial carcinoma of the bladder as well as carcinoma in-situ which may permit bladder preservation with cure of tumor.

Project Description

Professional Personnel Engaged on Project:

W. M. Linehan	Senior Investigator	SB, NCI
M. Walther	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR

Objectives

This is a Phase I trial designed to determine the feasibility of treating patients with superficial bladder carcinoma with a combination of hematoporphyrin derivative (H_pD) and laser light, and to judge tumor response.

Methods Employed

Eligible patients receive hematoporphyrin derivative by intravenous injection. They will subsequently undergo a cystoscopy at which time light is delivered to the bladder. Following treatment, both cystoscopy and urine cytology will be done regularly to assess response to treatment. If partial responses are observed without serious side effects, repeat treatment will be performed. Patients who develop recurrence or invasive bladder cancer will be taken off protocol and referred for appropriate treatment.

Major Findings

Nine patients have been entered on the protocol since 7/89. Two patients remain free of disease, 3 and 20 months after treatment. Five patients have recurred, 4 in the urinary bladder, and 1 in the prostatic urethra out of the light field, all were superficial transitional cell carcinoma of the urinary bladder. Treatment response is pending in 2 patients. All patients have had transient significant bladder irritation secondary to treatment which has been managed symptomatically. This has been less prevalent since we have reduced the sensitizer dose from 2.0 mg to 1.5 mg/kg given 48 hours prior to treatment. One patient who received 2.0 mg/kg of photosensitizer and the highest total light dose to the entire bladder (5,032 joules) developed a contracted bladder and vesico-ureteral reflux after treatment. One patient treated with 2.0 mg/kg of photosensitizer and 3500 joules developed bilateral vesico-ureteral reflux, but he is asymptomatic and free of disease. Since switching to the lower photosensitizer dose of 1.5 mg/kg and light doses of less than 3500 joules, we have not seen any late complications of treatment.

We have developed a light monitoring device utilizing very thin optical fibers which can be passed through the cystoscope and permits measuring of light dose received

at the bladder wall during photodynamic therapy. This is a substantial improvement in dosimetry and should have wide applicability.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially useful mode of curative therapy for selected patients with superficial carcinoma of the bladder. If this is achievable without requiring that the patients have their urinary bladder removed, this will represent a major advance in treatment of superficial carcinoma of the bladder.

Proposed Course

We propose to study a total of 12 patients on the protocol. Once we have evaluated the response to treatment in these 12 patients, we will decide whether to pursue additional studies using this modality in patients with superficial carcinoma of the bladder.

Publications

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06390-03 RO

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bifunctional Chelates for Gallium (III)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. J. McMurry Senior Staff Fellow ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The synthesis and evaluation of bifunctional chelating agents designed to sequester gallium (III) isotopes define the general scope of the project. Taking advantage of the extensive base of chemical literature describing the coordination chemistry of Ga(III), we have established two chelating agents as synthetic targets. The first, a ligand incorporating three catechol binding subunits, forms a trianionic complex with trivalent ions and is expected to display considerable stability at physiological pH. The second ligand is the macrocyclic polyaminocarboxylate NOTA, which is known to form an exceptionally stable neutral complex with Ga(III). A comparison of the relative efficacy of the two very different ligands should assist the evolution of optimal chelating agents for gallium.

The C-14 labeled bifunctional tris-catechol ligand was synthesized and labeled to mAb B72.3. It has been determined that the synthesis of bifunctional NOTA cannot be performed using the techniques developed for the larger macrocycles DOTA and TETA. Therefore, an alternative synthesis of this interesting chelating agent is underway.

Professional Personnel Engaged on the Project:

G. Pippin	Staff Fellow	ROB, NCI
O. Gansow	Senior Investigator	ROB, NCI
M. Brechbiel	Chemist	ROB, NCI

Objectives

We plan to evaluate the utility of the new bifunctional tris (catecholate) chelate for labeling of monoclonal antibody with Gallium (III). This broad objective includes the evaluation of the stability of the metal complex, conjugation with protein, and eventual in vivo studies.

The thermodynamic stability of the Gallium complex will be determined by classical techniques and the metal exchange properties investigated. This data will help us predict whether or not the integrity of the metal complex will be compromised in vivo. Conditions for optimal conjugation of the chelate to antibody will be investigated as will the techniques for labeling with several Gallium isotopes (Ga-66, 67, 68).

Since the Ga-68, 66 radionuclides could be useful for diagnosis by PET and for therapy, respectively, parallel in vivo studies on animal tumor models will be performed with Ga-67, a readily available gamma emitter.

One specific goal of the project is to make the Gallium isotopes useful for PET imaging and consequent accurate dosimetry when delivered to tumor by monoclonal antibody. Thus, when large doses of Ga-66 are subsequently used for tumor therapy, an accurate correlation between dose and therapeutic efficacy may be made.

These new chelating agents are also potentially useful for linkage of the 10.6 hour lead-212 isotope which could deliver alpha-particles to tumors when linked to monoclonal antibody.

We anticipate that these new methodologies will be most useful for the treatment of AIDS-related lymphoma and other blood borne malignancies.

Methods Employed

Standard organic and inorganic synthetic techniques are required for the preparations of the chelate. Evaluation of the labeling efficiency will be achieved using radiochemical tracers (C-14, Ga-67) and UV-VIS spectroscopy.

Major Findings

The C-14 labeled macrocyclic tris-catechol chelating agent was conjugated in high yield to mAb B72.3 and labeled efficiently with ^{111}In and ^{67}Ga. The radiolabeled B72.3 conjugates suffer loss of immunoreactivity, apparently due to the nature of the chelate employed. Preliminary studies with mAb CC-49 suggest this antibody does not suffer the same fate when conjugated with the tris-catechol ligand.

Significance to Biomedical Research and the Program of the Institute

Several Gallium isotopes have desirable properties for applications in nuclear medicine. In particular, Ga-67 78.3 hr, (EC 100%, 93(38%), 185(24%) KeV) and Ga-68 (68 min., B^+, 90% (1.89 MeV, 100%) are suitable for gamma imaging and PET scanning, respectively, while the energetic positron emission of Ga-66 (9.45 hr, B^+,56%(4.2 MeV,51.2%), EC 44%) combined with its half-life of 9.5 hours make it an attractive candidate for radioimmunotherapy. While simple inorganic complexes (e.g., Ga-67 (citrate)) of Ga-67 and Ga-68 are used clinically, it is anticipated that conjugation with monoclonal antibody will greatly enhance the utility of Gallium isotopes. By developing a selective and stable bifunctional chelate for attachment of Gallium to monoclonal antibody, we hope to contribute to the development of site-specific radiopharmaceuticals, in particular, for the treatment of AIDS-related lymphoma.

Publication

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06391-02 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

IUdR as a Radiosensitizer in Unresectable Sarcomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Goffman	Head, Clinical Therapy Section	ROB, NCI
Others:	J. Mitchell	Deputy Chief	ROB, NCI
	A. Russo	Head, Exp. Phototherapy Sec.	ROB, NCI
	J. Cook	Senior Staff Fellow	ROB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC
	S. Rosenberg	Chief	SB, NCI
	S. Steinberg	Head	BDMS, NCI

COOPERATING UNITS *(if any)*

Cancer Nursing Service, CC
Surgery Branch
Biostatistics and Data Management Section

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5-6	4	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This protocol has accrued 16 patients. We have had no problem with patient refusal to be randomized. We have had problems in that many patients have some element of metastatic disease at presentation which makes them ineligible for randomization. We have sent out a flyer and had responses, and we have been able to convince several major universities to send up patients for study. There have been no significant toxicities to date. No publication planned at this time with such small numbers. A new flyer is planned for better "advertising".

Project Description:

Professional Personnel Engaged on the Project:

J. Mitchell, Ph.D.	Deputy Chief	ROB, NCI
A. Russo, M.D., Ph.D.	Head, Exp. Phototherapy Sec.	ROB, NCI
J. Cook, Ph.D.	Senior Staff Fellow	ROB, NCI
R. Smith, R.N.	Cancer Nursing Specialist	CNS, CC
S. Rosenberg, M.D.	Chief	SB, NCI
S. Steinberg, Ph.D.	Head	BDMS, NCI

Objectives: a. The main objective is to assess the results of patients treated with IUdR as a radiosensitizer in the management of patients with unresectable sarcomas and compare the result to similarly irradiated patients without the radiosensitizer; local control and survival will be the important endpoints.

b. In selected patients, to obtain a biopsy after infusion to allow for cell kinetic quantification using flow cytometric techniques and thymidine replacement estimates.

Methods Employed

Patients with non-metastatic unresectable sarcomas of various types will be randomized to be treated with radiation therapy plus Iododeoxyuridine (IUdR), a radiosensitizer.

Major Findings

Too soon to show.

Proposed Course

Continuation of current studies.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06392-02 RO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

IUdR as a Radiosensitizer in Unfavorable Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Goffman	Head, Clinical Therapy Section	ROB, NCI
Others:	J. Mitchell	Deputy Chief	ROB, NCI
	A. Russo	Head, Exp. Phototherapy Sec.	ROB, NCI
	J. Cook	Senior Staff Fellow	ROB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC
	H. Pass	Senior Investigator	SB, NCI
	S. Steinberg	Head	BDMS, NCI

COOPERATING UNITS *(if any)*

Surgery Branch
Biostatistics and Data Management Section
Cancer Nursing Service, CC

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3-6	4	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Overall, we have continued to accrue considerable numbers of patients on this protocol: the focus this year has been on obtaining biopsies as delineated in the original protocol for thymidine replacement. The results have been variable, but no toxicity was encountered in biopsy of these patients. We continue to control a majority of unresectable sarcomas, many of whom now have received prior chemotherapy, for whom there is no practical option. These have been published this year in Cancer. A long-term follow-up of the glioblastoma results has been accepted with revisions in the Journal of Clinical Oncology.

We have started a small pilot study for IUdR in locally advanced head and neck cancers and have documented high IUdR uptake and high response rates.

Project Description

Professional Personnel Engaged on the Project:

J. Mitchell, Ph.D.	Deputy Chief	ROB, NCI
A. Russo, M.D., Ph.D.	Head, Exp. Phototherapy Sec.	ROB, NCI
J. Cook, Ph.D.	Senior Staff Fellow	ROB, NCI
R. Smith, R.N.	Cancer Nursing Specialist	CNS, CC
H. Pass, M.D.	Senior Investigator	SB, NCI
S. Steinberg, Ph.D.	Head	BDMS, NCI

Objectives: a. The objective is to assess the results of patients treated with IUdR as a radiosensitizer in the management of patients who have gross residual cancers, but are not being studied as disease-oriented protocols by the rest of the Cancer Institute. Comparisons will be made with historical controls by emphasizing local control and survival.

b. In selected patients whose tumor is accessible to biopsy, to obtain a biopsy after infusion to allow for cell kinetic quantification using flow cytometric techniques and thymidine replacement estimates.

Methods Employed

Patients with unresectable cancer of relatively low expected responsiveness to radiation therapy will be treated with the radiosensitizer, Iododeoxyuridine, (IUdR) plus irradiation and compared to historical controls.

Major Findings

Too soon to show.

Proposed Course

Continuation of current studies.

Publications

1. Goffman TE, Raubitschek A, Mitchell JB, Glatstein E. The emerging biology of modern radiation oncology, Cancer Research 1990;50:7735-7744.

2. Goffman TE, Tochner Z, Glatstein E. Primary treatment large and massive adult sarcomas: with Iododeoxyuridine and aggressive hyperfractionated irradiation, Cancer 1991;67:572-576.

3. Goffman TE, Dachowski LJ, Bobo H, Oldfield EH, Steinberg SM, Cook J, Mitchell JB, Katz D, Smith S, Glatstein E. Long-term follow-up on NCI phase I/II study of glioblastoma multiforme treated with IdUdr and hyperfractionated irradiation, J Clin Oncol, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06393-02 RO

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Surgery and Photodynamic Therapy for Intraperitoneal Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. F. DeLaney Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*
Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR; Laboratory of Pathology, NCI; Diagnostic Radiology Department, CC; Radiation Oncology Branch, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The patients with tumors diffusely seeding the peritoneal cavity have a poor prognosis with conventional treatment. Ovarian cancer is one tumor which presents with advanced disease diffusely involving the peritoneal surface in approximately 70% of the 23,000 women who develop ovarian cancer annually in the United States. The disease free survival in these patients at 5 years is less than 10% with conventional management, which involves initial debulking surgery followed by multi-agent chemotherapy. We have been able to sterilize an ovarian tumor similar to this in a mouse model using intraperitoneal photodynamic therapy, i.e. a photosensitizer which localizes in tumor and can be activated by light to destroy cancer cells. We have been interested in incorporating this strategy into the management of patients with ovarian cancer and have thus initiated this Phase I trial.

U.S. GOVERNMENT PRINTING OFFICE 1991 O - 864 939

Project Description

Professional Personnel Engaged on Projects:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR
L. Elwood	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	DR, CC
E. Glatstein	Branch Chief	ROB, NCI

Objectives

This is a Phase I study designed to assessed the toxicity and effectiveness of surgical debulking and hematoporphyrin derivative photodynamic therapy at the time of laparotomy in patients with primary or metastatic malignant tumors involving the peritoneal cavity.

Methods Employed

Patients with tumors seeding the surface of the peritoneal cavity who have no known curative options for their particular disease or stage receive Photofrin II photosensitizer by intravenous administration 1.5-2.5 mg/kg. 48-72 hours later the patients undergo laparotomy with surgical debulking of tumor. If tumor can be resected to less than 5 mm thickness, light is delivered to the entire peritoneal surface using appropriate optical fibers connected to lasers. Light dose and drug dose will be subsequently escalated.

Major Findings

This study has been completed. The maximum tolerated photosensitizer/light dose that can be delivered at the time of debulking surgery at laparotomy has been determined. Fifty-four patients have received the photosensitizer and have gone on to laparotomy. Twenty-two patients have had ovarian cancer, 13 have had sarcomas, 8 have had carcinomas of gastro-intestinal organs, 8 have had low grade mucinous tumors,1 patient had an adrenal tumor, and 1 patient each with fallopian tube carcinoma and mesothelioma. Resection/light delivery was successful in 18/22 with ovarian cancer, 12/13 patients with sarcoma, 7/8 patients with a low grade mucinous carcinomas, and 2 of 8 patients with GI primaries. PDT dose was sequentially escalated by increasing the DHE dose from 1.5 to 2.5 mg/kg, shortening the interval between DHE injection and surgery from 72 to 48 hours, and increasing the light dose. Initially, 630 nm red light alone was used and was subsequently combined with 514 nm green light (twice as effective per joule at 630 nm red light, available in higher power from the lasers but only able to penetrate 2-3 mm in tissue).

Later patients received boost doses of 10-15 J/cm^2 with 630 nm red light or 5-7.5 J/cm^2 with 514 nm green light to the diaphragms, abdominal gutters, and/or pelvis. The follow-up range is 1-40 months and median 19 months. Three major PDT complications were seen with red light; small bowel perforations in patients who had undergone bowel resections or tumor resection within enterotomy followed by 2.8-3.0 J/cm^2. Small bowel complications were not seen after switching to less penetrating green light. Dose limiting toxicities were encountered in 2 of the 3 patients at the highest light dose of 5.0 J/cm^2 green light with boost; both patients had pleural effusions that required taps and necessitated postoperative respiratory support for 7-9 days, while 1 of these 2 patients experienced a gastric perforation requiring re-operation. We concluded the maximum, safely tolerated dose of PDT to the entire peritoneal surface after debulking surgery is 3.75 J/cm^2 with 514 nm green light to bowel and mesenteric surfaces. Both diaphragms can be boosted safely to 7.5 J/cm^2 with 630 nm red light, while the abdominal gutters and pelvis can be boosted safely to 10-15 J/cm^2. Twelve of the 39 patients remain free of disease.

Significance to Biomedical Research and the Program of the Institute

Because photodynamic therapy has shown curative potential in an animal model, we are quite interested in bringing this modality in to the clinic for the treatment of patients with ovarian cancer. We would like to incorporate this treatment strategy in to the second look laparotomy which is often done in these patients, currently without known therapeutic benefit. Over the course of this study we have developed an on-line light dosimetry system which we feel we'll have applicability to treatment with light activated compounds at various other sites, including the pleural cavity and bladder.

Proposed Course

We propose Phase II/III studies at these doses in patients with ovarian cancer undergoing second look laparotomy and in patients undergoing resections of primary retroperitoneal sarcomas.

Publications

1. Sindelar WF, DeLaney TF, Tochner Z et. al. Technique of photodynamic therapy for disseminated intraperitoneal malignant neoplasms: Phase I study, Arch Surg 1991;126:318-324.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06394-02 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developing insights into Radiolabelled Antibody dosimetry by computer simulation & experimental procedures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	T. Goffman	Radiotherapist	ROB, NCI

COOPERATING UNITS *(if any)*

	J. Carrasquillo	Nuclear Medicine Physician	NM, CC

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10.0	8.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Radiolabelled monoclonal antibodies are meant to carry radioactivity to be deposited in tumor cells. The pharmaceutical and physiologic processes ultimately governing the actual distribution of antibodies, and thereby the associated dosimetry are complex. Quantitative imaging, both spatial and temporal, of radioactive nuclides is a key element as a basis of clinical dosimetry. Our group is engaged in efforts to examine the potential of combining gammacamera and CT or MRI images as a basis for quantitative clinical dosimetry. Gammacamera images are 2-dimensional (2-D) condensations of information, with relatively low resolution, while sets of CT or MRI slices provide in principle high resolution 3-D information on the underlying anatomy. Our efforts involve

a) the development of computer linkage between the two, so that areas of interest on a gammacamera scan can be located over the 3-D anatomy;
b) establishing the potential for determining the distribution of radioactivity in the layers underlying an area of interest.

Project Description

Objectives: To determine the potential of combining gammacamera and CT or MRI imaging as a basis for clinical dosimetry of radiolabelled monoclonal antibody therapy.

Methods Employed

1. Quantitative linkage of gammacamera and CT images using a Macintosh II computer system.

2. Experimental determination of the "response resolution" of a gammacamera, that is, the response to thin-layer radioactivity, in terms of distance from the detector and away from the line of view, and also as a function of thickness of tissue equivalent overlaying material.

3. Determination of the capability to compute the specific activity in a number of distinct layer-like, simple geometry compartments across the line of view.

4. Determination of the capability to compute the specific activity in a number of distinct organ-shaped compartments, each of different, but uniform specific activity.

5. If still meaningful, extension to patient data.

Special attention will be paid to the influence of counting statistics.

Major Findings

1. The linking of CT and gammacamera (GC) images has been completed. It is now possible to display a camera image, mark "landmarks, find the respective Ct slice(s), find the marks, thus calibrating the GC image, define an area of interest in the GC image and see the "column" in the appropriate CT slice.

2. The GC count reponse in air is essentially independent of distance. The volume from which reponse is contributing is essentially a cylinder with a diameter about equal to that of the area of interest.

The other experiments are in progress. Access to the equipment is too limited because of heavy service obligations in the Nuclear Medicine division.

Significance to Biomedical Research and the Program of the Institute

Quantitation of dosimetry, with reasonable spatial resolution is essential to the evaluation of clinical applications of radiolabelled mAbs. Sofar, the calculation methods are far too crude. The GC is the imaging tools fairly readily available for imaging of isotope of practical interest in the present

context. Its resolution is intrinsically poor, and the image is essentially 2-D. CT and MRI can provide 3-D information, so that tissues and organs under the ROI can be identified. The present project aims at establishing the realistic potential of the combination CT + GC as imaging tools toward clinically useful radiolabelled antibody dosimetry. Much will depend on the uniformity of specific activity per layer, or organ, the number of distinct layers or organs involved, and therefore the number of independent areas of interest needed, the differences in specific activity, and the counting statistics available. Whatever the outcome as to practical clinical value, it will have practical implications.

Proposed Course

This project is to be continued, with the above steps 3 - 5.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06395-02 RO

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Solution Chemistry of Metal-Ions Used in Radioimmunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	C. G. Pippin	Staff Fellow	ROB, NCI
Others:	T. J. McMurry	Senior Staff Fellow	ROB, NCI
	O. A. Gansow	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The prime objective of this project is to develop an understanding of the fundamental solution chemistry of metal ions utilized in radioimmunotherapy, especially bismuth and lead. To accomplish this objective, we need to understand the coordination chemistry of the metal ions and, in particular, their kinetic and thermodynamic behaviors in aqueous solutions. This information may be used to develop radiolabeled monoclonal antibody (mAb) systems and to assess the factors which control the retention of the metal ions bound to mAb conjugates
in vivo. Two systems investigated during the past year were:

1. Pb(II) Reactions with DOTA (H_4DOTA = 1,4,7,10-Tetraazacyclododecane-N,N',N'',N'''-tetraaceticacid). The rates of formation of the 1:1 complexes of $Pb(DOTA)^{2-}$ were studied by stopped-flow spectrophotometric techniques. The empirical form of the rate law was found to be: rate = a[Pb(II)] [DOTA]/1+b[Pb(II)] at constant acidity and $[Pb]_{total} >> [DOTA]_{total}$. A mechanism consistent with this result is the fast formation of a Pb(DOTA) intermediate which rearranges during the rate-limiting-step. This kinetic information was successfully applied in the preparation of Pb-203 mAb conjugates of the mAb B72.3-DOTA. In collaborative studies described elsewhere, the biodistribution and images of these conjugates were obtained in tumor bearing mice.

2. Bi(III) Reactions with Polyaminocarboxylate Ligands. The bismuth(III) complexes of polyaminocarboxylate ligands EDTA, DTPA, DCTA, CyDTPA were prepared and characterized by physical methods (elemental analysis, NMR, FAB mass spec.). The Bi(III) complex of EDTA formed crystals of two structural modifications, one of which has been characterized in the solid-state by x-ray diffraction. Currently, the coordination chemistry of these complexes is being investigated.

The significance of the project is the ability to probe the chemistry of the metal ions and translate the chemical information to radioimmunotherapy systems.

Publication

1. Clem R, Lambrecht RM, Mirzadeh S, Pippin CG, Brechbiel MW, Roselli M, Gansow OA, Colcher D. Radiochemistry of Pb-203 for radiolabelling antibody conjugates, J Labeled Compds and Radiopharm 1991;30:327.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06396-01 RO

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Invasive Carcinoma of the Bladder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L. Pierce Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch (Urology), NCI; Medicine Branch, NCI; Biostatistics and Data Management Section, NCI; Surgical and Cytopathology, NCI; Diagnostic Radiology, CC

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	2	0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with localized carcinoma of the bladder will be treated with external beam irridiation followed by an implant of the bladder tumor with iridium-192 following cystotomy and modified pelvic node dissection. Patients will receive cisplatin chemotherapy as a radiation sensitizer concurrent with external beam therapy and, following removal of the implant, will receive chemotherapy consisting of methotrexate, cisplatin, vinblastine (MCV) in an attempt to sterilize any potential residual disease. The feasibility, therapeutic efficacy and toxicity of this treatment approach will be evaluated. This is a pilot study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03800-21 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surgical Consultants & Collaborative Research Involving Surgical Services at NIH

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.A. Rosenberg	Chief of Surgery, NCI	SURG, NCI
Others:	Entire Staff	Surgery Branch	SURG, NCI

COOPERATING UNITS *(if any)*

GD Aurbach (NIAMDD), JL Doppman (CC), E Glatstein (NCI), J Robbins (NIAMDD), L Liotta (NCI), C Myers (NCI), R Wittes (NCI), P Pizzo (NCI), J Gardner (NIAMDD)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency, as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital. Increasing surgery in AIDS patients is being performed.

Secondly, the Surgery Branch collaborates in the procurement of tissue for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissue.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I.

Over 1000 consultations were received last year from other NCI Branches as well as other NIH institutes.

1. Perry RR, Rosenberg RK, Pass HI. Tracheoesophageal fistula in the patient with lymphoma: case report and review of the literature, Surgery 1989;105:770-7.

2. Perry RR, Nieman LK, Cutler GB Jr, Chrousos GP, Loriaux DL, Doppman JL, Travis WD, Norton JA. Primary adrenal causes of Cushing's Syndrome: diagnosis and surgical management, Ann Surg 1989;210:59-68.

3. Haas GP, Pittaluga S, Gomella L, Travis WD, Sherins RJ, Doppman JL, Linehan WM, Robertson C. Clinically occult Leydig cell tumor presenting with gynecomastia, J Urol 1989;142:1364-8.

4. Fraker DL, Norton JA. The role of surgery in management of islet cell tumors, Gastroint Endocrino 1989;18:805-30.

5. Sheppard BC, Norton JA, Doppman JL, Maton PN, Gardner JD, Jensen RT. Management of islet cell tumors in patients with multiple endocrine neoplasia: a prospective study, Surgery 1989;106:1108-17.

6. Friedman B, Darling G, Norton J, Hamby L, Metcalfe D. Splenectomy in the management of systemic mast cell disease, Surgery 1990;212:621-8.

7. Perry RR, Keiser HR, Norton JA, Wall RT, Robertson CN, Travis W, Pass HI, Walther MM, Linehan WM. Surgical management of pheochromocytoma with the use of metyrosine, Ann Surg 1990;212:621-8.

8. Norton JA, Shawker TH, Doppman JL, Miller DL, Fraker DL, Cromack DT, Gorden P, Jensen RT. Localization and surgical treatment of occult insulinomas, Ann Surg 1990;212:615-20.

9. Doherty GM, Norton JA. Preoperative and intraoperative localization of gastrinomas, Problems Gen Surg 1990;7:521-32.

10. Norton JA, Jensen RT. Unresolved surgical issues in the management of patients with Zollinger-Ellison syndrome, World J Surg 1991;(15):151-9.

11. Danforth DN, Fraker DL. Splenectomy for the massively enlarged spleen, Am Surgeon 1991;57:108-13.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03801-21 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies in Cancer Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S.A. Rosenberg	Chief of Surgery, NCI	SURG, NCI
Others:	Entire Staff	Surgery Branch	SURG, NCI

COOPERATING UNITS *(if any)*

Others: None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant diseases. The major emphasis of these studies is in the treatment of soft tissue sarcomas, osteogenic sarcomas, colorectal cancer, gastric cancer, renal cell, mesothelioma, and melanoma with emphasis on the use of combined treatment modalities in addition to surgery.

1. Sindelar WF. Clinical experience with regional pancreatectomy for adenocarcinoma of the pancreas, Arch Surg 1989;124:127-32.

2. Ward BA, Miller DL, Frank JA, Dwyer AJ, Simmons JT, Chang R, Shawker TH, Choyke P, Chang AE. Prospective evaluation of hepatic imaging studies in the detection of colorectal metastases: correlation with surgical findings, Surgery 1989;105:180-7.

3. Edington HD, Evans S, Sindelar WF. Reconstruction of a functional hemidiaphragm with use of omentum and latissimus dorsi flaps, Surgery 1989;105:442-5.

4. Sindelar WF, Kinsella TJ. Intraoperative radiation therapy for locally advanced cancers, South Med J 1989;82:358-63.

5. Ahn C, Sindelar WF. Bilateral radical neck dissection: report of results in 55 patients, J Surg Oncol 1989;40:252-5.

6. Pass HI, Delaney T, Smith PD, Bonner R, Russo A. Bronchoscopic phototherapy at comparable dose rates: early results, Ann Thorac Surg 1989;47:693-9.

7. Jablons D, Steinberg SM, Roth J, Pittaluga S, Rosenberg SA, Pass HI. Metastasectomy for soft tissue sarcoma. Further evidence for efficacy and prognostic indicators, J Thorac & Cardiovasc Surg 1989;97:695-705.

8. Bock SN, Lee RE, Fisher B, Rubin JT, Schwartzentruber DJ, Wei JP, Callender DPE, Lotze MT, Pizzo PA, Rosenberg SA. A prospective randomized trial evaluating prophylactic antibiotics to prevent triple-lumen catheter-related sepsis in patients treated with immunotherapy, J Clin Oncol 1990;8:(91):161-9.

9. Danforth DN JR, Lippman ME, McDonald H, Bader J, Egan E, Lampert M, Steinberg SM, Swain SM. Effect of preoperative chemotherapy on mastectomy for locally advanced breast cancer, Am Surg 1990;30:342-50.

10. Ward B, McGarvey C, Lotze MT. Excellent shoulder function is attainable after partial or total scapulectomy, Arch Surg 1990;50:2463-9.

11. Glenn GM, Choyke PL, Zbar B, Linehan WM. Von Hippel-Lindau disease. Clinical review and molecular genetics. Problems in Urol, 1990;4(2):312-30.

12. Pastakia B, Chang V, McDonald H, Danforth DN. Immediate postexcision mammography for occult noncalcified breast lesions, Southern Med J 1990;83(1):30-3.

13. Alexander HR, Candela FC, Dershaw D, Kinne DW. Needle-localized mammographic lesions. Arch Surg 1990;125:621-8.

14. Griffith KD, Chang AE, Sugarbaker PH. Second hepatic resections in patients with liver metastases from colorectal carcinoma. In: Jakesz R, Rainer H, eds. Progress in regional cancer therapy. Berlin-Heidelberg: Springer-Verlag, 1990;46-51.

15. Norton JA. Carcinoembryonia antigen. New applications for an old master, Ann Surg 1991;213:95-7.

16. Lotze MT. Repeat hepatic resections for colorectal metastases. [Letter to the Editor], Surg 1991;109:347-8.

SURGICAL SERVICES DEPARTMENT

ANNUAL STATISTICS

April 1990 - March 1991

TOTAL PROCEDURES	HOURS	INSTITUTES/OTHERS	TOTAL PROCEDURES
386	1227.75	Ward (NCI)	76 Emergencies
765 1/2	1776.75	Consult (NCI)	145 Add-Ons
54 1/2	95.75	Med.Br. (NCI)	348 Cancellations
1206	3100.25	TOTAL (NCI)	303 OPD's
			2WCSR
1206	3100.25	NCI	ICU-2J
17	27.50	NHLBI	2 MICU-10D
167 1/2	780.50	NINCDS	59 Radiation
125	172.75	Med. Neuro	
79	169.00	NEI	
112 1/2	196.75	ENT	
38 1/2	123.75	NIDR	1,798 Total Cases
7 1/2	24.00	Orthopedics	4,666 Total Hours
33	50.75	NICHD	
12	20.75	Other (outside consults)	

MONTHLY SUMMARY

April	152	Total Procedures	October	145	Total Procedures
	364.75	Total Hours		384.25	Total Hours
May	156	Total Procedures	November	150	Total Procedures
	378.25	Total Hours		377.75	Total Hours
June	152	Total Procedures	December	130	Total Procedures
	390.00	Total Hours		291.00	Total Hours
July	146	Total Procedures	January	143	Total Procedures
	386.25	Total Hours		419.75	Total Hours
August	170	Total Procedures	February	159	Total Procedures
	476.00	Total Hours		404.75	Total Hours
September	150	Total Procedures	March	145	Total Procedures
	425.25	Total Hours		368.00	Total Hours

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03811-17 SURG

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Immunotherapy of Animal and Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.A. Rosenberg	Chief of Surgery	SURG, NCI
Others:	P. Hwu	Clinical Associate (CO)	MB, NCI
	A. Asher	Staff Fellow	SURG, NCI
	O. ElBadry	NCI General Fellow	SURG, NCI
	R. Zakut	Expert	SURG, NCI
	J. Weber	Senior Investigator	SURG, NCI
	J. Yannelli	Expert	SURG, NCI
	Y. Kawakami	Visiting Associate	SURG, NCI
	A. Kasid	Visiting Scientist	SURG, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
14	8	6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attempts are being made to develop new immunotherapy and gene therapy of patients with advanced cancer. A variety of animal models are being used to test the effects of lymphokine activated killer (LAK) cells, tumor infiltrating lymphocytes (TIL) and combinations of lymphokines including interleukin-2, tumor necrosis factor and alpha-interferon in the treatment of experimental animal tumors. Current research is attempting to define the factors necessary for achieving successful adoptive immunotherapy in experimental animal models. Efforts are directed at transducing new genes into tumor infiltrating lymphocytes that can increase their therapeutic effectiveness. Marker genes coding for neomycin phosphotransferase have been transduced into TIL. More recently genes coding for tumor necrosis factor (TNF) have been transduced and expressed in TIL. Cytokine genes are being inserted into tumors to increase their immunogenicity. Attempts to use adoptive immunotherapy approaches to the treatment of patients with AIDS are being explored.

A variety of clinical trials are also in progress exploring the application of new adoptive immunotherapies to patients with advanced cancer. Clinical trials are exploring the value of lymphokine activated killer cells and interleukin-2, high-dose interleukin-2 alone, combinations of cytokines, tumor infiltrating lymphocytes and gene modified tumor infiltrating lymphocytes.

1. Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF, Rosenberg SA. Human gene transfer: characterization of human tumor infiltrating lymphocytes as vehicles for retroviral mediated gene transfer in man, Proc Natl Acad Science 1990;87:473-7.

2. Linehan WM, Robertson CN, Rosenberg SA. Adoptive immunotherapy of renal cell carcinoma using lymphokine activated killer cells and recombinant interleukin-2. In: Williams RD ed. Advances in urologic oncology: treatment perspectives, New York: Pergamon Press, 1990;37-54.

3. Bock SN, Lee RE, Fisher B, Rubin JT, Schwartzentruber D, Wei JP, Callender DPE, Yang JC, Lotze MT, Pizzo PA, Rosenberg SA. A prospective randomized trial evaluating prophylactic antibiotics to prevent triple-lumen catheter-related sepsis in patients treated with immunotherapy, J Clin Oncol 1990;8:161-9.

4. Rosenberg SA. Biologic therapy of cancer using recombinant cytokines: Current status and future possibilities, Editorial, Mediscript 1990;11-8.

5. Cameron RB, Spiess PJ, Rosenberg SA. Synergistic antitumor activity of tumor infiltrating lymphocytes, interleukin-2, and local tumor irradiation: Studies on the mechanism of action. J Exp Med 1990;171:249-63.

6. Haas GP, Solomon D, Rosenberg SA. Tumor infiltrating lymphocytes from non-renal urologic malignancies, Cancer Immunol Immunother 1990;30:342-50.

7. Barth RJ, Bock SN, Mule JJ, Rosenberg SA. Unique murine tumor associated antigens identified by tumor infiltrating lymphocytes, J Immunol 1990;144:1531-7.

8. Mule JJ, McIntosh JK, Jablons DM, Rosenberg SA. Antitumor activity of recombinant interleukin-6 in mice, J Exp Med 1990;171:629-36.

10. Rosenberg SA. Adoptive immunotherapy for cancer, Scientific American 1990:262:62-9.

11. Wiebke EA, Custer MC, Rosenberg SA, Lotze MT. Cytokines alter target cell susceptibility to lysis: I. Evaluation of non-MHC restricted effectors reveals differential effects on natural and lymphokine-activated killing, J Biol Resp Mod 1990;9:113-26.

12. Eisenthal A, Cameron RB, Rosenberg SA. Induction of antibody-dependent cellular cytotoxicity in vivo by IFN-alpha and its antitumor efficacy against established B16 melanoma liver metastases when combined with specific anti-B16 monoclonal antibody, J Immunol 1990;144:4463-71.

13. Topalian SL, Kasid A, Rosenberg SA. Immunoselection of a human melanoma resistant to specific lysis by autologous tumor infiltrating lymphocytes: Possible mechanisms for immunotherapeutic failures, J 1990;144:4487-95.

14. Hauser GJ, McIntosh JK, Travis WD, Rosenberg SA. Manipulation of oxygen radical scavenging capacity in mice alters host sensitivity to the toxicity of recombinant human tumor necrosis factor toxicity, but does not interfere with its anti-tumor efficacy, Cancer Res 1990;50:3503-8.

15. Jablons D, Bolton E, Mertins S, Rubin M, Pizzo P, Rosenberg SA. Lotze MT. Interleukin-2 based immunotherapy alters circulating neutrophil Fc receptor expression and chemotaxis, J Immunol 1990;144:3630-6.

16. McIntosh JK, Mule JJ, Travis WD, Rosenberg SA. Studies of the effects of recombinant human tumor necrosis factor on autochthonous tumor and transplanted normal tissue in mice, Cancer Res 1990;50:2463-9.

17. Kragel AH, Travis WD, Feinberg L, Pittalugia S, Striker LM, Roberts WC, Lotze MT, Yang JJ, Rosenberg SA. Pathologic findings associated with interleukin-2 based immunotherapy for cancer: A postmortem study of 19 patients, Human Path 1990;21:493-502.

18. Lotze MT, Custer MC, Bolton ES, Wiebke EA, Kawakami Y, Rosenberg SA. Mechanisms of immunologic antitumor therapy: Lessons from the laboratory and clinical applications, Human Immunol 1990;28:198-207.

19. Topalian SL, Rosenberg SA. Cellular immunotherapy of cancer, Critical Care Med 1990;18:S144.

20. Belldegrun A, Kasid A, Uppenkamp M, Rosenberg SA. Lymphokine mRNA profile and functional analysis of a human CD4+ clone with unique antitumor specificity isolated from renal cell carcinoma ascitic fluid, Cancer Immunol. Immunother. 1990;31:1-10.

21. Yang JC, Perry-Lalley D, Rosenberg SA. An improved method for growing murine tumor infiltrating lymphocytes with in vivo antitumor activity, J Biol Resp Modif 1990;9:149-59.

22. Huang CM, Elin RJ, Rudel M, Sliva C, Elin RJ, Lotze MT, Rosenberg SA. Changes in laboratory results for cancer patients treated with interleukin-2, Clinical Chemistry 1990;36:431-4.

23. Topalian SL, Rosenberg SA. Tumor infiltrating lymphocytes (TIL). Evidence for specific immune reactions against growing cancers in mice and humans. In: DeVita VT, Hellman S, Rosenberg SA eds. Important advances in oncology. 1990;19-41.

24. Skornick Y, Topalian S, Rosenberg SA. Comparative studies of the long-term growth of lymphocytes from tumor infiltrates, tumor-draining lymph nodes and peripheral blood by repeated in vitro stimulation with autologous tumor, J Biol Resp Mod 1990;9:431-8.

25. Knazek RA, Wu YW, Aebersold PM, Rosenberg SA. Culture of human tumor infiltrating lymphocytes in hollow fiber bioreactors, J Immunol 1990;127:29-37.

26. Rosenberg SA. Immunotherapy with recombinant cytokines and activated lymphocytes in patients with advanced cancer: review of Surgery Branch, NCI experience. In: Salmon SE, ed. Adjuvant therapy of cancer, Philadelphia: WB Saunders, 1990;33-8.

27. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, Merino MJ, Culver K, Miller ADE, Blaese RM, Anderson WF. Gene transfer into humans: immunotherapy of patients with advanced melanoma using tumor infiltrating lymphocytes modified by retroviral gene transduction, N Engl J Med 1990;23:570-8.

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29. Robertson CN, Linehan WM, Pass HI, Gomella LG, Haas GP, Berman A, Merino M, Rosenberg SA. Preoperative cytoreductive surgery in patients with metastatic renal cell carcinoma treated with adoptive immunotherapy with interleukin-2 or interleukin-2 plus LAK cells, J Urol 1990;144:614-8.

30. Alexander RB, Rosenberg SA. Long term survival of adoptively-transferred tumor infiltrating lymphocytes in mice, J Immunol 1990; 145:1615-20.

31. Rosenberg SA. The immunotherapy of human cancer: From laboratory to bedside. In: Lotze MT, Finn OJ, eds. Cellular immunity & the immunotherapy of cancer. New York: Wiley-Liss, Inc. 1990;383-93.

32. Mule JJ, Rosenberg SA. Murine sarcomas: definition of elements responsible for successful immunotherapy. In: Lotze MT, Finn OJ, eds. Cellular immunity and the immunotherapy of cancer. New York: Wiley-Liss, Inc. 1990;223-34.

33. Kragel AH, Travis WD, Steis RG, Rosenberg SA, Roberts WC. Myocarditis or acute myocardial infarction associated with interleukin-2 therapy for cancer, Cancer 1990;66:1513-6.

34. Hom SS, Topalian SL, Rosenberg SA. MHC class I antigen restriction of tumor recognition by lymphocytes infiltrating human melanomas, Surg Forum 1990;41:428-9.

35. Weber JS, Rosenberg SA. Effects of murine tumor I major histocompatibility complex expression on antitumor activity of tumor-infiltrating lymphocytes, J Natl Cancer Inst 1990;82:755-61.

36. Aebersold P, Kasid A, Rosenberg SA. Selection of gene-marked tumor infiltrating lymphocytes from post-treatment biopsies: a case study, Human Gene Therapy 1990;1:373-84.

37. Fox BA, Spiess PJ, Kasid A, Puri R, Mule JJ, Weber JS, Rosenberg SA. In vitro and in vivo antitumor properties of a T-cell clone generated from murine tumor-infiltrating lymphocytes, J Biol Resp Mod 1990;9:499-511.

38. Lefor AT, Rosenberg SA. The specificity of lymphokine-activated killer (LAK) cells in vitro: fresh normal murine tissues are resistant to LAK-mediated lysis, J Surg Res 1991;50:15-23.

39. Rosenberg SA. Biologics in diagnosis and treatment of human disease: portents of things to come. In: Harrison DC, Osterweis M, Rubin ER, eds. Preparing for science in the 21st century. Assoc Academic Health Centers, Washington, D.C., 1991;34-9.

40. Morecki S, Karson E, Cornetta K, Kasid, Aebersold P, Blaese RM, Anderson WF, Rosenberg SA. Retrovirus-mediated gene transfer into CD4+ and CD8+ human T cell subsets derived from tumor infiltrating lymphocytes and peripheral blood mononuclear cells, Cancer Immunol Immunother 1991;32:342-52.

41. Mule JJ, Jicha DL, Aebersold PM, Travis WD, Rosenberg A. Disseminated human malignant melanoma in congenitally immune-deficient (Bg/Nu/Xid) mice, J Natl Cancer Inst 1991;83:350-5.

42. Barth RJ, Mule JJ, Spiess PJ, Rosenberg SA. Interferon gamma and tumor necrosis factor have a role in tumor regressions mediated by murine CD8+ tumor infiltrating lymphocytes, J Exp Med 1991;173:647-58.

43. Culver K, Cornetta K, Morgan R, Morecki S, Aebersold P, Kasid A, Lotze M, Rosenberg SA, Anderson WF, Blaese RM. Lymphocytes as cellular vehicles for gene therapy in mouse and man, Proc Natl Acad Sci USA 1991;88:3155-9.

44. Wong RA, Alexander RB, Puri RK, Rosenberg SA. In vivo proliferation of adoptively transferred tumor-infiltrating lymphocytes in mice, J Immunol 1991;10:120-30.

45. Karpati RM, Banks SM, Malissen B, Rosenberg SA, Sheard MA, Weber JS, Hodes RJ. Phenotypic characterization of murine tumor-infiltrating T lymphocytes, J Immunol 1991;146:2043-51.

46. Rosenberg SA. Development of adoptive cellular immunotherapies for the treatment of cancer. In: Broder S, ed. Molecular foundations of oncology. Baltimore: Williams & Wilkins, 1991;153-93.

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51. Rosenberg SA. The development of new approaches to the immunotherapy of cancer using recombinant cytokines and gene transfer. In: Najarian JS, Delaney JP, eds. Progress in cancer surgery. Philadelphia: JB Lippincott Co, 1991;1-6.

52. Mulé JJ, Rosenberg SA. Interleukin-2: Preclinical trials.In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott Co, 1991;142-158.

53. Lotze MT, Rosenberg SA. Interleukin-2: Clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott Co, 1991;178-96.

54. Yang JC, Rosenberg SA. Adoptive cellular therapy: Preclinical studies. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott Co, 1991;197-213.

55. Rosenberg SA. Adoptive cellular therapy: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott Co, 1991;214-36.

56. Aebersold P, Hyatt C, Johnson S, Hines K, Korcak L, Sander M, Lotze MT, Topalian SL, Yang JC, Rosenberg SA. Lysis of autologous melanoma cells by tumor infiltrating lymphocytes is associated with clinical response, J Natl Cancer Inst; in press.

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61. Spencer WF, Linehan WM, Walther MM, Lotze MT, Topalian SL, Yang JC, Merino MJ, Lange JR, Pockaj BA, Rosenberg SA. Immunotherapy with interleukin-2 and alpha interferon in patients with metastatic renal cell cancer with in situ primary cancers: A pilot study, J Urol; in press.

62. Pockaj BA, Rosenberg SA. Lack of antihypertensive effect of interleukin-2 administration in humans, Ann Int Med; in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06654-14 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Studies in Malignant Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: W.F. Sindelar Senior Investigator SURG NCI

COOPERATING UNITS *(if any)*

Others: Radiation Oncology Branch

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Patients with gastrointestinal carcinomas have been studied for evidence of reac- tivity against tumor-associated determinants expressed on both fresh and cultured syngeneic or allogeneic tumor cells using immunoperoxidase staining techniques. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and have been investigated for possible applications to immunotherapy or methods of immunodiagnosis. Monoclonal antibodies have been developed to tumor-associated determinants in both hamster and human pancreatic cancers. Tolerance of various normal and surgically-manipulated tissues to intraoperative radiotherapy has been investigated in dogs to determine both acute and long-term toxicity from radiation effects. Clinical trials of intraoperative radiotherapy have been per-formed including feasibility and developmental studies, randomized trials in resectable and unresectable pancreatic carcinoma, randomized trials in gastric carcinoma, and randomized trials in retroperitoneal sarcomas. Tolerance of normal and surgically-manipulated tissues to photodynamic therapy using hematoporphyrin derivatives and laser light has been investigated in dogs to determine toxicity and to establish dose levels applicable for clinical practice. Clinical trials of assessing the feasibility of intraperitoneal photodynamic therapy have been performed for the treatment of peritoneal carcinomatosis and peritoneal surface malignancies, including ovarian carcinoma, metastatic gastrointestinal carcinoma, and sarcomatosis. Randomized trials of intraperitoneal photodynamic therapy in retroperitoneal sarcomas have been initiated.

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2. DeLaney TF, Sindelar W, Smith P, Friauf W, Pass H, Russo A, Thomas G, Dachowski L, Cole J, Glatstein E. Initial experience with photodynamic therapy for intraperitoneal carcinomatosis. In: Sharp F, Mason WP, Leake RE, eds. Ovarian Cancer. Biological and Therapeutic Challenges. New York: WW Norton. 1990;371-82.

3. Kinsella TJ, Sindelar WF. Normal tissue tolerance to intraoperative radiation therapy. Experimental and clinical studies. In: Vaeth JM, Mayer JL, eds. Radiation Tolerance of Normal Tissues. New York: S Karger. 1990;202-14.

4. Jensen JC, Pass HI, Sindelar WF, Norton JA. Recurrent or metastic disease in select patients with adrenocortical carcinoma. Aggressive resection vs chemotherapy, Arch Surg 1991;126:457-61.

5. Sindelar WF, DeLaney TF, Tochner Z, Thomas GF, Dachoswki LJ, Smith PD, Friauf WS, Cole JW, Glatstein E. Technique of photodynamic therapy for disseminated intraperitoneal malignant neoplasms. Phase I Study, Arch Surg 1991;126:318-24.

6. DeLaney TF, Tochner Z, Sindelar WF, Pass HI, Thomas GF, Harrington FS, Smith PD, Mitchell JB. A light diffusing device for intraoperative photodynamic therapy in the peritoneal or pleural cavity, Lasers Med Surg 1991; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06657-09 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Studies with Cytokines and Clinical Studies with Endocrine Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Norton	Senior Investigator	SURG, NCI
Others:	H.R. Alexander	Expert	SURG, NCI
	C. Buresh	Biologist	SURG, NCI
	G. Doherty	Clinical Associate (CO)	SURG, NCI
	M. Block	Clinical Associate (CO)	SURG, NCI
	A. Thom	Clinical Associate (CO)	SURG, NCI
	M. Zeiger	Clinical Associate (CO)	SURG, NCI
	J. Lange	Clinical Associate (MSF)	SURG, NCI
	S. Carty	Clinical Associate (MSF)	SURG, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Surgical Metabolism Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Repetitive intraperitoneal sublethal doses of human tumor necrosis factor α (TNF) protect rats against the lethality, hypotension and hypothermia of gram negative sepsis induced by cecal ligation and puncture. The protective effects of TNF is associated with induction of the gene for manganous superoxide dismutase (MnSOD). Similarly, a single iv dose of either interleukin-1(IL-1) or TNF produces similar protection through a similar mechanism. Moreover, a specific receptor antagonist to IL-1 protects mice against the lethality of endotoxin (LPS) when given iv ½h following a lethal dose of endotoxin. This finding implies that IL-1 (in addition to TNF) is responsible for the lethality of LPS and that the IL-1 receptor antagonist may be a new treatment strategy to reverse lethal endotoxemia. Furthermore, interferon-γ may also be responsible for the lethality of LPS and the cachexia of cancer. Specific antibodies to it reverse the lethality of LPS when administered 6h before LPS and the late cachectic decline of tumor-bearing rats resulting in prolonged survival. Pentoxifyline inhibits TNF secretion by macrophages both in vitro and in vivo. It inhibits secretion at the level of TNF gene transcription. It may have promise in conditions that are mediated through excessive TNF secretion.

Since 1980, 73 patients who presented with localized disease had surgery to resect all gastrinoma as part of a prospective strategy to manage patients with Zollinger-Ellison syndrome (ZES). The immediate cure-rate was 58% and the long-term (5-year) cure rate was 38%. Of the patients who presented with liver metastatic disease, the 5 year survival was 20%. This study suggests that there are 2 groups of patients with ZES: localized disease, the majority of patients who can be cured, and distant disease, approx ¼ of patients who need more aggressive anti-tumor treatment.

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5. Langstein HN, Doherty GM, Fraker DL, Buresh CM, Norton JA. The roles of interferon-gamma and tumor necrosis factor in an experimental rat model of cancer cachexia, Cancer Res 1991;51:2302-6.

6. Sheppard BC, Norton JA. Tumor necrosis factor and interleukin-1 protection against the lethal effects of tumor necrosis factor, Surgery 1991;109:698-705.

7. Axiotis CA, Merino MJ, Ain K, Norton JA. Papillary endothelial hyperplasia in the thyroid following needle aspiration, Arch Pathol Lab Med 1991;115:240-2.

8. London JB, Shawker TH, Doppman HL, et al. Prospective assessment of abdominal untrasound in patients with Zollinger Ellison syndrome, Radiology 1991;178:763-7.

9. Doppman JL, Miller DL, Chang R, Gorden P, Norton JA. Insulinomas localization with selective intraarterial injection of calcium, Radiology 1991;178:237-41.

10. Bale AE, Norton JA, Wong EL, et al. Allelic loss on chromosome II in hereditary and sporadic tumors related to familial multiple endocrine neoplasia type 1, Cancer Res 1991;51:1154-57.

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12. Langstein HN, Norton JA. Mechanisms of cancer cachexia, Hematol/Oncology Clinics of NA 1991;5:103-23.

13. Norton JA. Cachectin/tumor necrosis factor and cancer cachexia. In: The role of nutrients in cancer treatment. Ross Conference, 1991;51-9.

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15. Fraker DL, Travis W, Merendino J, et al. Locally recurrent parathyroid neoplasms as a cause for recurrent and persistent hyperparathyroidism, Ann Surg 1991;213:58-65.

16. Norton JA, Jensen RT. Unresolved surgical issues in the management of patients with Zollinger-Ellison syndrome, World J Surg 1991;15:151-159.

17. Doherty GM, Jensen JC, Alexander HR, Buresh C, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription, Surgery 1991; in press.

18. Norton JA, Doppman JL, Jensen RT. Curative resection in Zollinger-Ellison syndrome: results of a 10 year prospective study, Ann Surg 1991; in press.

19. Thom AK, Norton JA, Axiotix CA, Jensen RT. Location, incidence and malignant potential of duodenal gastrinomas, Surgery 1991; in press.

20. Zeiger MA, Nieman LK, Cutler GB, et al. Primary bilateral adrenocortical causes of Cushing's syndrome, Surgery 1991; in press.

21. Doherty GM, Doppman JL, Shawker TH, Eastman RC, Gorden P, Norton JA. Results of a prospective strategy to diagnose, localize and resect insulinoma, Surgery 1991; in press.

22. Carty SE, Norton JA. Management of patients with persistent or recurrent primary hyperparathyroidism, World J Surg 1991; in press.

23. Salomon GD, Kasid A, Cromack DT, et al. The local effects of cachectin/tumor necrosis factor on wound healing, Ann Surg 1991; in press.

24. Carty SE, Buresh C, Norton JA. Cellular cytokine resistance occurs at a post-transcriptional level, J Surg Res 1991; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06659-09 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tumor Suppressor Genes in Genitourinary Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	W. M. Linehan	Head, Urologic Oncology Section	SURG, NCI
Others:	M. M. Walther	Senior Investigator	SURG, NCI
	G. H. Weiss	Senior Investigator	SURG, NCI
	J. R. Gnarra	Senior Staff Fellow	SURG, NCI
	M. W. Ewing	NCI Biotechnology Fellow	SURG, NCI
	J. P. Long	Clinical Associate (CO)	SURG, NCI
	S. C. Liu	Chemist	SURG, NCI
	E. E. Trahan	Medical Technician	SURG, NCI

COOPERATING UNITS *(if any)*

Others:	B. Zbar	Laboratory of Immunology	DCDBC, NCI
	P. Steeg	Laboratory of Pathology	DCDBC, NCI
	C. E. Myers	Clinical Pharmacology Branch	COP, DCT, NCI

LAB/BRANCH

Surgery Branch

SECTION

Urologic Oncology Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7 1/2	5 1/2	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sporadic renal cell carcinoma In studies performed to isolate and clone the tumor suppressor genes associated with initiation and progression of renal cell carcinoma (RCC) we have described DNA sequence deletions on the short arm of chromosome 3p in tumor tissue as well as tumor derived cell lines from 88% of patients with sporadic renal cell carcinoma and have localized the gene to the 3p21-26 region. In a study of tumor suppressor genes involved in progression of this malignancy we have described loss of heterozygosity at chromosomal loci of the Wilms tumor gene, the retinoblastoma gene, p53 gene and nm23.

Familial renal cell carcinoma In order to more precisely localize the disease gene for the familial form of RCC we have performed clinical evaluation and genetic linkage analysis on over 240 individuals from over 40 kindred with von Hippel-Lindau disease. We have localized the disease gene to the 3p26 locus. A prospective evaluation determined that preclinical DNA polymorphism analysis could accurately determine which individuals in this familial cancer syndrome carry the disease gene. Chromosomal walking as well as sequencing of cDNA probe candidate genes is currently underway.

Prostate Carcinoma We are evaluating the antitumor effect of a number of antineoplastic agents in human prostate carcinoma as well as evaluating tumor suppressor gene abnormalities in this malignancy. We have demonstrated that suramin has in vitro antitumor effects which are synergistic with other agents such as tumor necrosis factor. We participate in the clinical trials evaluating the antitumor effect of suramin in patients with advance prostate carcinoma and have initiated in vitro gene transfer studies to evaluate the role of tumor suppressor genes in initiation and progression of this malignancy. The significance of this project lies in the identification of the tumor suppressor genes associated with kidney cancer and prostate cancer as well as in the evaluation and development of antineoplastic agents for use in therapy of patients with advanced forms of these neoplasms.

1. Hosoe S, Brauch H, Latif F, Glenn G, Daniel L, Bale S, Choyke P, Gorin M, Oldfield E, Berman A, Goodman J, Orcutt ML, Hampsch K, Delisio J, Modi W, McBride W, Anglard P, Weiss G, Walther MM, Linehan W.M, Lerman TI, Zbar B. Localization of the von Hippel-lindau disease gene to a small region of chromosome 3, Genomics 1990;8:634.

2. Hartge P, Harvey EB, Silverman DT, Sullivan JW, Hoover RN, Linehan WM, Fraumeni JF. Explaining the male excess in bladder cancer risk, JNCI 1990;82:1636.

3. Anglard P, Tory K, Branch H, Weiss GH, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM. Molecular analysis of genetic changes in the origin and development of renal cell carcinoma, Cancer Res 1991;51:1071.

4. Leone A, McBride OW, Weston A, Wang M, Anglard P, Cropp CS, Gorpel J, Liederau R, Callahan R, Linehan WM, Rees RC, Harris C, Liotta LA, Steeg PS. Somatic allelic deletion of nm23 in human cancer, Cancer Res 1991;51:2490.

5. Liu S, Ewing MW, Trahan E, LaRocca RV, Myers CE, Linehan WM. The effect of suramin, tumor necrosis factor and interferon-g on human prostate carcinoma, J Urology 1991;145:389.

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7. Filling-Katz MR, Choyke PL, Oldfield E, Charnas L, Patronas NJ, Glenn GM, Gorin MB, Morgan JK, Linehan WM, Seizinger BR, Zbar B. Central nervous system involvement in von Hippel-lindau disease, Neurology 1991;41:41.

8. Miller DL, Choyke PL, Walther MM, Doppman JL, Kragel PJ, Weiss GH, Linehan WM. Von hippel-lindau disease: Inadequacy of angiography for identification of renal cancers, Radiology 1991;179:883.

9. Glenn GM, Daniel LN, Choyke P, Linehan WM, Oldfield E, Gorin M, Hosoe S, Latif F, Weiss G, Walther M, Lerman M, Zbar B. von Hippel-lindau disease: Distinct phenotypes suggest more than one mutant allele at the VHL locus, Human Genetics, in press.

10. Brauch H, Tory K, Hosoe S, Anglard P, Lerman M, Linehan WM, Zbar B. Molecular analysis of DNA sequences on chromosome 3 in patients with renal cell carcinoma, in press.

11. Wu YW, Chik CL, Albertson BD, Linehan WM, Knazek RA. Inhibitory effects of gossypol on adrenal function, Acta Endocrinologica, in press.

12. Linehan WM, Walther MM, Rosenberg SA. Metastatic renal cell carcinoma. In: Resnick MI, Kursh ED, (eds). Current Therapy in Genitourinary Surgery. Philadelphia: BC Decker, in press.

13. Jensen JC, Choyke P, Rosenfeld M, Pass HI, Keiser H, White B, Travis W, Linehan WM. A report of familial carotid body tumors and multiple extra-adrenal pheochromocytomas, J Urology, in press.

14. Myers CE, Trepel J, Neckers L, Linehan WM. Potential roles for growth factors, their agonists and antagonists in adjuvant therapy. In: Sixth International Conference on the Adjuvant Therapy of Cancer. Philadelphia: WB Saunders, in press.

15. Wade TP, Kasid A, Stein CA, LaRocca RV, Sargent ER, Gomella LG, Myers CE, Linehan WM. Suramin interferes with transforming growth factor-beta induced inhibition of human renal cell carcinoma in culture, J Surg Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06660-08 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Study of Interleukin-2 Based Immunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.C. Yang	Senior Investigator	Surg, NCI
Others:	D. Perry-Lalley	Microbiologist	Surg, NCI
	S. Marcus	Clinical Associate (MSF)	Surg, NCI
	R. Sherry	Staff Fellow	Surg, NCI
	B. Averbook	Clinical Associate (MSF)	Surg, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory is investigating ways to improve the efficacy of tumor-infiltrating lymphocytes (TIL), and trying to delineate the mechanisms of their action in vivo. We have been studying the use of cytokine adjuvants incorporated in slow-release collagen matrix to enhance the host antitumor response. We have described that interleukin-6 utilized in this fashion can generate TIL with enhanced in vivo activity. In addition, we are studying the migration of TIL in response to various stimuli. We have recently found that the fresh tumor preparations secrete a soluble substance which is chemo-attractant to TIL. We are attempting to identify the product(s) responsible for this, and determine its role in TIL function. These studies are associated with on-going clinical trials trying to determine the role of cyclo-phosphamide in the localization of TIL to sites of tumor. Early data suggest that cyclophosphamide may contribute to successful TIL localization in patients.

Other clinical trials have investigated the role of chemotherapy and interleukin-2 in the treatment of colorectal cancer, as well as the benefit of long-term maintenance lymphokine therapy (using PEG-IL2) in the immunotherapy of melanoma and renal cell carcinoma. A new trial investigating autoimmunization with tumor and growth of TIL from these cutaneous inoculation sites is being investigated as a way to improve a patient's immune response against their cancer.

1. DeLaney TF, Yang JC, Glatstein E. Adjuvant therapy for adult patients with soft tissue sarcomas, Oncology 1991;5:105-18.

2. Sherry RM, Rosenberg SA, Yang JC. Relapse after response to IL-2 based immunotherapy: Patterns of progression and response to retreatment, Journal of Immunotherapy; in press.

3. Barth RJ, Merino MJ, Solomon D, Yang JC, Baker AR. A prospective study of the value of TRU-CUT^{R} needle biopsy and fine needle aspiration in the diagnosis of soft tissue masses, Surgery; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06662-05 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Phototherapy and Free Radical Lymphokine Relationships

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	H.I. Pass	Senior Investigator	SURG, NCI
Others:	W. Matthews	Chemist	SURG, NCI
	G. Chaudri	Visiting Fellow	SURG, NCI
	H. Pogrebniak	Clinical Associate (MSF)	SURG, NCI
	T. Prewitt	Clinical Associate (CO)	SURG, NCI

COOPERATING UNITS *(if any)*

Others:	J. Mitchell	Deputy Branch Chief	ROB, NCI
	A. Russo	Head, Experimental Photo-therapy Section	ROB, NCI

LAB/BRANCH

Surgery Branch

SECTION

Thoracic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory has continued investigation of photodynamic therapy (PDT) by sensitization of malignant cells with dihematoporphyrin ether followed by illumination by 630 nm light. Since October 1990, we have established an in vitro, in vivo model of PDT for cells transformed with the k-ras oncogene which is frequently activated in lung cancer. The model was found to exhibit (1) classic sensitizer retention characteristics of other models, and (2) predictable tumor regression with PDT. The model have been used to investigate the use of monoclonal antibody conjugated to the sensitizer, and superior PDT effects as well as greater sparing of normal tissue has been demonstrated. A murine model of solid peritoneal carcinomatosis, investigating sensitizer delivery, and maximal tolerated dose of PDT was published. A large animal model of PDT investigating the effects on intrathoracic organs revealed that focal PDT up to 40 Joules/cm^2 was tolerated by heart, esophagus, chest wall, and lung. A Phase I human trial which combines radical debulking to 5 mm thickness of pleural malignancies followed by intrathoracic-intraoperative delivery of PDT for incurable, localized thoracic malignancies has been ongoing. Light dose escalation in cohorts of three patients has been performed from 15 Joules/cm^2 to 32.5 Joules/cm^2. We continue to treat patients with obstructing endobronchial malignancies with PDT delivered by bronchoscopy. As a followup on studies revealing PDT induction of tumor necrosis factor, our laboratory has demonstrated that free radical stress itself with hydrogen peroxide or superoxide can induce macrophages to produce TNF, and that radical-inducing chemotherapies can increase TNF production. Moreover, we have demonstrated that SOD-mimics can, at least, in vitro, abrogate TNF cytotoxicity. Investigations of other compounds which may be useful in protecting against cytokine toxicity is continuing in our section with in vitro and in vivo models.

1. Perry R, Matthews W, Mitchell JB, Russo A, Evans S, Pass HI. Sensitivity of different human lung cancer histologies to photodynamic therapy, Cancer Res. 1990;50:4272-6.

2. Pass HI, Evans S, and Matthews W. Kinetics of tumor necrosis factor production by phototherapy stimulated macrophages. In: Dougherty T (ed). SPIE Proceedings: Progress in Medical Optics-Photodynamic Therapy. Mechanisms II: Los Angeles, 1990;160-8.

3. Pass HI, Tochner Z, De Laney T, Smith P, Friauf W, Travis W. Surgical resection and intrapleural photodynamic therapy for mesothelioma. Ann Thor Surg. 1990;50:687.

4. Pass HI and Reed C: Lasers in the Management of Malignant Aerodigestive Disease. In DeVita, V., Hellman, S., and Rosenberg S.A.(eds). Important Advances in Oncology. Philadelphia: Lippincott, 1990.

5. Pogrebniak H. Matthews W, and Pass HI. Reactive oxygen species can amplify macrophage tumor necrosis factor production, Surg Forum XLI: 1990;101.

6. Pass HI, Evans S, Matthews W, and Perry R. In vitro, in vivo, phototherapy of transfected human lung cancer, J Thorac Cardiovasc Surg 1991;101:795-9.

7. Perry RR, Smith PD, Evans S, Pass HI. Intravenous vs. intraperitoneal sensitizer: implications for intraperitoneal photodynamic therapy, Photochem-Photobiol 1991;53:335-8.

8. Pogrebniak HW, Matthews W, Russo A, Mitchell J, and Pass HI: Spin trap protection from tumor necrosis factor cytotoxicity, J Surg Res 1991;50: 469-74.

9. Tochner Z, Pass H, Smith P, et al. Intrathoracic Photodynamic Therapy: A canine normal tissue tolerance study and early clinical experience, Int J Rad Onc Biophys 1990; in press.

10. Pass H and De Laney T: Innovative Photodynamic Therapy at the National Cancer Institute. In: Henderson B and Dougherty T, (eds). Photodynamic Therapy: Basic Principles and Clinical Aspects. Marcel Dekker; in press.

11. Pogrebniak HW, Matthews W, and Pass HI: Chemotherapy amplifies production of tumor necrosis factor, Surgery, in press.

12. Pogrebniak HW, Matthews W, Prewitt T, and Pass HI: Tumor necrosis factor alters lung cancer cell resistance to free radical stress, J Thorac Cardiovasc Surg; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06663-02 SURG

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effect of IL-1, IL-6, and TNF on Breast Cancer Cell Growth and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.N. Danforth	Senior Investigator	SURG NCI
Others:	M. Sgagias	NCI Biotechnology Fellow	SURG NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the effects of the cytokines IL-1, IL-6 and tumor necrosis factor alone and in combination to determine synergy, cell growth inhibition, and regulation of estradiol stimulated metabolism of breast cancer cells. We found that all three cytokines inhibit cell growth in vitro with the following efficacy: TNF > IL-1 > IL-6. IL-1 in combination with IL-6 causes greater inhibition and greater antagonism of E_2 stimulation of growth of ER+ cells MCF-7 and T47D than either IL-1 or IL-6 alone. Growth inhibition/antagonism by this combination is dose dependent for both IL-1 and IL-6, and estradiol. Both IL-1 and IL-6 down-regulate the estrogen receptor. The combination has a greater effect than either IL-1 or IL-6 alone. TNF down-regulates the ER and upregulates the PR without altering steady-state levels of ER or PR mRNA. TNF increases insulin-like growth factor secretion, which may explain PR upregulation. IL-1 stimulates secretion of TGFß, which may explain IL-1 growth inhibition. Stimulation is dose-dependent for IL-1 and time-dependent. The combination of IL-1 and IL-6 produces a greater increase in TGFß secretion than IL-1 alone. Estradiol antagonizes IL-1 stimulation of TGFß secretion in a dose-dependent manner. IL-1 stimulates production of TNF mRNA in breast cancer cells. IL-1 induces the 26 kD membrane bound form of TNF protein, which is confirmed by Western blot analysis. IL-1 does not induce secretion of TNF. We are currently studying the effect of IL-1 ± IL-6, and TNF in vivo on breast cancer cell growth and metabolism, and the kinetics, cellular distribution, and modulation of estradiol regulated metabolism of IL-1 induced TGFß in vitro. We are also determining the role of IL-1 induced 26kD TNF protein in mediating IL-1 induced inhibition of cell growth, and, using retrovirally transfected cells with 26kD and 17 kD constructs, we are studying the regulation and kinetics of TNF protein in hormone-dependent and hormone-independent breast cancer cells.

1. Danforth DN Jr, Sgagias MK. Interleukin-1 α blocks estradiol-stimulated growth and down regulates the estrogen receptor in MCF breast cancer cell in vitro, Cancer Research 1991;51:1488-93.

2. Danforth DN Jr, Sgagias, MK. Tumor necrosis factor alpha (TNFα) blocks estradiol stimulated and modulates ER and PR Content in MCF-7 Human Breast Cancer Cells. The Endocrine Society Annual Meeting, 1991, Washington, D.C. (Abstract #573).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06664-02 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immune Recognition of Autologous Tumor by Human Tumor Infiltrating Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.L. Topalian	Senior Investigator	SURG, NCI
Others:	D.J. Schwartzentruber	Senior Investigator	SURG, NCI
	S.S. Hom	Clinical Associate (MSF)	SURG, NCI
	M. Mancini	Biologist	SURG, NCI
	R. Zakut	Expert	SURG, NCI
	H. Stotter	Visiting Associate	SURG, NCI

COOPERATING UNITS *(if any)*

HLA Laboratory, Department of Transfusion Medicine, Clinical Center, NIH
Laboratory of Pathology, NCI, NIH

LAB/BRANCH

Surgery Branch

SECTION

Human Tumor Immunology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tumor infiltrating lymphocytes are currently under investigation in Surgery Branch clinical protocols for the adoptive immunotherapy of patients with advanced cancers. Responses to therapy have been observed in select patients with melanoma. In vitro studies have shown that melanoma TIL are T cells which can specifically recognize autologous tumor. Identifying tumor Ag and mechanisms by which TIL destroy tumor is essential to optimizing current clinical protocols and devising new therapeutic strategies. Areas of study are:

1. Isolation of a melanoma-specific Ag recognized by TIL. A melanoma tumor line resistant to TIL lysis has been immunoselected from a TIL-sensitive melanoma. A subtracted cDNA library has been prepared from these two tumor lines and transfected into the TIL-resistant tumor. TIL lysis is being used to screen for positive transfectants, preliminary to isolating a gene coding for the relevant tumor Ag in this system.

2. Multiplicity and distribution of melanoma-specific Ag. Melanoma-specific cytolytic TIL were used to screen HLA-matched tumors for the presence of specific Ag. Some Ag were broadly expressed among many melanomas while others had more limited expression. Multiple HLA-A, B, C determinants could function in Ag recognition.

3. Specific cytokine release by TIL in response to autologous tumor. Studies have shown that 18/30 melanoma TIL as well as 3/12 breast carcinoma TIL cultures secreted TNF-α, GM-CSF, and/or IFN-g specifically on contact with autologous or some HLA-matched tumors. $CD8^+$ as well as $CD4^+$ T cells were capable of secretion. We are currently studying this phenomenon as a possible predictor of therapeutic response.

1. Skornick Y, Topalian SL, Rosenberg SA. Comparative studies of the long-term growth of lymphocytes from tumor infiltrates, tumor-draining lymph nodes, and peripheral blood by repeated in vitro stimulation with autologous tumor, J Biol Response Mod 1990;9:431-8.

2. Hom SS, Topalian SL, Rosenberg SA. MHC class I antigen restriction of tumor recognition by lymphocytes infiltrating human melanomas, Surg Forum 1990;41:428-9.

3. Morecki S, Topalian SL, Myers WW, Okrongly D, Okarama TB, Rosenberg SA. Separation and growth of human $CD4^+$ and $CD8^+$ tumor infiltrating lymphocytes and peripheral blood mononuclear cells by direct positive panning on covalently attached monoclonal antibody coated flasks, J Biol Response Mod 1990;9:463-74.

4. Hom SS, Topalian SL, Simonis T, Mancini M, Rosenberg SA. Common expression of melanoma tumor-associated antigens recognized by human tumor infiltrating lymphocytes: Analysis by HLA restriction, J Immunotherapy 1991;10:153-64.

5. Schwartzentruber DJ, Topalian SL, Mancini M, Rosenberg SA. Specific release of GM-CSF, TNF-α and IFN-g by human tumor infiltrating lymphocytes following autologous tumor stimulation, J Immunol 1991;146:3674-81.

6. Topalian SL, Rosenberg SA. Adoptive cellular therapy: Basic principles. In: DeVita V, Hellman S, Rosenberg SA, (eds). Biologic Therapy of Cancer: Principles and Practice. Philadelphia: JB Lippincott Co., 1991;178-196.

7. Aebersold P, Hyatt C, Johnson S, Hines K, Korcak L, Sanders M, Lotze M, Topalian S, Yang J, Rosenberg SA. Lysis of autologous tumor cells by tumor infiltrating lymphocytes correlates with clinical response in patients with malignant melanoma, J Nat Cancer Inst; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06665-02 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Preclinical Studies of Antitumor Efficacy of Adoptive Immunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J.J. Mulé	Microbiologist	SURG, NCI
Others:	R.J. Barth, Jr.	NCI Biotechnology Fellow	SURG, NCI
	S.N. Bock	Staff Fellow	SURG, NCI
	D.L. Jicha	Clinical Associate (MSF)	SURG, NCI
	N.P. Restifo	NCI Biotechnology Fellow	SURG, NCI
	M.G. Sanda	Clinical Associate (MSF)	SURG, NCI
	M. Custer	Microbiologist	SURG, NCI
	S. Karp	Visiting Associate	SURG, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pathology, NCI (W.D. Travis)

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	6.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Preclinical studies in mice were undertaken to investigate the antitumor effects and mechanisms of action of recombinant cytokines and effector cells in adoptive immunotherapy models of established cancer.

A. Tumor Infiltrating Lymphocytes (TIL). We have studied the mechanisms whereby adoptively transferred murine TIL mediate tumor regression. Noncytolytic, $CD8^+$ TIL eradicated established lung tumors in irradiated mice. Many cytolytic and noncytolytic $CD8^+$ TIL cultures specifically secreted interferon-gamma and tumor necrosis factor when stimulated with tumor cells in vitro. The antitumor effectiveness of TIL in vivo correlated better with their ability to specifically secrete lymphokines than with their cytotoxicity in vitro.

B. Human Tumors in Immunodeficient Mice. We have developed a model of disseminated fresh human malignant melanoma in congenitally immune deficient mice. We have found that the adoptive transfer of autologous melanoma-specific human TIL in conjunction with IL-2 could significantly prolong the survival of mice with disseminated human disease.

C. Macrophage Colony Stimulating Factor (M-CSF). We have investigated the biologic and antitumor effects of M-CSF in mice. The systemic administration of high-dose M-CSF elicited a profound localization/ proliferation of macrophages in the livers of treated mice. The combined administration of tumor-specific monoclonal antibody plus M-CSF resulted in synergistic anti-tumor effects against established hepatic metastases from the B16 melanoma.

D. Tumor Antigen Processing and Presentation. We have studied whether tumor cells can escape recognition by CTL and subsequent immune eradication by suppressing presentation of endogenous antigen. Certain murine sarcomas failed to present endogenously synthesized viral antigens to specific CTL. The deficiency in presentation by tumor was attributed to a markedly reduced rate of synthesis of MHC class I molecules.

1. Mulé JJ, Rosenberg SA. Murine sarcomas: Definition of elements responsible for successful immunotherapy. In: Lotze MT, Finn OJ, eds. Cellular immunity and the immunotherapy of cancer. UCLA symposia on molecular and cellular biology, new series. New York: Wiley-Liss, Inc., 1990;223-34.

2. Barth RJ Jr, Mulé JJ, Spiess PJ, Rosenberg SA. Interferon-gamma and tumor necrosis factor have a role in tumor regressions mediated by $CD8^+$ tumor infiltrating lymphocytes, J Exp Med 1991;173:647-58.

3. Mulé JJ, Jicha DL, Aebersold PM, Travis WD, Rosenberg SA. Disseminated human malignant melanoma in congenitally immune-deficient (Bg Nu Xid) mice, JNCI 1991;83:351-5.

4. Bock SN, Cameron RB, Kragel P, Mulé JJ, Rosenberg SA. Biologic and antitumor effects of recombinant human macrophage colony stimulating factor in mice, Cancer Res 1991;51:2649-54.

5. Mulé JJ. Preclinical studies in the treatment of advanced cancer with recombinant lymphokine combinations. In: Fair WR, ed. Immunotherapy with interleukin-2. Issues in urology: Current therapy and future alternatives. New York: Park Row, 1991;3-7.

6. Mulé JJ, Rosenberg SA. Interleukin-2: preclinical studies. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JP Lippincott Co., 1991;142-58.

7. Mulé JJ, Rosenberg SA. Combination cytokine therapy: Experimental and clinical studies. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JP Lippincott Co., 1991; 393-416.

8. Restifo NP, Esquivel F, Asher AL, Stotter H, Barth RJ, Bennink JR, Mulé JJ, Yewdell JW, Rosenberg SA. Defective presentation of endogenous antigens by a murine sarcoma: Implications for the failure of an anti-tumor response, J Immunol 1991; in press.

9. Barth RJ Jr., Mulé JJ, Asher AL, Sanda MG, Rosenberg SA. Identification of unique murine tumor-associated antigens by tumor infiltrating lymphocytes using tumor specific secretion of interferon-gamma and tumor necrosis factor, J Immunol Meth 1991; in press.

10. Jicha DL, Mulé JJ, Yannelli JR, Custer M, Rosenberg SA. Use of immunodeficient mice for the study of human peripheral blood mononuclear cell reconstitution, human tumor infiltrating lymphocyte persistence and growth of human colon adenocarcinoma, J Immunother 1991; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06667-01 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Detection of Tumor Reactive T Cells by Determination of Intracellular Calcium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)*

PI:	R. B. Alexander	Senior Investigator	SURG, NCI
Others:	E. S. Bolton	Microbiologist	SURG, NCI
	D. A. Shulman	Microbiologist	SURG, NCI

COOPERATING UNITS *(if any)*

Navy Medical Research Institute, Bethesda, Maryland (CH June)
Laboratory of Immunoregulation, NIAID (S. Koenig)
Neuroimmunology Branch, NINDS (W. Biddison)

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Tumor infiltrating lymphocytes (TIL) are undergoing evaluation in the Surgery Branch as a therapy for advanced human malignancy. We are attempting to identify the subset of cells within bulk TIL culture which have specific immune reactivity to the tumor itself. We have developed a method of detecting intracellular calcium in individual TIL after exposure to tumor. The method involves the use of flow cytometry and cell sorting to detect and recover specifically reactive anti-tumor lymphocytes.

We showed that in human T cell clones that we could detect an increase intracellular calcium after exposure to specific target cells. We could demonstrate conjugation of the T cell to its target cell as well as signalling with an increase in intracellular calcium in this system using both $CD4^+$ and $CD8^+$ T cells. These cloned populations of T cells, when bound to the target, demonstrated an increase in intracellular calcium and could be sorted. We have begun preliminary experiments in patients TIL and have found that we can also detect tumor T cell conjugates by flow cytometry and that in a subpopulation of these conjugates the signal of an increase in intracellular calcium can be detected. The biologic relevance of such cells is undergoing evaluation with sorting and subculturing experiments.

The significance of the project lies in identification of specific anti-tumor reactive T cells in the tumors of patients. We suspect that if these cells are present in the patients tumor and can be enriched and subcultured that such cells may have increased efficacy compared to bulk whole populations of TIL in the therapy of patients with cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06668-01 SURG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Cancer Immunotherapy with Tumor Infiltrating Lymphocytes and Interleukin-2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Ettinghausen Senior Investigator SURG, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Prior work has shown that tumor infiltrating lymphocytes (CTL) may be expanded in vitro and, when transferred to tumor bearing mice in combination with interleukin-2 (IL-2), can reduce lung metastases and prolong host survival. Studies have been undertaken to optimize the efficacy of in vivo TIL function.

1. In vitro growth of TIL in low dose (20 u/ml) versus high dose (1000 u/ml) IL-2.
During in vitro expansion, TIL require IL-2 and repeated syngeneic tumor stimulation. TIL growth in vitro at low dose IL-2 was found to be at least 3-fold more potent in vivo in the treatment of 3 day lung metastases than TIL grown continuously in high dose IL-2 or TIL switched from low dose to high dose IL-2 as little as 4 days before adoptive transfer.

2. TIL potency and nitric oxide synthase inhibitors (NOSI)
N-methyl-arginine (NMA), a NOSI, has been shown to augment the allo-reactivity of murine splenocytes in vitro. After confirmation of these findings in this laboratory, NMA was found to minimally augment TIL cyto-toxicity in vitro. This result does not appear to be explained by significant differences in cytokine secretion by TIL in response to tumor.

Although significant responses to immunotherapy of established cancers using TIL and IL-2 have been observed in animal models and in patients, further optimization of in vivo TIL function is necessary to improve the results in ongoing clinical trials.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM

DIVISION OF CANCER TREATMENT

OCTOBER 1, 1990 - SEPTEMBER 30, 1991

I. INTRODUCTION

In 1982 the Radiation Research Program (RRP) was established in the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH) to develop research program for the extramural community in which radiation and related forms of energy are used in the diagnosis, staging, treatment and post-treatment evaluation of the patient with cancer. The RRP is an extramural program having two branches: the Diagnostic Imaging Research Branch (DIRB) and the Radiotherapy Development Branch (RDB). The scientific mission includes the planning, development, administration, and evaluation of an extramural radiation research program. This is accomplished by establishing program priorities, allocating resources, maintaining project integration, evaluating program effectiveness, and representing the program in the administrative and scientific decision-making processes of the National Cancer Institute.

For scientific and administrative direction, the RRP relies heavily on the advice of the DCT Board of Scientific Counselors. The Program coordinates research activities with related programs at NCI and NIH, other federal agencies, and national and international research organizations. The RRP provides a radiation research focal point for national and international extramural investigators.

II. PERSONNEL

A. Staffing

1. Office of the Associate Director
   Michael A. Friedman, M.D., Acting Associate Director
   Sandra Zink, Ph.D., Cancer Expert
   Wendy R. Fredericks, Biologist
   Richard V. Stepney, Computer Specialist

2. Administrative Office
   Berit Fitzpatrick, Acting Administrative Officer

3. Diagnostic Imaging Research Branch
   Faina Shtern, M.D., Chief
   Matti Al-Aish, Ph.D., Program Director
   Roger Powell, Program Director

4. Radiotherapy Development Branch
   Francis Mahoney, Ph.D., Acting Chief
   Thomas Strike, Ph.D., Program Director

B. Recruitments

Associate Director, Radiation Research Program
Administrative Officer, Radiation Research Program
Chief, Radiotherapy Development Branch
Radiation Biologist, Radiotherapy Development Branch
Program Director, Radiotherapy Development Branch
Program Director, Diagnostic Imaging Research Branch

III. MAJOR ACTIVITIES

The Radiation Research Program continues to stimulate, develop, administer and evaluate basic science and clinical research areas in radiation therapy, nuclear medicine, diagnostic imaging, and their related subspecialty areas.

The Radiotherapy Development Branch (RDB) stimulates and supports research in the scientific areas of conventional photon radiation therapy, 3-D conformal radiotherapy, 3-D treatment planning, fast neutron radiation therapy, proton beam radiation therapy, hyperthermia, radiation sensitizers, radiation protectors, systemic radiation therapy (SRT), photodynamic therapy (PDT), boron neutron capture therapy (BNCT), radiobiology, radiation physics, and the application of medical informatics in the radiologic sciences. In addition, RDB supports the Patterns of Care study, a survey of radiotherapy practices in the US to correlate types of equipment, methods of patient management and type of facility with patient outcome. The study includes 5-, 10- and 15-year follow-up on patients treated for cancer in five disease sites.

In the Diagnostic Imaging Research Branch (DIRB), research activities include: magnetic resonance imaging (MRI), computerized tomography (CT), conventional X-ray procedures, nuclear medicine studies, including positron emission tomography (PET), single photon emission computerized tomography (SPECT) and radio-immuno-diagnosis (RID). A major DIRB goal is the development of non-invasive, tissue specific diagnostic procedures and techniques. Multi-institutional clinical trials in diagnostic radiology conducted by the Radiologic Diagnostic Oncology Group (RDOG). The objectives of the trials are to use single or multiple new imaging technologies to diagnose, stage, and monitor cancers and to develop an algorithm for the appropriate sequential, cost effective selection of diagnostic procedures. Institutions participate in the group through regular working group meetings and by accruing patients to the imaging protocols. Data from the first trial, RDOG 1, on lung and prostate cancer are now being analyzed. A manuscript has been submitted for publication. RDOG 2, now in progress, is examining the diagnosis, staging, and monitoring of colorectal and pancreatic tumors. The third trial will study musculo-skeletal and head and neck tumors. Dr. Antoine reiterated that the data from all of the three trials would be brought to the Board for review as they become available.

The Diagnostic Radiology Coordinating Committee (DRCC) is centered in the Office of the Director of the NIH as mandated by Congress. The committee is charged with developing a five-year research plan for diagnostic radiology/imaging at the NIH. Additional functions of the DRCC include improving the NIH database and information dissemination. Extramural scientific advisors will be involved in the development of the research plan.

Exciting areas of research supported by the RRP include the following:
In the Radiotherapy Development Branch:

Continued excellent results in the control of clival chordomas, base of skull chondrosarcomas and uveal melanomas are reported by the proton beam research team at the Harvard Cyclotron Laboratory, Cambridge, Massachusetts.

Because of these encouraging results there is increasing radiation oncology interest in the use of proton beams for the treatment of malignant disease. A dedicated clinical proton research and treatment facility has begun treating patients at the Loma Linda Medical Center in Riverside, California. Interest in proton beam therapy is increasing not only in the United States of America but also in the international radiation research community. Congress made available $1.5 million for proton beam facility planning in FY1990 and $4.0 million in FY1991. Two planning grant were awarded in FY1990 to Massachusetts General Hospital and to the Lawrence Berkeley Laboratory of the University of California. One or two continuation awards are expected in FY1991.

Data from the Heavy Ion Project at the Lawrence Berkeley Lab are consistent with data being obtained from the Harvard Cyclotron Proton Beam Project. The heavy ion beam and the proton beam projects demonstrate that there is a definite place for this type of precision radiotherapy in the treatment of well-defined localized cancers.

Neutron therapy clinical trials have been an ongoing project of the Radiation Research Program since 1979. Phase III studies in head-and-neck, prostate and lung cancers were closed in the spring of 1991 with nearly 1200 patients accrued to the Phase II/Phase III studies. More than 2000 patients were treated with neutrons during this time. Preliminary analyses show no difference in survival between the neutron patients and those patients treated with conventional radiotherapy. Final evaluation of the results of the recent Phase III studies, however, must await long-term follow-up.

Intraoperative radiation therapy continues to be clinically investigated and appears to be effective in the treatment of advanced local gynecological and rectal tumors, retroperitoneal sarcomas and gastric cancers. Phase II and III Clinical Trials are being performed in the United States and internationally.

Radiation modifiers: The radiation sensitizer contracts of RRP continue to identify and develop substances with radiosensitizing properties. Encouraging results with SR-2508 have been followed by Phase II and Phase III Clinical Trials presently being carried out by the Radiation Therapy Oncology Group (RTOG), and other cooperative groups. The sensitizer program is being reevaluated and there is hope that a large scale automated radiation sensitizer screening program can be developed. The expertise, experience and advice of the Developmental Therapeutics Program (DTP) is being utilized in the development of a new screening program.

The radioprotector, WR-2721, continues to show a normal tissue protective effect, not only when used with radiation, but also with chemotherapy. Encouraging clinical results are being reported in the use of WR-2721 with chemotherapeutic agents (e.g., melanoma). Larger doses of chemotherapy can be given with the normal tissues protected. Improved therapeutic ratios are being reported but this scientific observation requires substantiation by further clinical investigation.

Hyperthermia continues to show promise in the management of malignant disease. In addition to being used with radiation for the improved control of local tumors it is being investigated as an adjunct to chemotherapy in the treatment of systemic disease. In the treatment of localized neoplasms thermometry remains an invasive procedure requiring multiple probes be inserted into the patient's tumor. Research on the development of non-invasive thermometry is needed. Magnetic Resonance Imaging techniques may make non-invasive thermometry a reality. Persistent difficulties with adequate local deep heating may be overcome by the use of ultrasound techniques. A RRP workshop addressing these problems was held May 12 - 13, 1988. A Recommendation that NCI hyperthermia efforts be coordinated largely through RTOG were made to RRP by the participants of this workshop and this advice has been followed by the Program during 1988, 1989, and 1990.

The exciting field of photodynamic therapy (PDT) is a research field in which systemically administered tumor seeking light sensitive compounds are used in conjunction with activating light, usually generated by a laser. Improvements in the light sensitizing compounds are being made and several new compounds are now entering the Decision Network of DCT, NCI. The potential for the treatment of closed space neoplasms such as carcinoma of the ovary, mesothelioma and bladder cancer is being explored. The effectiveness of this therapy in the reestablishment of airway in totally occluded bronchi caused by lung cancer has been demonstrated. Hopefully, this research area will mature into a treatment modality giving improved results in the treatment of tumors which commonly recur following conventional therapy , e.g., ovarian cancer. Industry-supported Phase III clinical trials are now being performed in lung, esophageal, and bladder cancer.

Dosimetry studies: Research in determining optimal radiation treatment planning is ongoing. These activities include the dosimetry of interstitial radiation therapy, x-ray, electron, and particle beams.

A "Patterns of Care" study to evaluate radiation therapy practices in the US and Puerto Rico is in progress. This retrospective analysis evaluates treatment outcomes for cancer patients treated for breast, cervix, prostate, and recto-sigmoid cancer and Hodgkin's Disease and correlates the results with kind of facility, type of equipment, management practices and other variables associated with good radiation therapy practice. Previous patterns of care studies have proven helpful in identifying methods for the improvement of patient treatment. The results are widely disseminated to the radiation therapy community through newsletters, educational symposia, scientific presentations and professional society meetings.

SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

For the current fiscal year, the Radiation Research Program is funding 34 Phase I SBIR grants and 31 Phase II. In addition, the program has 3 Phase II SBIR contracts. The funded research in the SBIR program spans a broad spectrum. In Phase I, a number of imaging projects are focused at improved methods for delineation of tumors, detecting air emboli, imaging boron isotopes in tissue, non-invasive thermometry and mammography. Therapy is the focus of several other Phase I projects, namely, gynecological hyperthermia, dynamic electron arc collimator, optimization of complex multi-field radiation treatments and photochemotherapy. The Phase II efforts in imaging include several projects for radiographic display on computer-based workstations, improved methods for MRI, Ultrasound and radiolabeled monoclonal antibodies. Phase II therapy projects include neutron and photodynamic therapy dosimeters, lung cancer hyperthermia, an accelerator for delivering epithermal neutrons for BNCT and real-time 3D visualization for radiotherapy planning. Funded research impacts photodynamic therapy, fast neutron therapy, boron neutron capture therapy, photon therapy, electromagnetic and ultrasonic hyperthermia, expert systems for Radiation Oncology and real time portal scanning for Radiation Oncology.

IV. SCIENTIFIC OVERVIEW

DIAGNOSTIC IMAGING RESEARCH BRANCH

The Diagnostic Imaging Research Branch (DIRB) of RRP, DCT, NCI continues to develop and administer basic and clinical diagnostic imaging research. DIRB consists of two major sections, Non-Ionizing Radiation Section supporting research in the area of magnetic resonance imaging (MRI) and spectroscopy (MRS), MR microscopy, ultrasound, instrumentation development and image perception and Ionizing Radiation Section supporting nuclear medicine and X-ray computerized tomography (CT) research. Other research areas include digital radiography, novel methods of acquiring, sorting, viewing, archiving and communicating diagnostic imaging data. The growth of DIRB continues to be satisfactory. Starting with a modest budget of $3.5 million in 1982, the DIRB budget has grown to an estimated $39.9 million in 1991.

Magnetic resonance imaging/spectroscopy and nuclear medicine research continue to be two major areas of funding at DIRB. Areas of increasing interest and significance are the use of monoclonal antibodies in imaging and the collaborative clinical diagnostic imaging research. The following is a summary of the DIRB actual budget FY90 and estimated budget FY91.

FY90 AND 91 BUDGETS

			$(in thousands)	
GRANTS	FY90	FY91	FY90	FY91
Coop. Agree.(U01)	11	17	1,617	1,715
Traditional (R01)	93	96	21,378	23,333
Program Projects (P01)	10	9	8,223	7,487
Conf. & New Investigator (R13 & R23)	2	1	11	5
SBIR*	25	29	2,780	3,685
First Awards	8	12	623	1,003
Request for Applications (RFAs)	11	10	1,561	2,095
TOTAL GRANTS	162	170	36,618	39,765
CONTRACTS				
SPECT Contract	1	1	179	196
SBIR	1	0	223	0
TOTAL CONTRACTS	2	1	402	196
TOTAL DIRB BUDGET			37,020	39,961

NON-IONIZING SECTION

MAGNETIC RESONANCE IMAGING (MRI) AND SPECTROSCOPY (MRS)

Instrumentation and Technique Development:

The frontiers of research in this area have been extended both by continued progress in existing projects and by the appearance of new developments. Work on the SBIR-supported program at Advanced NMR Systems, Inc. in Woburn, Massachusetts has provided one of the fastest available MRI systems, which can depict any portion of the body in any plane in real time. Individual images can be made in as little as 25 milliseconds and "assembled" sequentially to create a moving picture of the beating human heart, lung and diaphragmatic motion, or the action of the temporomandibular joint, knees, ankles, elbows, and shoulders. Because MRI provides good grey scale images of soft tissues and because it provides dynamic imaging, the application of MRI to sports injuries is growing rapidly.

Another project on fast MRI at the Mayo Foundation provides real-time imaging and reconstruction in a manner analogous to x-ray fluoroscopy. Another novel fast scanning sequence has been developed at Stanford University to minimize motion artifacts when using MRI imaging of the chest and abdomen for tumor diagnosis and staging.

Advances at the Massachusetts General Hospital in proton NMR chemical shift imaging have now been used to study the fat and water fractions and their relaxation times in bone marrow. Several leukemic patients have been under study to determine if these MRI parameters can be used to monitor therapeutic response. The same group of investigators have pioneered proton lactic acid imaging of normal and diseased tissue. MR imaging of lactate may enhance this

modality as an in vivo tool for tissue characterization (e.g. differentiation of treatment-induced necrosis from viable tumor).

Outstanding physics and engineering contributions to the clinical applications of MRI have been made at the Medical College of Wisconsin at Milwaukee in the design, construction, clinical evaluation, and use of specially designed MR body and surface coils to fit around or next to different body parts. These coils are now routinely used to increase image quality in diagnostic images of the head, neck, spine, and abdominal organs as well as the shoulder, wrist, knee, and ankle joints. MR arthrography of the joints performed in combination with surface coils is a valuable new development in diagnostic medicine and in sports medicine (see above). Special coils have also been developed for use in research and clinical studies in MR spectroscopy to study metabolism and function and to analyze and monitor selected nuclei in cancer patients undergoing therapy.

Special coil and instrumentation developments at Johns Hopkins University have aided in the achievement of magnified images (MR microscopy). Another SBIR project with Tecmag, Inc., in Houston is aimed at the development of an MR microscope capable of a spatial resolution of only ten microns.

Excellent progress has been made at the Brigham and Women's Hospital in the development of 2D and 3D MR imaging of brain tumors in conjunction with computer-assisted laser therapy. MRI can thus be used to monitor laser-tissue interactions and separate reversible from irreversible tissue damage. Another advance in 3D imaging of radiation dose distribution has been made in a project at Yale University. These applications demonstrate well the combined uses of MRI for both diagnosis and treatment planning of cancer. Magnetic resonance spectroscopy (MRS) is being used to study the phosphate metabolism of human breast at Northwestern University Hospital.

Special MR techniques have been perfected at the University of California at Irvine for measurement of the flow velocity of blood and other body fluids in each voxel of a 3D MR image in the regimes of bulk flow, perfusion, and diffusion. An interesting mathematical improvement in MR image processing has come from the development of "eigenimage filtering" at the Henry Ford Hospital, in which a feature of interest in the image can be enhanced while the background noise is suppressed.

Valuable advances have been made at the University of Utah in the development of highly refined NMR techniques for determining lung water content and distribution in a variety of clinical pathologies. These techniques are providing new avenues for understanding of lung physiology on a microscopic scale at air-water interfaces and the relative role of alveolar recruitment and distention in human adults with lung injury or adult respiratory distress syndrome. These NMR methods, which can detect and characterize pulmonary disease such as pulmonary edema, emphysema, and fibrosis, have the advantage of being noninvasive, relatively rapid, easily reproducible, and less susceptible to motion artifacts caused by respiration, heart motion, or blood flow in the chest cavity than current conventional approaches. Thus they may provide an optimal standard for measuring and monitoring a variety of lung pathologies in the future.

Investigators at Harvard University have been investigating magnetic field effects on iron oxide-loaded lung macrophages in order to understand cytoplasmic viscosity and cell organelle motion at the "microscopic" level. Many subtle rheological, chemical, and physical properties of cell tissues and fluids and their changes have been investigated as a function of temperature and of their mechanical motion measured magnetometrically. Cell activity is maximal around 37 degrees C. Biochemical changes below that temperature are consistent with reversible inhibition of enzymatic processes. Above that temperature the inhibition appears to be irreversible.

A new technique for imaging oxygen concentrations in living mouse tissues has been developed at the University of Chicago in a specially designed low frequency electron spin resonance (ESR) spectrometer. The injection of nitroxide spin labels (free radicals) into the tissues enables a sensitive determination and mapping of oxygen concentrations. This technique may eventually be extended to humans and marks one of the earliest practical possibilities for the use of ESR imaging in medicine.

MAGNETIC RESONANCE SPECTROSCOPY (MRS) AND MULTINUCLEAR STUDIES:

Many investigators are now carrying out in vivo laboratory research in animals using magnetic resonance spectroscopy (MRS) to measure and to follow the concentration of particular magnetic elements (such as hydrogen-1, fluorine-19, sodium-23, and phosphorus-31) which occur naturally in the body or signal which can be enhanced by administration of contrast agents or treatment pharmaceuticals. Important progress has been made at Wayne State University in analysis of tumor metabolic products by phosphorus-31 MR spectroscopy in order to predict chemotherapeutic response. At Memorial Hospital in New York a number of phosphorus-31 MRS studies have been carried out to follow the metabolism of sarcomas under chemotherapy and the radioresistance and radiosensitivity of tumors under radiation treatment. Fluorine-19 MRS measurements have assisted in the in vivo monitoring of 5-fluorouracil metabolism after methotrexate administration.

With high magnetic fields, it is also possible to obtain MR images of some of the above mentioned magnetic nuclei for research and clinical studies. At the University of Pennsylvania, pharmaceuticals containing fluorine-19 have been used to measure the vascular concentration and distribution of oxygen, and sodium-23 MR has aided in evaluating the in vivo progression of human neuroblastomas implanted in nude mice. At the University of North Carolina, analytical MRS techniques with phosphorus-31 and fluorine-19 are being perfected to attempt to predict the metastatic potential of prostatic tumors in mice. This work may be extended to all types of tumors.

Pioneering work carried out on sodium-23 MR imaging at Columbia University at high magnetic fields of 2.0 and 3.0 Tesla has been extended to provide a novel methodology for noninvasive differentiation of intracellular sodium and extracellular sodium signals. It has thus become possible to differentiate tumor from normal and edematous tissue, radiation necrosis from tumor recurrence, and to obtain an in vivo index of malignancy. Pre-clinical validation of this technique is being carried out. Preparations are also under way to implement a 5.0 Tesla facility for MR imaging and spectroscopy. This will permit new spectroscopic studies and biologic imaging to be undertaken that heretofore were not possible. These investigators have also reported

that sodium-23 MR imaging examinations of patients with AIDS has permitted accurate diagnosis and characterization of distinct pathological features of brain lymphoma in all five patients.

Outstanding fundamental research studies have been pursued at Fox Chase Cancer Center using MR phosphorus-31, carbon-13, and proton spectroscopy to elucidate metabolic pathways in malignant solid tumors, especially arterially perfused hepatomas, and to study phospholipid metabolism in transformed cells. This group has used phosphorus-31 NMR to study phosphate metabolites in order to evaluate nutritional repletion in subjects suffering from cancer-induced cachexia. Using the technique of chemical shift imaging (CSI), exceptional progress has been made in developing the first color-coded metabolic NMR mapping of the human brain in vivo and human muscle physiology.

New NMR spectroscopic imaging techniques are being developed at the University of California at San Francisco to measure lactate and other metabolites in evaluating regional ischemia and malignancy. Phosphorus-31 and carbon-13 NMR studies at Evanston Hospital in Illinois are seeking to correlate steady state concentrations of phosphate-containing metabolites with the levels of several proto-oncogenes found in human breast tumor cells, with a particular focus on post-menopausal women with node negative and node positive cancers.

MR CONTRAST AGENTS AND PHARMACOKINETICS:

Contrast agents for MRI are of increasing importance not only to the improvement of image quality and the clinical ability to distinguish boundaries and differentiate one type of tissue from another, but with the development of new paramagnetic, superparamagnetic, and ferromagnetic contrast materials, it is becoming increasingly possible to carry out dynamic imaging and vascular and organ function studies using these contrast materials as pharmacokinetic agents. A notable SBIR program at Advanced Magnetics, Inc., in Cambridge, Massachusetts has produced new superparamagnetic iron oxide particles which are more effective as contrast agents than either paramagnetic ions or ferromagnetic particles. They are non-toxic, and the iron is biodegradable in the body. Recent imaging applications include tumors of the brain and gastrointestinal tract, pyogenic liver abscess, and micrometastases of the liver and spleen.

Pharmacokinetic studies and development and evaluation of new contrast agents based on gadolinium and nitroxide free radicals are being carried out at the University of California at San Francisco. The currently FDA approved Gd-DTPA serves as a standard for comparison with new agents. A program at the University of Arizona is concentrating on the development of new liposome-entrapped contrast agents for the detection of hepatic and splenic metastases. These agents look promising in their ability to deliver the desired magnetic contrast material to the desired sites in the body without prior degradation or dilution. Contrast agents have also been used pharmacokinetically at the Pittsburgh NMR Institute to study tissue perfusion in the spleen and placenta of rats and rabbits. Agents employed include Gd-DTPA, Gd-DTPA-albumin, colloidal gadolinium oxide, and magnetite microspheres.

Another study at the University of California at San Francisco has concentrated on the development of iron chelates and their toxicity and clinical effectiveness for many diagnostic uses, including tumor detection and enhancement. A group at the University of Illinois is examining liposomes containing paramagnetic materials as well as nitroxides as agents. Work is continuing on the characterization of various gadolinium complexes at the Brigham and Women's Hospital. Fundamental mechanisms in tissue relaxation properties, which are the major determinants of intrinsic contrast differences in MRI, is the subject of basic research at Yale University.

MR BIOEFFECTS:

Research has continued on the thermophysiologic effects of magnetic resonance imaging at Cedars-Sinai Medical Center in Los Angeles. Theoretical and experimental studies have focused on the possible slight heating effects that occur in tissues subjected to radiofrequency signals employed in the MR imaging system as well as any effects which might arise from static and gradient magnetic fields. The FDA has also examined this subject continually on all new systems submitted for approval. At present, it appears that no hazardous or adverse biological effects have been observed from present clinical exposure levels. The greatest hazard in present day clinical MRI systems is that of loose flying ferromagnetic objects (such as tools or laboratory hardware, including carts or compressed gas tanks), which may be attracted rapidly into the magnetic field as unintended projectiles. This hazard is preventable.

A three day workshop was recently conducted on the potential bioeffects of high MRI magnetic fields and high rates of gradient switching under the organization provided by the New York Academy of Sciences. The proceeds of this workshop will be made available in due course.

Work is continuing at the University of Michigan on a computer-based system for detecting lesions and microcalcifications in X-ray mammograms. The radiographs are first digitized electronically so that specially designed algorithms can be employed to provide automatic feature discrimination as an aid to the mammographer.

MICROWAVE RADIOMETRY

Another approach to breast cancer detection has been demonstrated by an SBIR grantee, Microwave Medical Systems, Inc., of Littleton, Massachusetts, in which a multiple antenna microwave radiometry system has been able to detect "hot spots" in a two-layer tissue phantom. Initial trials of this instrument on 183 volunteer women with mammary gland disease at the Nippon Medical School have shown the true negative rate for benign cases at 81.7% and the true positive rate for malignancies at 63.4%. Another SBIR grantee has been studying solutions to the inverse scattering problem in order to try to utilize extremely low levels of microwave radiation to provide dielectric imaging of the breast.

ULTRASOUND IMAGING, TISSUE CHARACTERIZATION, & BIOEFFECTS

Instrumentation:

Progress continues to be made on the development of a number of advanced ultrasound imaging systems for clinical use on various organ systems of the body. The high speed scanner at Duke University, by virtue of parallel computer processing of data and linear phased arrays of transducers, can transmit on 32 interlaced channels and receive with dynamic focussing on 16 channels, and can produce real time 3-D ultrasound images. Another highly promising new technical approach at Duke University is the development and study of a real time, adaptive phased array imager, which should generate very high quality images of diffraction-limited resolution, providing the ultimate possible anatomic detail. A consortium led by Mayo Clinic Foundation is developing a new class of transducers based on composite materials.

A novel reflex transmission imaging system has been developed at SRI International for medical imaging of various body parts, such as the extremities. A high resolution scanner (less than one millimeter spatial resolution) for imaging of abdominal organs and breast tissue is under development by TechniScan, Inc., under an SBIR (small business innovation research) contract at Salt Lake City. The system takes advantage of theoretical and applied research performed under a grant at the University of Utah, which uses very high speed computation to correct errors and artifacts which otherwise would normally occur as ultrasound energy travels through the tissues. The consequent improvement in image quality is dramatic in simulation studies to date. New ultrasound scanning arrays for operation at 0.5 and 2.0 megahertz have been developed.

Basic Research Studies:

Success in applied imaging system development requires fundamental knowledge about the propagation of ultrasound energy in the body and scattering and absorption phenomena which ultimately affect image quality. To add to this fund of understanding, basic research studies have been carried out for many years at the University of Illinois, Stanford University, Mayo Foundation, SRI International, the University of Wisconsin, and other institutions. Development of an ultrasound anthropomorphic breast tissue phantom at Madison over the past several years has resulted in its adoption by many clinics for use in quality control and training. The same group of investigators have also been developing a tissue-equivalent phantom for general quality control and training use in Magnetic Resonance Imaging systems.

Ultrasound Tissue Characterization:

One of the most active research and development areas in ultrasound imaging today is tissue characterization, where improvements in the understanding of such factors as texture, speckle reduction, angular distribution of reflectivity, speed of sound, attenuation, and other parameters lead to new and improved ways of displaying the image and of determining noninvasively the nature or state of the tissue seen in each area of the image. Highly quantitative studies have been carried out at the University of Texas at Houston on the properties of speed of sound and attenuation of sound (ultrasound) as it applies to clinical examinations of organs such as liver.

Quantitative measurements on freshly excised human breast tissue at SRI International have provided most accurate evaluation of interactions of ultrasound energy with breast tissues and improved understanding of how to interpret clinical breast images.

Remarkable progress over many years in high frequency ultrasound research at Riverside Research Institute in New York City, working in collaboration with Columbia and Cornell Universities, has permitted the depiction of dynamic, colored, 3-D images of tumors of the eye and other ocular structures in videotape displays which rotate the image. Measuring three different ultrasound characteristics (or parameters) of each little volume element of tissue permits specific colors to be assigned to different tissues. For example, the distribution of the primary tumor can be mapped in one color and the metastasized tumor in another. They have now been applying the same noninvasive and multiparametric display techniques at lower frequencies to imaging and characterization of liver and spleen tumors as well as human breast tumors in vivo.

A novel approach to the detection of tumors based on ultrasound Doppler detection of shifts in the velocity of blood under tumor-induced vascular changes is being pursued with success at Yale University in studies of tumors of pancreas, breast, kidneys, and liver in women.

Contrast Agents:

Good results have been achieved in enhancing the contrast of ultrasound images and the detection and delineation of tumors in the liver by extensive investigations at the University of California at San Diego in animals with perfluorochemical agents, e.g., perfluoro-oxybutylene (PFOB). Suspensions of iodipamide ethyl ester (IDE) particles are being investigated as contrast agents in the livers and spleens of animals at the University of Rochester. This material provides high contrast in ultrasound images because of its large impedance mismatch with water and tissues. At the University of Texas at Houston, the research team has emphasized aqueous chemical mixtures as renal contrast agents. Development studies of microbubbles as potential ultrasound contrast agents were first supported by NIH contract more than ten years ago and will soon be on the market in Europe for human use. Further research on a modified design of the original microbubble approach is being continued under an SBIR grant. Many other investigators are now studying new ways to form microbubbles and to modify them for specific clinical applications.

Bioeffects:

Extensive long term research continues to be supported on the potential bioeffects of exposure to ultrasound. It is known that bioeffects can be produced at very high exposure intensities because ultrasound is used regularly to produce high temperatures in tissues (hyperthermia) for cancer treatment. However, it is important to continue to study and monitor the absence of such effects at diagnostic levels. Understanding of the effects of ultrasound energy in different biological tissues continues to increase as the result of research supported at the University of Rochester, the University of Illinois, SRI International, Battelle Northwest Labs, Yale University, and in many other institutions and countries.

Present day research includes work on cells, bacteria, Drosophila, and animals and is concentrated especially on understanding the process called "cavitation" (or the collapse of microbubbles) in fluids. This phenomenon has not been observed in mammals at diagnostic levels of ultrasound exposure. It is still possible to say that in over 25 years of ultrasound exposure to millions of patients all over the world, no adverse effects have ever been observed in humans or animals when the exposures are kept below well-defined levels specified for clinical diagnostic use.

A workshop on Image Processing, Analysis, and Display resulted in a fair degree of interactive stimulation of the administrators of extramural research from several Institutes of NIH. Topics included image perception and enhancement, pattern recognition, and high definition TV projection.

OPTICAL TECHNIQUES

A new method of screening for melanoma is being developed and tested at Oregon Health Sciences University based on detection of skin changes sensed optically, digitized, and fed into a computer which analyzes the data for malignant characteristics.

One of the most interesting new fields that is developing as a spectroscopic tool and potentially as a new imaging modality is the technique of pulsed time-resolved spectroscopy based on picosecond pulses of light and analysis of the diffusely scattered light passing through tissues. (A picosecond is a millionth of a microsecond and can now be timed with almost incredible precision.) Several laboratories are investigating this new technological capability at the University of Pennsylvania, the University of Illinois, the City University of New York, the University of Utah and elsewhere. Time-resolved spectroscopy can now quantitatively assess the degree of hypoxia in an externalized animal tumor. It is hoped that time-of-flight measurements of the diffusely scattered light in the visible or near infrared spectra regions may permit the reconstruction of images of the breast.

A new optical system for viewing, recording, and reproducing black and white and color recordings of diagnostic ultrasound images is being developed on an SBIR program. This capability is not yet available anywhere.

IONIZING RADIATION/NUCLEAR MEDICINE SECTION

The Ionizing Radiation Section of the Diagnostic Imaging Research Branch (DIRB), Radiation Research Program (RRP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI) supports and administers research leading to the advancement of basic, applied, and clinical diagnostic imaging research with emphasis on cancer diagnosis. The ultimate goal of this section is a non-invasive anatomical [x-ray, CT,{1} MRI{2}] and functional tissue characterization (SPECT,{3} PET,{4} MRS{5}). This section supports meritorious research leading to the advancement of imaging research, technology transfer and evaluation and implementation of the newly developed technology for cancer diagnosis. The Ionizing Radiation Section consists of two major parts: (1) X-Ray Imaging; and (2) Nuclear Medicine Research.

There are three major mechanisms of research support in the Ionizing Radiation Section: (1) traditional grants (R01); (2) program project grants (P01); (3) cooperative agreements; and (4) contracts. Traditional grants constitute the major part of this section portfolio. Nine out of twelve DIRB program projects grants are supported by this section. Five program projects deal with nuclear medicine research, while remaining POI's cover wide range of research in physics, engineering and computer science, including digital radiography, (3-D) imaging, instrument development, and technology transfer. There is only one contract supported by this section, "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization," which deals with the development of Tc-99m radiolabeled compounds for use in SPECT. This section supports cooperative research group Radiological Diagnostic Oncology Group (RDOG) involving 17 institutions at an estimated cost of $1,752,000 in FY'91. Furthermore, this section established an interactive network of three institutions to conduct clinical research in the area early diagnosis of prostate cancer using ultrasonography and other methods.

NEW PROJECTS

Three new initiatives (RFA) have been announced and 13 grants were funded by the Ionizing Radiation section during FY'91.

1. RFA 90-CA-05 entitled "National Collaborative Imaging Trial Projects", RDOG III, was announced in FY'90 and funded in early FY'91. Seven institutions have been awarded grants at the first year total cost of $900,000. These new institutions will be added to the currently supported clinical trials research projects (RDOG I and II). The specific objective of RDOG III is to develop an optimal strategy for diagnosis, staging and monitoring head and neck and musculoskeletal tumors.

2. RFA 90-CA-12 entitled "Clinical Diagnostic Studies of Brain Tumors Using PET and Other Imaging Modalities" was announced, and 23 applications were received and reviewed in November, 1990. Three applications were funded in January, 1991 NCAB with the total annual budget of $900,000. The objective of this RFA is to advance PET, MRS and other functional modalities to study essential features of brain tumor metabolism. Ultimately, this research is expected to improve our knowledge of tumor growth and response to therapy. Steps are currently being taken to establish an interactive working group among the funded institutions.

3. RFA 90-CA-21 entitled "Digital Imaging of Chest X-Ray" was issued and three grants (annual budget $600,000) were funded in FY'91. The objective of this RFA is to support meritorious research in the area of digital chest radiography in order to enhance early detection and characterization of solitary lung lesions. The ultimate goal of digital radiography is to improve image transmission and archiving and to reduce cost of patient care.

In addition, an initiative entitled "Clinical Diagnostic studies of AIDS-Affected Brain Using PET and Other Imaging Modalities" is projected to be announced in late Fall'91. The objective of this RFA is to support neuroimaging research in order to improve understanding of the disease processes associated with HIV encephalopathy.

RADIOLOGIC DIAGNOSTIC ONCOLOGY GROUP

NARRATIVE

Radiologic Diagnostic Oncology Group (RDOG) was formed in September, 1987, in response to an RFA. The RDOG objective is timely evaluation of current and emerging imaging modalities in the management of patients with cancer. The development of multi-institutional clinical trial groups allows for rapid patient accrual within a short period of time. This in turn assures rapid evaluation and optimization of imaging techniques for diagnosis, staging and serial monitoring of cancer.

Since the time of its establishment, RDOG clinical research has been important for the development of optimal imaging algorithms for prostate and lung cancer (RDOG I), pancreatic and colon cancer (RDOG II). Four protocols are currently underway in ten academic centers in this country. Recently, a new RFA (RDOG III) has been issued to study musculoskeletal and head and neck tumor imaging, and five or six new institutions are expected to be funded. The results of each RDOG study should have a direct and immediate impact on patient care. Additionally, considerable cost saving is expected due to elimination of unnecessary diagnostic studies.

RDOG has had significant impact on clinical research in Radiology. This is the first time that multi-institutional clinical trials in diagnostic imaging have been conducted in a centrally coordinated fashion with strict quality control and analysis of cost-effectiveness. Ultimately, RDOG study findings would be useful for design of therapeutic protocols, in formulating clinical and reimbursement policy. Moreover, the proposed clinical trials will stimulate spin-off projects addressing a number of questions that are not within the scope of the RDOG grant. Indeed, potential research projects may involve detailed studies of MR tissue characterization, prognostic factors, and many other important areas in clinical Radiology research.

PROGRESS

A. RADIOLOGIC DIAGNOSTIC ONCOLOGY GROUP (RDOG I): LUNG CANCER IMAGING

Timely detection and accurate staging of lung cancer should have a direct and immediate impact on patient management. Indeed, in patients with lung cancer the anatomic extent of tumor at the time of diagnosis is of primary importance for treatment planning. In patients with chest wall or mediastinal invasion, the extent of invasion determines whether or not tumor is considered resectable. Extensive mediastinal fat invasion or invasion of mediastinal vessels, trachea or esophagus indicate unresectability. With peripheral tumors, chest wall invasion does not indicate unresectability, but a knowledge of its presence is important for planning surgery. In patients with superior sulcus tumors, the extent of chest wall invasion and the involvement of the vertebral body or vessels near the lung apex determines resectability. Thus, it is important to optimize imaging strategy for the detection and staging of lung cancer.

The RDOG cooperative lung studies in 170 patients with non-small cell bronchogenic carcinoma (NSCBC) have shown a significant difference in the relative accuracies of conventional X-ray Computed Tomography (CT) and

Magnetic Resonance Imaging (MRI) in diagnosing mediastinal invasion, with conventional MRI being more accurate. The estimates of the areas under the receiver-operated curves were significantly ($p<0.05$) higher for MR imaging [.924 (std. error=.034)] as compared to CT [.832 (std. error=.041)]. Because of the importance of this finding in determining surgical treatment, the study of additional patients is warranted. Additionally, the RDOG lung team recently proposed to study the role of the state-of-the-art high-resolution CT and high-resolution MRI, in addition to conventional CT and MRI, in the diagnosis of mediastinal invasion.

In contrast to the disparity between conventional CT and MRI in the detection of mediastinal invasion, these techniques have been found to have similar accuracy in diagnosing chest wall invasion, bronchial involvement and lymph node metastases. The sensitivity and specificity of CT for the determination of the true tumor stage were 63% and 84% respectively, while these values for MR imaging were not significantly different (56% and 80%). Recently, RDOG investigators proposed to study the state-of-the-art high-resolution CT and high-resolution MRI, which may offer improved accuracy in diagnosing chest wall invasion as compared to previously evaluated conventional CT and MRI. In summary, within the next two years, RDOG studies are expected to formulate the optimal imaging approach to diagnosing mediastinal and chest wall invasion in patients with lung cancer.

B. RDOG I: PROSTATE CANCER IMAGING

Prostatic cancer is the most common malignancy in North American males. Curability of prostatic carcinoma is closely related to the stage and extent of disease at the time of diagnosis. Indeed, when prostatic carcinoma is >1 cm in diameter or 1 cm 3 in volume, the risk of extracapsular invasion increases, and the cure rate diminishes dramatically. Thus, it is important to detect smaller prostatic lesions prior to capsular invasion.

Conventional diagnostic tests used for staging prostate cancer (e.g. digital rectal examination) often fail to detect the full extent of disease. More invasive studies such as lymphangiography and radical pelvic lymphadenectomy yield a higher accuracy in the detection of lymph node metastases, but are more invasive and associated with morbidity and even mortality. Development of non-invasive imaging strategy for the detection of early prostatic cancer (frequently amenable to radiation and hormonal therapy), is extremely important.

A number of non-invasive imaging techniques are now available for the evaluation of prostate gland and surrounding tissues. The specific goal of the RDOG research was to develop an optimal imaging approach for the timely detection of the localized prostatic cancer. While RDOG studies have shown in a series of 230 patients that whole-body MRI is slightly more accurate as compared to endorectal ultrasound in staging prostate carcinoma (77% of patients with advanced disease and 57% of those with localized disease were diagnosed by MR imaging, while corresponding figures for ultrasound were 66% and 46%), the accuracy of MRI in this series of experiments was lower compared to previous reports. Consequently, RDOG researchers had proposed to evaluate the novel MRI approach, utilizing endorectal surface coil, to diagnosis and staging of prostate cancer. Within the next year, it is expected that the RDOG studies will demonstrate comparative accuracy of whole-body MR imaging

using conventional parameters versus fat-suppression techniques (chemical shift imaging) and MR imaging using endorectal surface coils.

CURRENT PLANS

A. RDOG IV: OVARIAN CANCER AND PEDIATRIC SOLID TUMORS
(annual budget $800,000, for three years)

Ovarian Cancer

Cancer of the ovary is the leading cause of death among gynecologic malignancies in the United States. The major challenge in the management of ovarian cancer is the early detection of the disease which is usually asymptomatic in its early stages and usually diagnosed after the peritoneal spread has occurred. The five year survival in these patients remains dismal (about 15-20%). Thus, there is a great need to improve diagnostic imaging of ovarian cancer.

In FY'90 in the Diagnostic Imaging Research Branch (DIRB) there were no funded grants in the area of ovarian cancer diagnosis. Indeed, DIRB staff identified ovarian cancer as one of the undersupported areas in diagnostic imaging research. Consequently, in early August, 1990, DIRB staff proposed to expand funds for the existing Radiologic Diagnostic Oncologic Group (RDOG) in order to include clinical studies in the area of optimization of ovarian cancer detection and characterization . One of the important expected outcomes of the proposed research would be the evaluation of the impact of the improved early diagnosis of ovarian cancer on the management of these patients. This proposal was supported by the Division of Cancer Treatment Board of Scientific Counsellors in October, 1990, and awards are expected to be issued in FY'91 for a period of three years.

PEDIATRIC SOLID TUMORS

Non-CNS pediatric solid tumors (e.g. neuroblastoma, Ewings sarcoma, rhabdomyosarcoma) represent another high priority research area in clinical Radiology. The difficulties with formulating an optimal imaging approach to these tumors stem at least in part from the limited number of patients at a given institution. It is expected that the multi-institutional nature of RDOG studies would allow for enhanced patient accrual and development of the optimal approach to staging of pediatric tumors. The DIRB proposal to expand RDOG in order to include pediatric solid tumors was supported by the Division of Cancer Treatment Board of Scientific Counsellors in October, 1990.

B. FUTURE PLANS FOR RDOG: THE QUALITY ASSURANCE CENTER AND RDOG V.

RDOG has been rapidly expanding, involving rapidly growing number of participating institutions. Consequently, one of the highest priority areas for RDOG in FY'93 will be to establish the Quality Assurance Center (Estimated increase in the RDOG budget: $300,000 per year).

Additionally, it is planned that RDOG V, which will be focused on breast cancer imaging, will be added to the current cooperative group (Estimated increase in budget: $900,000 per year for three years).

NIH PROSTATE ULTRASOUND WORKING GROUP

In addition to RDOG I, multi-institutional NIH Prostate Ultrasound Group was funded in fiscal year 1988. The ultimate goal of this project is the improvement in the accuracy of prostatic cancer detection and staging.

Three institutions (Cleveland Clinic, Baylor College of Medicine, University of Utah) formed Prostate Ultrasound Group which has been investigating the capability of ultrasound (U/S) and ultrasonography-guided biopsy, along with biological markers, to detect prostate cancer, to determine location, volume, extent and invasiveness, and to assess the impact of U/S on morbidity, disease progression, and survival in a group of patients at high risk for having clinically important prostate cancer.

PROGRESS

The investigators of the NIH Prostate Ultrasound Group have observed that ultrasound significantly improved the accuracy of needle biopsies of the prostate. Approximately 25% more cancers have been identified in 85 patients by the combination of U/S and digital guidance for needle biopsy. In patients with no palpable nodes, 20% of patients who underwent U/S and guided biopsy were found to have cancer (R. Shabsigh, S. Carter, S. Egawa, C.D. Wright, C.E. Carlton, P.T. Scardino. American Urology Association, 1989 Annual Meeting). Total annual budget for this project is about $400,000.

PLANS

The multi-institutional working group entitled "Imaging-Guided Tissue Diagnosis of Prostate Cancer" is expected to be funded in FY'93 (estimated budget: $600,000 per year for three years). This project will focus on ultrasound and MRI-guided interventional procedures for early diagnosis and (in some instances) treatment of prostate cancer.

SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS

Examples of the successfully completed SBIR grants can be illustrated by the two products described below.

Science Research Laboratory, Inc., a small business concern in New England, constructed a low cost accelerator during the two years of SBIR funding. The low cost facility based on Cascade Tandem Accelerator (CTA) is designed for the production of PET radioisotopes. It is projected that a CTA facility may be purchased, installed and operated in a hospital for a fraction of the conventional cyclotron facility cost. The prototype instrument funded by NCI was designed for the production of oxygen-15 (O-15) and demonstrated the capacity to deliver sufficient deuteron current at an energy of 3-7 MeV for saturated yield of 2 curies of O-15. The success of a small scientific company in developing this accelerator is an example of a major role SBIR grants can play in the advancement of health care research. Future CTA production and marketing will help to reduce the cost of medical care in diagnostic imaging.

The Western Research Co., a small scientific business company in Tucson, Arizona, was successful in designing and developing a digital imaging colposcope system, which allows for a visual examination of the cervix and its surroundings using a specially designed microscope. During the two years of SBIR funding, investigators were able to develop the state-of-the-art, two dimensional image detector system integrated into the design of the conventional colposcope. The new device is a low cost digital imaging colposcope; this product makes it possible to include colposcopic images in the medical picture achieving and communications systems (PACS), provides useful teaching tool to medical students, and improves early cervical cancer detection in women.

NUCLEAR MEDICINE

The National Institute of Health has been supporting research in nuclear medicine for over three decades. Since 1982 the number of the NCI funded grants in nuclear medicine has been increasing rapidly. The development of new radiolabeled compounds for PET and SPECT is just one example illustrating the wide range of this program.

Program project grants deal with various aspects of nuclear medicine research. The first POI deals with the development and evaluation of promising radiolabeled compounds which have the potential to scintigraphically diagnose and (at higher doses) treat cancer. The second program project continues to explore the enhancement of image-based information by the administration of a novel radiopharmaceutical, as well as the improvement of the qualitative and quantitative aspects of SPECT imaging. An important goal of another program project is to design and develop a scintillation probe for intraoperative tumor detection. This newly constructed probe effectively discriminates against background radiation improving detection of small metastases when compared to external imaging or other surgical probes. It is expected that a commercial probe will be constructed based on this prototype for routine use in surgery.

At Harvard, a novel system for positron emission tomography (PET) is under development in order to improve resolution and sensitivity for animal and human studies. The single ring analog system currently achieves 4.5 mm resolution and produces very useful images in animal research. Further, Harvard researchers are designing an advanced version of this instrument, which will provide 3 mm resolution while retaining adequate sensitivity. Algorithms are being developed to reconstruct three-dimensional images. Animal studies are currently being carried out in animal models of cancer, stroke, myocardial infarct and ischemia.

At Purdue University, novel radiopharmaceuticals are being developed for PET. This effort focuses on the use of radionuclides that can be obtained from parent/daughter generator systems, most notably copper-62, since suitable tracers labeled with such a nuclide could help free PET imaging from the expense associated with its current dependence on isotopes that require in-hospital cyclotron production. High-level (>300 mCi) Cu-62 generators have been developed to prepare copper-62 labeled pyruvaldehyde bis (N4-methylthiosemicarbazonato)-copper (II), [62 Cu]Cu(PTSM), for PET imaging

experiments. In animal models this copper-62 radiopharmaceutical appears promising for PET studies of cerebral, myocardial and renal perfusion.

At the University of Arizona, imaging instrumentation development have brought successful results this year, where two SPECT imaging systems based on modular scintillation cameras have been completed. Cardiac imaging device using 16 modular cameras in a ring configuration with a multiple-pinhole coded aperture is operational, and initial phantom images are quite encouraging. Resolution of 5-6 mm has been achieved, and high-quality tomographic images have been obtained. A hemispherical brain imaging device has been producing good phantom images as well. When complete, this system will use 20 modular cameras. For both of these systems, image reconstruction is accomplished by the specially developed algorithm, which has been now implemented on a parallel computer system designed and constructed by this scientific group. With this algorithm, the University of Arizona system is approximately 50 times faster than currently used VAX 8600. During a recent site visit, the high quality real-time images obtained in this laboratory received enthusiastic approval. This prototype upon completion is expected to advance imaging of the human brain and chest.

One of our grantees is successfully investigating radiotoxicity of several commonly used gamma emitters (e.g. Tc-99m and I-125) attached to pharmaceuticals of biological or chemical interest. These investigators are using murine models in their experimental radiobiological and theoretical investigations to evaluate the in vivo effect of gamma emitters on DNA molecules. The findings of these investigators suggest that free radicals play a major role in the biological damage caused by radionuclides.

A sophisticated simulation system is under investigation at the University of Washington in Seattle to study many imaging problems (e.g. scatter, artifacts) and to develop techniques to improve image quality in SPECT and PET. This work is built on the development of a software system designed to look at complex three dimensional objects. A very efficient Monte Carlo calculation (reduced variance Monte Carlo) recently implemented provides significant (factor of 1000) improvement in calculation efficiency over traditional simulation techniques used in medical imaging. With this high efficiency, two distinct problems can be addressed. First, techniques can be developed to allow for a conventional scintillation camera to image therapeutic dose of labeled antibody in the treatment of tumors (up to 800 mCi doses to date). This capability allows verification of the therapeutic dose distribution and is proven to be of enormous value to the antibody projects. Second, a new approach to scatter correction can be developed. With the enormous reduction in computer time needed to perform Monte Carlo calculations, it is quite feasible to use initial emission scans as the basis for Monte Carlo computation of the scatter portion of the image. The preliminary results produced are most encouraging. Additional simulations were also performed this year on detector modules used in the Scanditronix SP-3000 PET system, with a significant improvement in currently available version.

Researchers at George Washington University are conducting research leading towards the evaluation of radiolabeled lymphocyte migration in tumor bearing mice. Six different populations of lymphocytes were labeled with In-111 and injected intravenously in C3H/OuJ mice with spontaneous mammary carcinoma. The highest concentration of labeled lymphocytes in tumor was observed with

tumor infiltrating lymphocytes, either freshly harvested, or cultured in interleukin-2. Lower tumor concentrations were found with splenic lymphocytes, natural killer (NK) cells and lymphokine activated killer (LAK) cells. Such studies of native and cultured lymphocyte migration will help to explain the variable success of adoptive immunotherapy in the treatment of advanced human malignancies.

MONOCLONAL ANTIBODIES

Research leading to the development and evaluation of new monoclonal antibodies (MoAb) for diagnostic imaging in both human and laboratory animals has been encouraged by this section for the past decade. The number of grants supported in this field has increased since the issuance of an RFA several years ago. Noticeable progress has been made and important publications resulted from this effort. The need for more effective human tumor specific MoAb, however, continues to be the deciding factor for any future progress in this field. It is imperative that more NCI or private funds become available for the development of tumor specific MoAb's to advance this promising field in both diagnostic imaging and tumor therapy.

In another research project, our grantees have completed extensive preclinical trials using intact MoAb 1A3 linked to Indium - 111 by a novel linking agent (developed at the University of Washington and the University of Texas.) Preclinical testing for toxicity, efficacy, safety and dosimetry of MoAb 1A3 has been promising. Phase I clinical study devoted to imaging of tumor deposits in advanced colorectal cancer is planned for the near future.

The NMR evaluation of the phosphocreatinine/inorganic phosphate ratio (PCr/pi) reported by another grantee established bioenergetic indices for monitoring and predicting tumor response to I-131 Lym-1 radioimmunotherapy (RIT). This human anti-lymphoma monoclonal antibody was injected I.V. (200 - 500 uCi) in athymic mice bearing human lymphoma xenografts. P-31 NMR spectroscopy was performed on test and control mice at regular intervals before and after RIT. Thirty six percent of tumors responded by exhibiting regression or arrested growth, which correlated well with increases in PCr/Pi. The tumor PCr/Pi ratio thus prove useful in planning more efficient dose fractionation schedules and for predicting response to RIT.

Preliminary animal data obtained in another DIRB-supported laboratory showed that monoclonal antibody CO117-1A labeled with beta-emitting yttrium-90 (Y-90) via a new bifunctional chelate reagent (BCR), 2-p-aminobenzyl-1,4,7,10-tetraazacyclododecanetriacetic acid (p-NH2-DOTA-3A), which was conjugated site specifically to the oligosaccharide portion of CO17-1A, gave a higher tumor and a lower bone uptake than had been observed previously for DTPA-based BCRs. The uptake by SW 948 human colorectal carcinoma xenografts in nude mice increased with time, reaching 26% of the injected dose per gram of tumor at 48 hours and 39.4% at 168 hours. Adjunctive use of granulocyte colon stimulating factor with Y-90-labeled McAb /co17-1A showed the potential in the nude mouse model for reducing radiation-induced myelotoxicity, which is the dose-limiting factor in the use of Y-90-labeled McAbs for radioimmunotherapy of cancer. Such treatment may permit safe use of larger and more efficacious doses of Y-90-labeled MoAbs.

Research by another grantee is focused on methods to improve radioiodination of anti-tumor monoclonal antibodies so that deiodination is decreased and tumor retention of the radiolabel is increased. The most successful method has been based on coupling radioiodinated tyramine cellobiose to the antibody. In experimental tumors, this method has doubled tumor retention of radioiodine compared to radioiodination by chloramine T. The second goal is to develop methods to quantitate in-vivo biodistribution of radiolabeled anti-melanoma antibody and to select patients with sufficient tumor concentration for therapy. Successful methods to measure organ and tumor concentrations of antibody have been developed. Because of the relatively low MoAb concentration achieved in tumor, high dose therapy has been achieved by using simultaneous autologous bone marrow transplant to avoid irreversible marrow aplasia.

DIGITAL RADIOGRAPHY AND OTHER TENOLOGY DEVELOPMENTS

Research in areas of digital electronic communication technology, PACS image acquisition and sorting, 2-D, 3-D, 4-D imaging, dual-energy systems, and image enhancement has been actively supported by this section. This year's 90-CA-21 RFA entitled "Digital Imaging of Chest X-Ray" generated great enthusiasm in the scientific community. Three radiology research project grants were funded in this area to bring electronic imaging technology to clinic and to advance early diagnosis of cancer.

At the University of California in Los Angeles research is in progress on many aspects of PACS in radiology. A digital viewing station with six 1K by 1K monitors was designed and developed several years ago to be used in pediatric radiology. The prototype station was successfully utilized for case review, radiology conferences and consultations. The success of the pediatric viewing station led to the development of a more sophisticated 2K by 2K monitor display station for primary clinical diagnosis. These modules will be evaluated in clinic. The most significant undertaking, however, is the development of PACS modules for chest radiography, neuroradiology and the intensive care unit (ICU). The ICU module focuses on neonatal, pediatric and coronary diagnoses. The final results of the PACS research at UCLA will determine whether or not the newly designed digital-based systems can replace the conventional film-based approach for use in radiology departments. Using the valuable experiences gained in the past few years in the development of pediatric radiology and coronary care units stations, the grantees are expanding their investigations to include other areas of radiological examination. These experiments are planned to prove the hypothesis that PACS represents suitable replacement of the conventional film-based radiological examinations in clinical environment.

At the University of North Carolina, DIRB-supported grantees are developing high quality improved image display systems. They are utilizing data obtained from conventional x-ray, computed tomography (CT), magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT) imaging systems. Among the activities of this group is the development of software and hardware, stereo, kinetic, depth effect, and hard-mounted displays. They are also involved in the assessment of clinical applications of the developed systems in diagnostic imaging and radiation treatment planning. A major effort of this group is directed towards the development of the appropriate software for rapid automated image computation for both 2D and 3D. Images

produced by this system will be evaluated by experienced radiologists in clinical setting. In addition, an improved software is being developed for direct dynamic display and immediate clinical evaluation. Another significant aim of this research is the development of algorithms for optimal image quality. Other studies are directed towards the reduction of noise-to-signal ratio and the improvement of spatial resolution. The primary objective of this research program is to develop improved means of medical image processing utilizing data obtained from various imaging modalities. Efforts have been made to produce better gray scale 2D images by using regionally adaptive contrast enhancement techniques and targeting context-sensitive human vision, to develop dynamic presentation of 3D images both for anatomical and functional information and to define anatomic objects for fast 2D/3D object definition.

Our grantees at the University of Pittsburgh are also involved in the development and assessment of PACS. Their goal is to develop PACS capable of being the primary technological tool for clinical imaging in the radiology department. These studies will assess the quality of images of the newly developed PACS for clinical imaging in daily routine examinations in the department of radiology. These investigators plan to observe, examine and evaluate the technical aspect of PACS and to assess the electronic imaging performance in a traditional setting at the hospital. In addition to the evaluation of image quality, they will determine the cost effectiveness of the system. The extensive and continuous evaluation and improvement of the electronic imaging is essential for the development of digital imaging equal or better in quality than conventional imaging. A series of workstations were assembled for clinical use. The newly developed display system have the following advantages over other display systems: 1) it is extremely user-friendly (all functions are controlled by a trackball and three push buttons); 2) very high image quality; and 3) high speed (it displays up to 400 images at full resolution in 1/3 of a second). Another major objective of this project is to evaluate cost-effectiveness of the digital imaging system. Preliminary multi-institutional financial studies were conducted to assess the potential impact of PACS on health care delivery and cost effectiveness. Preliminary results showed that electronic imaging is currently competitive with conventional imaging and with refinement and improvement of technology, the electronic imaging will eventually surpass conventional imaging in the quality of examinations. Eventually, PACS may reduce patient care costs.

3-D IMAGING DEVELOPMENT

A number of research grants supported by DIRB are concerned with the manipulation and representation of digital images in 3D surface and volume renderings, as well as using holographic techniques. Research is also being carried out to clinically evaluate new digital representations of anatomy to determine if they are acceptable and useful to busy clinicians. Methods are under development to index 3-D images with temporal indices for analysis in a 4-D environment. Tools such as these will provide diagnosticians with tools, for example, for the detection of congenital heart defects or the changes over time of a tumor in a patient undergoing treatment. Automatic delineation of normal anatomy or auto-segmentation is a research effort that is under exploration by several researchers, enabling the rapid generation of organ volumes and normal tissue structures so vital to radiotherapy treatment planning. Efforts are also being investigated as to the efficacy of the

filmless radiology department, e.g., all diagnostic images to be obtained, archived and viewed as digital images, making the x-ray film a tool of the past.

DIAGNOSTIC RADIOLOGY COORDINATING COMMITTEE (DRCC)

This committee (DRCC) was created in 1989 to replace the NIH Inter-Institute Diagnostic Imaging Group (IDIG). DRCC was established to promote collaboration among NIH institutes and to facilitate the dissemination of information concerning diagnostic imaging research. There is general agreement that a large amount of information is available concerning NIH resources and activities (both intramural and extramural) that can be shared and disseminated among the various Institutes. The Committee is responsible for the coordination of diagnostic imaging research at NIH, developing a NIH-wide long-range research plan for diagnostic radiology, and reporting on a regular basis to the Director, NIH.

In order to have a focal point to oversee the coordination of NIH-wide imaging activities, the Director of NIH designated the National Cancer Institute to be the lead Institute responsible for the development of the new committee (DRCC). The Radiation Research Program, Division of Cancer Treatment has been identified by the NCI to direct this committee.

The DRCC has representatives from each of the Institutes and other NIH component groups, both intramural and extramural, which have significant interests or programs in diagnostic imaging. The Imaging Planning Panel consisting of leading scientific experts and NIH representatives has been formed to identify the most important directions in radiologic science for the next five years. The first meeting of the panel took place on May 30-31, 1990. The important outcome of this meeting is the preliminary list of important new areas of diagnostic research. The next meeting is planned for late summer-fall, 1991, at which time a detailed report is expected to be produced. This report will provide valuable information about the technological and clinical aspects of all of the diagnostic imaging modalities and prioritize future research directions. The Imaging Planning Panel report will have a two-fold impact: I) it will help various institutes to facilitate and corrdinate support of the most important areas of radiologic science; and 2) it will educate and stimulate extramural radiologic scientific community.

FUTURE DIRECTIONS (DIRB)

1. Multi-institutional Imaging Trials for optimization of tumor detection and staging will need to be expanded beyond their current activity. As research in this area expands, both the malignant disease sites to be studied and the number of institutions participating will be increased. Additional funding is thus necessary. New advances in technology will be clinically tested. RDOG III (Head and Neck and Musculoskeletal tumors) has been funded in FY91. In October, 1990, RDOG IV (pediatric solid tumors and ovarian carcinoma) has been approved for funding in FY'92.

2. One of the scientific highlights of the DIRB in FY90 was the recent BSC/DCT approval of the development of in vivo ultrasound microscopic

device by the Board of Scientific Counselors (BSC). This concept is planned to be funded in FY92.

3. A DIRB workshop entitled "Imaging-Guided Stereotactic Tumor Diagnosis and Treatment" was held in May, 1991. A new initiative based on this workshop will be presented to the DCT-BSC in FY92.

4. MRI-guided localized MRS has important potential for early detection and prediction of tumor response to treatment. A new initiative entitled "MRS and Cancer Treatment" based on the DIRB workshop of September, 1989 was approved by BSC as a Program Announcement in October, 1990.

5. New progress in the development of picture archiving and communication systems (PACS) have brought about the need for new software management tools from the field of medical informatics, a new and growing science concerned with the development of decision support tools, data management and physician workstation environments that increase the efficiency and personal productivity of the diagnostic radiologist. New initiatives are expected that will stimulate research and development of knowledge-based systems directed at diagnostic imaging applications. These systems, coupled with PACS networks, will eliminate the need for the patient's traditional x-ray film file which is now tracked by each department care facility. These computer-based systems will improve efficiency, quality control and bring new capabilities to the physician. The first specific DIRB initiative in this area, "Digitization of Chest Radiography for Lung Cancer Detection", has been funded in FY91.

6. The "Clinical Applications of Positron Emission Tomography" was explored in the DIRB workshop held September 14-16, 1988. Advances in the use of this diagnostic modality make it possible to study tumor physiology and metabolism in vivo. Two new initiatives have been recently approved by the DCT-BSC: 1) Clinical Diagnosis of AIDS-Associated Brain Disease Using PET and Other Imaging Modalities (planned to be funded in FY'92); and 2) Diagnostic Studies of Brain Tumor Metabolism Using PET and Other Imaging Modalities (funded in FY'91).

7. Three-dimensional display and analysis of medical imaging can have significant impact on cancer treatment planning in several areas: 1) accurate 3D display; 2) stereotactic computer-assisted surgery; and 3) tumor volumetric analysis. The workshop entitled "3D Display and Analysis for Cancer Treatment Planning" was held in July, 1990. At this workshop, the state-of-the-art in the area of 3D imaging research was evaluated and the future directions prioritized. A new initiative entitled "Quantitation of Tumor Response to Treatment: a Three-Dimensional Approach" was supported by the DCT-BSC for funding in FY92 (annual budget $500,000, for three years).

8. The multidisciplinary DIRB Advisory Group met in January, 1991 in order to define the avenues to bridge the currently existing gap between cancer cell biology and basic biochemistry of MRS. Indeed, this group identified MRS as the unique in vivo tool to study multidrug resistance gene-related changes in chemotherapeutic agents' metabolism. An initiative based on this meeting will be presented to the October, 1991 DCT-BSC.

9. The first inter-agency agreement for RRP was established between DIRB and National Science Foundation. As the result of this agreement, a workshop entitled "NSFNET" was held in March, 1991.

10. The following workshops are planned for FY'92: 1) Receptor Imaging as the Means to Characterize Tumor Cell Biology"; 2) MRS as the Biochemical Basis of Cancer Patient Management; and 3) MR Contrast Agents for Quantitative Functional Tumor Imaging.

STAFF PUBLICATIONS

Shtern F, Garrido L, Compton C, Swiniarski JK, Lauffer RB, Brady TJ: MR Imaging of hematogenous liver metastases in mice; contrast enhancement with Fe-EHPG. Radiology 178: 83-89, 1991.

Shtern F: PET as a Clinical Tool: a Reassessment Based on the Literature Review. Investigative Radiology, in press.

Jolesz F, and Shtern F: Surgical Theater of the Future (NCI Workshop Report). Investigative Radiology, in press.

RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch (RDB) administers a large program of basic, developmental, and clinical research related to cancer treatment utilizing ionizing and nonionizing radiations. Radiation research encompasses a range of scientific disciplines including biology, chemistry, physics and clinical oncology as well as the specialized treatment modalities of photodynamic therapy and hyperthermia. More recently, the role of computer-based tools for the diagnosis, therapy selection and radiotherapy treatment planning processes have received increased emphasis in the Program. Research efforts range from the investigation of basic mechanisms at the atomic and cellular levels to controlled clinical trials for a multitude of diseases using single or multimodality treatment schemes.

Basic research supported by RDB has generated leads for promising new treatment modalities that are currently being tested in clinical trials. Major areas of funded research include particle radiotherapy, hyperthermia, and general radiobiology. Substantial support is also provided for the development of radiomodifiers, tagged antibody therapy, boron neutron capture therapy, photodynamic therapy, and radiation physics. Radiation modifiers are being explored as protective agents to reduce normal tissue morbidity, and as sensitizers to enhance the effects of radiation on tumors. Advanced treatment planning tools continue to be developed through a series of collaborative working groups that are bringing three-dimensional computer graphics and decision-support tools to the treatment planning process. An area of increasing interest and importance is Boron Neutron Capture Therapy (BNCT).

FY90 and FY91 RDB Budget

			$(thousands)	
Grants	FY90	FY91	FY90	FY91
Traditional (R01)	141	127	27,354	25,194
Program Projects (P01)	15	14	15,926	17,717
Conference and New Investigators	6	4	39	16
First Awards	21	22	1,901	2,050
Merit Awards	11	12	3,221	3,968
Cooperative Agreement (U10)	1	2	1,302	1,302
RFA, 90-CA-07	2	2	1,356	4,000
RFA, 90-CA-06	0	4	0	451
RFA, 90-CA-04	2	2	344	329
SBIR	16	16	2,755	2,465
Total Grants	215	204	54,198	57,492
Contracts				
Regular	10	9	3,067	2,191
SBIR	2	2	373	479
Total Contracts	12	11	3,440	2,670
TOTAL RDB BUDGET	227	215	57,638	60,162

PARTICLE RADIOTHERAPY

Radiotherapy with either charged or uncharged particles continues to receive a significant portion of the RDB budget. Neutron therapy Phase III trials compare fast neutrons against best conventional photon therapy for head and neck cancers, prostate and lung tumors, as well as tumors of radioresistant histologies, such as sarcomas of the soft tissue and bone and melanoma. Charged particle therapy with both protons and heavy ions are now successfully treating a variety of tumors in the lung, prostate and eye as well as lesions adjacent to the spinal cord that cannot be treated with any other therapy. Results of treatment of tumors such as uveal melanomas and chordomas and low grade chondrosarcomas of the base of the skull and the cervical spine show a major improvement over conventional x-ray treatment methods. Because of the sparing of adjacent normal tissue with protons or heavy ions, higher tumoricidal doses can be delivered to these lesions which are not attainable with conventional therapies.

The proton beam therapy experience at the Massachusetts General Hospital/Harvard Cyclotron Laboratory, based on the treatment of more than 2000 cancer patients, has confirmed the expectation that higher doses to the target volume and smaller treatment volumes can be used to treat and control selected tumors. Comparing the data to historical controls, proton treatment has resulted in a greater tumor control rate, comparable or lesser morbidity, and no increase in marginal failures. This experience is consistent with the concept that improved radiation dose distribution yields better therapeutic results.

A highlight of recent work with neon ions has demonstrated that shorter fractionation schemes with higher doses per fraction are feasible and safe. The treatment course has been shortened in many target sights to as little as 8-12 treatments given over 2-3 weeks. Acute reactions have been tolerated and local control results appear better than earlier Phase I studies. These sites include soft tissue and bone sarcoma, prostate tumors and some head and neck tumor sites. Further study of glioblastoma is ongoing but it is too early for evaluation.

The history of radiation therapy shows clearly that major improvements in dose distribution have yielded clinical gains. Further gains will come if worthwhile improvements in dose distributions can be realized. Major issues regarding the use of new techniques which yield further improvements in dose distributions are: the ability to image the target and non-target tissues/structures precisely; the ability to align the target and the beam and to define the uncertainties of that alignment; the ability to deliver the dose to all of the defined target (in 3 dimensions) at each treatment session, and the cost (personnel, equipment, space, etc.). To the extent that gains are demonstrated, the results will prompt efforts to test additional sites for proton beam therapy. Positive results will also support the study of radiation therapy strategies other than protons to improve dose distributions.

Charged particles may have a significant place in the armamentarium of cancer therapy. Further research is needed to establish their exact role. There is clearly a need for a small number of state-of-the-art, dedicated, hospital-based proton research and treatment facilities in the U.S. The

HYPERTHERMIA

The research community continues to express a high level of interest in hyperthermia an evidenced by the large number of grant applications received. Both pre-clinical and clinical studies are proposed in these applications. During the last year the major emphasis in the field of hyperthermia has been in several areas. In the preclinical area attention was directed to studies investigating the mechanisms of heat damage and the factors which modify this effect. Clinically the emphasis has been placed on developing and implementing quality assurance guidelines for clinical trials and investigating the usefulness adding radiation or chemotherapy to hyperthermia.

It is well known that cells which are repeatedly exposed to applications of heat develop a transient resistant to the effects of the heat. This phenomenon has been termed thermotolerance. The development of thermotolerance and its relationship to heat shock protein synthesis has been investigated in several studies. Investigators at Stanford University have shown that the magnitude of thermotolerance and the level of heat shock protein (HSP) expression were measured in chinese hamster ovary cells after gradual temperature transients from 37° or 39° to 42° or 43°C. When the level of thermotolerance was measured by clonogenic survival after challenging temperatures between 42° and 43°C, substantial thermotolerance was observed. However, when the challenging temperature was raised to 45°C, proportionally less thermotolerance was apparent. Scanning densitometry revealed that low levels of heat shock proteins were synthesized during the heating gradients, but less than after a heat shock at 45°C that delivered an equivalent heat dose. The immunoassay of HSP 70 levels measures both pre-existing and newly synthesized protein, and showed that there was no net increase in HSP 70 during two of the heating gradients tested, despite the increase in synthesis noted on the gels. Higher turnover of HSP 70 at the elevated temperatures possibly accounted for the failure to detect a net gain in total protein. In contrast, the total amount of HSP 70 doubled during the 6 hrs following a heat shock of 45°C for 10 min, an equivalent heat dose to one of the gradients where no net increase in HSP 70 was measured by immunoassay.

Studies in the University of California, San Francisco have demonstrated in 1) heat resistant variants 2) preheated cells 3) cells microinjected with purified HSP 70 and 4) rodent cells overexpressing an exogenous cloned humans HSP 70 gene, that mammalian cells having elevated levels of HSP 70 are more resistant to thermal stress. In these studies the cellular HSP 70 levels were qualified by 35S methionine labeling, enzyme-linked immunosorbent assay (ELISA) and flow cytometry and were positively correlated with thermotolerance. The data suggest that HSP 70 can be used as a predictive assay to monitor retained thermotolerance in heated tissues.

In studies conducted at Thomas Jefferson University it was shown that deuterium oxide (D2O - heavy water) protects G1 wildtype chinese hamster cells (CHO) and a heat sensitive variant HS-36 from heat cytotoxicity, as well as, further protects thermotolerant CHO cells against additional heat cytotoxicity. The investigators also did a comparative quantification of the heat-induced nuclear matrix associated protein with 1) the increased electron density of nuclear matrices using electrons microscopy and 2) the increased brightness of nuclear matrices stained with antibodies against hnRNPC proteins and PI antigens. The findings indicated that the C proteins represent a major

TER's. BCNU was most significantly enhanced by heat while 5FU was least. Cis-platinum and bleomycin were intermediate.

Earlier clinical hyperthermia trials have produced results which were difficult to interpret and whose validity was questionable. This situation arose because there trials lacked good quality assurance guidelines. Through the efforts of the Hyperthermia Physics Center, the Center for Devices and Regulatory Health, The North America Hyperthermia Group and The Radiation Therapy Oncology Group general guidelines for quality assurance have been developed, published and implemented. These guidelines have emphasized equipment reliability and reproducibility, superficial applications and microwave techniques. The current clinical hyperthermia trials are using the quality assurance guidelines and it is anticipated that these guidelines will have a positive impact on the trial results.

The long standing goal of clinical hyperthermia researchers is to heat deep tumors uniformly. Hyperthermia instrument development have focused on systems having this capability. Small business firms are attempting to develop such systems. The BSD Medical Corporation has introduced the Sigma Treatment System (BSD-2000 control console and Sigma-60 applicator) which is being tested by the Radiation Therapy Oncology Group in a Phase III clinical trial (90-02). Labthermics Technologies, Inc. has developed an ultrasound system with 56 ultrasound transducers mounted on a spherical shell which can focus a three dimensional beam of specified path and dwell time to produce a uniformly heated treatment volume at depth. A prototype of this hyperthermia system has been placed at the Dana Farber Cancer Institute where methods for treatment planning using various imaging and temperature probe insertions and localization techniques will be investigated to provide the therapy preparation methodologies necessary for practical clinical implementation of the system. A clinical trial using this system is expected in the near future.

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is continuing to gain acceptance as a potential treatment modality for some cancers. PDT is based upon the principal that systemically administered photsensitizers appear to be preferentially retained longer by tumor tissue than normal tissue. When tumor tissue containing the photosensitizer is exposed to visible light with an absorption wavelength that is near the maximum for the photosensitizer, single oxygen is produced in the tumor cells. Cell death and tissue necrosis result. The increased interest in PDT as a mode of cancer therapy has stimulated the search for new photosensitizers and has promoted basic research on the cellular mechanisms associated PDT.

Evaluation of the feasibility of synthesizing and spectrally characterizing a large variety of long-wavelength cationic dyes as possible photosensitizers is continuing. Characterization includes *in vitro* and *in vivo* toxicity and pharmacokinetic studies. All living cells have a negatively charged electrical potential across their plasma and mitochondrial membranes which cause positively charged (cationic), membrane-permeable dyes to concentrate within cells and mitochondria. Many types of malignant cells accumulate higher concentrations of these dyes and retain them for much longer than do normal cells. One investigator has synthesized over 40 dyes within four

different chemical classes and evaluated them for selective photodamage *in vitro* and *in vivo*.

The more promising compounds give selective killing ratios greater than 1000 between malignant and non-malignant cells in culture; a cell kill which compares favorably with antibody-targeted techniques. Using measurements of respiration in intact cells, these investigators have demonstrated that different cationic photosensitizers interact at different sites within the mitochondria and that many of the compounds can damage closed circular (e.g. mitochondrial) DNA, though the mechanisms of the photodamage appear to differ. Acceptable in vivo dose levels for some of their most promising cationic photosensitizers have been established and appropriate vehicles for their administration were devised.

New classes of photosensitizers continue to be synthesized, characterized spectrally, and evaluated biologically. These classes include purpurins, phthalocyanines, bacteriochlorophylls, napthalocyanines, benzoporphrins, chlorins, the far red absorbing iso-BOSiNC and metal complexes of octabatoxphthalocyanine. All of these classes appear to be potent photogenerators of single oxygen. The pharmacokinetics studies of iso-BOSiNC conducted on normal and tumor-bearing rats and mice have shown ten times more iso-BOSiNC in tumorous than in normal tissue at 24 hours post injection. Phototherapy of the tumor-bearing animals resulted in cure rates exceeding 80 percent. Tin purpurin, a compound synthesized at the University of Toledo has been licensed to Phototherapeutics, Inc., a small business which plans to obtain an investigational new drug (IND) approval to conduct clinical trials to evaluate this compound.

A precise understanding of the mechanism of tumor destruction by photodynamic therapy is critical to the further development of this treatment modality for clinical use. Several studies have been undertaken which contribute to the understanding of the mechanism of PDT.

At the University of Louisville investigators studying the effect of PDT on blood flow found that a marked redistribution off-actin in PDT treated cells resulted in a conformational change of the cells and a significant effect on capillary permeability and on the capillary endothelial cell surface receptors. Using computers modeling to evaluate intravital microscopic data from PDT treated microvessels, these investigators were able to identify vessels which respond differently to therapy.

Investigators at the University of Rochester have used magnetic resonance imaging and spectroscopy to study the mechanism of action of PDT in mammary tumors. The short time behavior of the spatially resolved NMR spectra indicated that mitochondrial involvement in vivo is a major factor in the efficiency of therapy. Using the delivery of various metabolites as a method of evaluating the microcirculation, it was noted that different agents were distributed differently but that generally the microcirculation is affected, as well, by the light activation at the surface of the tumor. Other investigators at this institution have studied the effects of modifying the laser light delivery on tumor growth in vivo. A significant delay in tumor growth was noted when the tumors were exposed to periodic irradiation (15 minutes light, 60 minutes dark, 15 minutes light) compared to continuous light of the same total fluence. It was also noted that the recovery of the

markedly reduced mitochondrial enzyme cytochrome C oxidase took longer to return to normal levels after periodic light irradiation.

The mechanism faction of Merocyanine 540 (MC 540) as a phototherapeutic sensitizing agent for ex vivo purging of neoplastic cells (leukemia, lymphoma and neuroblastoma) from autologous bone marrow grafts is being studied at the Medical College of Wisconsin. The photochemistry and photobiology of the dye is being investigated. Some of the preliminary findings are:

a) Quantum yields for single oxygen production by membrane-bound dye have been found to be at least 10-times greater than those measured in solution, supporting the importance of this species as a cytotoxic intermediate.

b) Competitive kinetic studies and identification of the 5 alpha- and 6 beta- hydroperoxides of cholesterol in model membranes and leukemia cells have confirmed that single oxygen is generated and attacks important membrane targets (lipids and proteins).

c) Dye-mediated lipid peroxidation in membranes is exacerbated by ascorbate and iron, which also enhance photokilling of cultured leukemia cells.

d) MC 540 undergoes rapid photobleaching in simple solvents and membranes; major photoproducts are being characterized and examined for possible cytotoxicity. The membrane reaction is strongly accelerated by free radical lipid peroxidation.

e) Certain less polar MC540 analogs are more cytotoxic (and possibly more therapeutically effective) than the parent dye.

f) Light delivered in split doses to MC540-sensitized cells is less lethal than continuously delivered light, suggesting that a repair mechanism (possibly acting co-operatively with detoxification) exists.

At the University of Southern California a series of studies was conducted to examine the basic mechanisms associated with PDT mediated tumoricidal action. The initial results reported were:

a) PDT sensitivity was examined in cell lines resistant to hyperthermia. Cross resistance between hyperthermia an PDT was not observed even though PDT induces the same family of heat shock proteins as hyperthermia.

b) Porphyrin mediated PDT induces transcriptional and translation expression of heme oxygenase. Cellular exposure to porphyrin and to photochemically generated single oxygen independently induce increased expression of this gene (which is thought to play a role in modulating oxidative damage in cells).

c) Four variants of the mouse RIF-1 cell line were isolated that are resistant to porphyrin mediated PDT. The resistance was not due to trivial differences in photosensitizer uptake nor did there appear to be significant differences in antioxidant enzymes levels or constitutive

stress protein levels. Two dimensional gel electrophoresis demonstrated several unique proteins being overexpressed in the resistant cell lines.

d) Glucose regulated protein (GRP) induction and function was evaluated following PDT. GRP induction correlated with PDT treatments in which cellular protein glycosylation is inhibited. In addition, overexpression of GRP correlated with cellular tolerance to PDT.

RADIATION MODIFIERS: SENSITIZERS AND PROTECTORS

The preclinical and clinical areas associated with radiation modifiers continued to be active. The preclinical studies emphasize chemopotentiation by radiosensitizers and chemoprotection by radioprotectors. Clinically, the evaluation of etanidazole (SR-2508) in a Phase III trial is continuing.

At the University of Wisconsin, investigators have focused on the development of chemosensitizing agents. They synthesized mixed-function compounds consisting of a 2-nitroimidazole functionality convalently linked to a chloroethylating species resulted in compounds with preferential hypoxic toxicity. Structure-activity studies with these compounds has provided leads for the synthesis of the next generation of compounds which could significantly improve antitumor selectivity. In collaboration with chemists at the University of Toronto, they have started to examine the molecular mechanism of chemosensitization by determining the biological properties of individual reductive metabolites of a model chemosensitizing 2-nitroimidazole. These experiments indicate that the one electron reduction intermediate (nitroso-) is a potent cytotoxic agent, reacts with and depletes cellular thiols, induces significant DNA damage and enhances the toxicity of the chemotherapeutic drug, melphalan. Further studies are in progress to help elucidate the active intermediates responsible for the biological effects of reduced nitroimidazole sensitizers.

A new approach for delivering high local doses of cisplatin to tumors using a biodegradable open-cell polylactic acid matrix has been developed at Dartmouth College. This delivery system has been evaluated in dogs undergoing limb sparing therapy and dogs with transplantable murine tumors. In the latter model, the higher levels of cisplatin measured in tumors without increased normal tissues toxicity resulted in therapeutic potentiation when combined with radiation therapy superior to that attainable using systemic administration of cisplatin. Both radiosensitization of hypoxic cells and radiopotentiation, by carboplatin pre- and post-irradiation, respectively, correlated with an increased production of DNA single strand breaks as measured using alkaline elution and DNA unwinding (FADU). However, there was no evidence for increased production of DNA double strand breaks in these protocols using neutral elution. This work has been extended to paraplatin a second generation platinum drug in combination with multifractions of radiation. The tumor regrowth delay results demonstrated a significantly improved enhancement ratio when the drug was delivered by the open-cell polylactic acid bio-degradable polymer rather then intervenously.

The new bioreductive drug, SR-4233 (NSC 130181), a benzotriazine di N-oxide, which was developed at Stanford University was shown to be highly effective in killing hypoxic cells in vitro and in vivo. It can also radiosensitize aerobic cells after hypoxic activation. This effect can be used to radiosensitize both aerobic and hypoxic cells in highly fractionated regimes (similar to those used in radiotherapy) under conditions in which classic hypoxic cell radiosensitizers do not work because of reoxygenation. The drug has to be activated by the fluctuating hypoxia in the tumors. No radiosensitization of normal tissues is seen resulting in therapeutic gain factors of ~2.0 for these fractionated protocols. As the drug doses are highly tolerated (even giving 5 x/week for 6 weeks), this drug appears to be ideal for clinical trials with radiotherapy. Further development of this agent is being done by Sterling Drug, Inc. the pharmaceutical company which recently licensed this agent.

The Phase III clinical trial of the radiosensitizer Etanidazole (SR 2508, NSC 301467), which is sponsored jointly by the NCI and Roberts Laboratories, is continuing to accrue patients. Patient accrual to this protocol is projected to be complete in 1991. This trial is evaluating Etanidazole in patients with locally advanced head and neck squamous carcinomas. An identical trial is being sponsored by Robert Laboratories in Europe and the results of both trials will be combined for analysis. Roberts Laboratories is expecting to obtain FDA market approval for Etanidazole under their trade name Radinyl.

At the Harvard Joint Center for Radiation Therapy, two protocols, one completed and the other being initiated, are evaluating Etanidazole as a hypoxic cell sensitizer. The Phase I study (RTOG 8605) attempted to determine the MTD, toxicity, pharmacokinetics and tissue levels of Etanidazole, continuously infused over two days in patients with histologically proved malignancies which were locally advanced metastatic or recurrent and had an expected local control rate of less than 80%. Interstitial or intracavitary brachytherapy was given to all patients. The study has closed. An MTD of 20 grams/m2 over 48 hours had been reached and reported toxicity was considered minimal. A Phase II trial to evaluated Etanidazole in locally advanced prostate carcinoma patients receiving conventional fractionated radiation therapy has been opened to accrual.

Several studies have been directed toward elucidation of the chemical/biochemical mechanisms of toxicity of radioprotector thiol compounds. Investigators at the Massachusetts General Hospital have shown that manipulations of enzymatic pathways for detoxification of oxygen radicals can influence toxicity of added thiols. It has been postulated that thiols are toxic because of the production of hydrogen peroxide during thiol autoxidation, but studies on eight different thiols show there is no simple correlation between thiol autoxidation rate and toxicity. These results are of practical relevance for use in establishing proper conditions for radioprotection studies with thiols and also are relevant to the current interest in oxygen radicals as damaging agents in chemotherapy, carcinogenesis, aging and arthritis.

At the University of California thiols of varying net charges were compared for their effectiveness in protecting V79 cells from radiation. When the results were summarized in terms of the intracellular thiol concentration it was clear that under aerobic conditions anionic thiols protect mainly by

hydroxed radical scavenging while cationic thiols protect mainly by chemical repair of DNA radicals. DNA radical repair seemed to be the dominant mechanism by which amino thiols i.e. WR-2721 and WR-1065 exhibit their marked radioprotection of DNA in vitro.

RADIOLABELED ANTIBODY DIAGNOSIS AND THERAPY

Radiolabeled monoclonal and polyclonal antibodies and their fragments directed against tumor cell surface antigens have shown promise as both diagnostic and therapeutic agents in-vitro and in human tumor implants in animals. These observations have led to research in supporting this technology that is only now receiving significant federal funding. There are at least 4 foci of interest in this NCI research area; Radiation Research Program (RRP), Radiation Oncology Branch (ROB), and Biological Response Modifiers Program (BRMP) in Division of Cancer Treatment (DCT), and the Cancer Immunology Branch (CIB), Division of Cancer Biology and Detection (DCBD). Research is ongoing in the development of radionuclides for imaging and therapy. For imaging tumors, a low energy gamma emitter of about 150 KeV is optimum. For therapy a medium energy beta or high energy alpha emitter is necessary to deposit the energy to kill tumor cells. Investigations are studying the chemistry of linking the radionuclides and antibodies for greatest stability in in vivo.

Clinical trials are being carried out at a small number of research centers. The Radiation Therapy Oncology Group (RTOG) is conducting a Phase I/II study using Dextran modified antiferritin labeled with Iodine-131 to treat nonresectable hepatocellular cancer is also being performed.

During the past year, an investigator at Duke University demonstrated in athymic mice with subcutaneous human glioma xenografts that tumor localized hyperthermia can increase the rate and magnitude of tumor uptake of a labeled antibody fragment of two. These results suggest that it may be possible to use tumor localized hyperthermia to increase the therapeutic utility of radiolabeled MAbs, particularly when labeled with short-lived nuclides such as At-211, an exciting prospect.

As more is understood about the inhomogeneous distribution of radiolabeled antibodies, it is apparent more research is needed on dosimetry. Two investigator-initiated grants are currently funded in this area and two additional grants were funded in FY89 in response to an RFA.

Strong central coordination of all of these research areas is necessary to thoroughly explore radiolabeled ligands/conjugates for the diagnosis and therapy of cancer.

RADIOTHERAPY TREATMENT PLANNING

The Radiation Research Program has made a major investment in radiation therapy treatment planning over the last decade, beginning with four contractors which formed the first Collaborative Working Group (CWG), Evaluation of Treatment Planning for Particle Beam Radiotherapy, funded from 1982-86. This group was then followed by Evaluation of High-Energy Photon External Beam Treatment Planning (1984-87); Evaluation of Dosimetry, Calculations and Afterloading Techniques for Interstitial Brachytherapy

(1985-88); Evaluation of High-Energy Electron External Beam Treatment Planning (1986-89); and the Radiotherapy Treatment Planning Tools CWG, funded from 1989-94. The last group of contractors consists of three institutions funded as a Collaborative Working Group to develop portable and transportable software to attack the time-consuming and labor-intensive tasks of 3-dimensional radiation therapy treatment planning. The group has developed new software and documentation standards which will yield software tools that can be exchanged and used at different institutions, irrespective of the hardware, operating systems and computer architecture of the different facilities. These contributions will have important consequences for the future development of sophisticated software that can then be adapted to community-based health care centers. Preliminary discussions with scientific investigators from the National Aeronautics and Space Administration (NASA) have identified common problems of scientific interest in the area of image processing and collaborative research projects between the NCI and NASA investigators are likely.

Prototype demonstrations of the new portable software tools were demonstrated in 1991 at all three institutions. The tools consist of the following:

1) an image management executive for handling CAT scan studies (multiple slices with contours defining normal anatomy and tumor);

2) image correlation software for registration of two imaging studies from different modalities (e.g., CAT scan registered to MRI; or MRI registered to PET);

3) target volume definition, based on accepted standards for margins around known tumor to account for patient motion and treatment set-up error and knowledge about micro-extensions of tumor growth;

4) a knowledge-based system to develop "first-guess" treatment plans from an existing database or library of plans used in the past for tumors of similar anatomical location and type;

5) treatment plan evaluation tools which combine data from diverse sources (dose-volume-histograms, tumor control probability, normal tissue complication probability, isodose distributions) to arrive at a recommendation for choosing an optimal treatment plan; and

6) treatment verification tools which compare the image of the actual patient in the treatment position with the image that is expected based on the treatment prescription and the treatment planning simulation.

The implementation of the software tools at all institutions is the next stage of the project for the purpose of clinical evaluation, followed by dissemination of the software to the radiotherapy community. Currently, 3-D treatment planning is a time-consuming and labor-intensive process and is practiced at relatively few institutions. With the implementation of the tools, many of the barriers to the use of this advanced technology will be

removed, enabling greater focusing of radiation on the tumor and target volumes. This improvement in the radiation dose distribution offers the opportunity for greater tumor dose and cell-killing, with no increase in radiation complications to the normal tissues.

PATTERNS OF CARE

The Radiation Research Program is currently funding a contract with the American College of Radiology with co-funding support from the Agency for Health Care Policy and Research to investigate "Patterns of Care in Radiation Oncology". The multi-faceted project will achieve a number of goals:

1) A survey of all radiation therapy facilities in the 50 states of the US plus Puerto Rico, will yield a facility master list. From this, stratification according to type of equipment, number of full-time physicians and physicists, method of treatment planning and other parameters, will be used to determine how these factors influence the survival and complication rates of radiotherapy patients.

2) A consensus of the best current management methodology in five cancer sites is to be determined by a panel of experts and the consensus published as a newsletters with widespread dissemination to the radiation therapy community. The five tumor sites include: breast, cervix, Hodgkin's disease, prostate and recto-sigmoid.

3) The Patterns of Care Study will then survey the radiation therapy community at a statistically representative number of facilities compliance to determine to what extent the facilities in the US conform to a "best" standard of care. Patient outcome will be compared as a function of the pre-determined facility parameters to yield recommendations leading to improved patient survival and/or reduced complications.

4) Long-term follow-up of patients included in previous patterns of care studies in 1973, 1978 and 1983 will be carried out to continue the study of how type and kind of facility, as well as treatment methodology, affects survival and outcome.

5) A new facet of the current Patterns of Care Study is the patterns of fractionation project. This aspect of the contract will examine patient outcome at a number of institutions practicing widely varying dose fractionation and treatment scheduling. There is evidence that hyperfractionation (two treatments per day) has a particularly efficacious effect in some tumors. The data is controversial, however, and the patterns of fractionation project will correlate differences in patient outcome with particular fractionation schedules.

6) Finally, the results of the Patterns of Care Study will be disseminated to the radiation therapy community through lectures, education symposia, professional meetings, seminars, workshops and newsletters to the widest possible audience to bring about an improvement in the quality of treatment for patient care in radiotherapy.

The contract is in its third year of performance and has completed the facility master list survey. Newsletters that describe best current management for the five tumor sites have been written and are in the process of being disseminated to the radiotherapy community. The other projects are in various stages of intensive effort.

RADIATION BIOLOGY

The NCI, primarily through the Radiotherapy Development Branch, RRP, DCT, continues to support a major portion of radiation biology research in the United States. This research is dedicated to improving radiation therapy as a treatment modality. Tumor and normal tissue radiobiology at the molecular, cellular and animal levels continues to be vigorously researched.

The following examples illustrate the breath and diversity of this program.

1) Researchers at Colorado State University have been working for a decade on a program project to define and use naturally occurring tumors in pet animals as models for experimental cancer therapy in humans. The program is based on experimental medicine, hyperthermia, radiotherapy and surgery. A major accomplishment of this program has been the development of cooperative multi-institutional comparative oncology research initiatives involving Duke University, North Carolina State University, and The Norris Cotton Cancer Center in New Hampshire. Second important accomplishment was the determination of the radiation dose response for oral carcinomas in dogs and comparison with the dose response of radiation combined with hyperthermia. This was the first clear clinical evidence of a major advantage of combining heat with radiation for local control of tumors. Another significant accomplishment was the evaluation of the influence of surface area and weight in determining an appropriate dose of melphalan by studying dogs of various sizes. It became clear that a weight based dose determination was more appropriate. This is of interest to pediatric oncologists because of greater variation in surface area and weight in children.

2) In research at The Massachusetts General Hospital on the chemical and biochemical mechanisms of toxicity of thiol compounds, results show "that serum dramatically alters toxicity of thiols and their oxidation" product, H_2O_2. In particular, glucose from serum is important because it is the ultimate source of the reducing equivalents which are used in cellular detoxification of peroxidation damage. Furthermore, copper, either in the free form or as the serum protein ceruloplasmin , greatly increases thiol toxicity, although many other metal ions do not alter the toxicity. The results are consistent with the hypothesis that thiols oxidize to their disulfides, producing H_2O_2, in a reaction that is copper-dependant. The H_2O_2 then reacts, also in a metal catalyzed reaction, to produce the ultimate toxin, "the OH radical". These data are important not only for the study of possible toxicity of thiols used clinically as radioprotectors, but have a much wider and potentially more important application to the fields of arthritis, oxygen where thiols may be used as therapeutic agents.

3) At The University of Utah the induction of cell death by an apoptotic mechanism was examined in a hybrid cell line (Hb 8-3). Apoptosis (endonucleolytic fragmentation of nuclear DNA followed by a loss of plasma membrane integrity and uptake of vital dyes) was induced in a number of ways and determined in a number of ways. Apoptosis began 12 to 14 hr. after treatment and was completed by 28 to 30 hr. It occurred in the G1 phase of the cell cycle subsequent to treatment. The results indicated that while DNA damage is not a prerequisite for the induction of apoptosis, DNA damage (pyrimidine dimers, DNA single and double strand brakes) can serve as a signal for this stress-induced cell response.

4) At Syracuse University a research effort is underway to elucidate the role of acentric chromosome fragments in gene amplification a mutation associated with drug resistant in cultured mammalian cells and also with oncogene overexpression and tumor progression in human cancer. A report has been published in the past year linking x-irradiation and gene amplification in cultured mouse cells which amplify their genes an double-minute chromosomes. Another report has shown that double-minute chromosomes can be used to quantify double-strand breakage of DNA when coupled with gel electrophoresis.

5) One of the most promising uses of intraoperative radiotherapy (IORT) is for abdominal cancers located near critical retroperitoneal structures. Aorta, vena cava, ureters, peripheral nerves, muscle and bone may be included in the field of irradiation. Tumors located around these structures are frequently difficult to resect completely. The tissues of the abdomen are sufficiently sensitive to limit the total dose of externally delivered radiation therapy. During IORT, the tumor can be removed and radiation directed to the tumor bed. By using electron beam radiotherapy, the depth of the dose can be controlled to spare underlying structures such as the spinal cord for example. Therefore, by using IORT the volume irradiated can be carefully defined and some sensitive structures excluded from the field.

   The research project Experimental Intraoperative Radiotherapy, was begun a decade ago at Colorado State University to estimate normal tissue tolerances to large single doses of radiation delivered to the para-aortic region. At the time of initiation of this project there was little information on the tolerance of retroperitoneal structures to large single doses of irradiation. This project has provided critically important guidelines for the use of IORT in the treatment of cancers in humans. Earlier clinical and experimental reports indicated that retroperitoneal structures tolerated relatively high IORT doses. A major finding of this research was that 15 Gy IORT combined with 50 Gy of external beam therapy is near the maximum tolerance dose for retroperitoneal structures. The peripheral nerves appear to have the least tolerance and larger doses cause significant injury to aorta, ureters, muscle and bone. The questions which have been asked are of more general interest in radiation therapy then just for IORT. The tissues evaluated are the entire vascular tree from aorta to capillaries, peripheral nerves, muscle, bone and ureters. There is little research specifically

directed at those tissues even though they include the most common tissues at risk in radiation therapy and in many cases are dose limiting. Questions concerning the ability of these tissues to repair radiation injury have not been answered and are of major concern in radiation therapy. Clinically relevant end points such as paralysis, ureteral stricture, aneurysms, thrombosis, bone necrosis and muscle atrophy are obviously important clinical consequences of irradiation. Careful histologic evaluations permit dose response comparisons and insight into the basic mechanisms and cellular interactions leading to late consequences of irradiation.

6) Slow but steady progress continues in the search for suitable compounds for boron neutron capture therapy (BNCT). At the University of California(San Francisco) research is underway to utilize low-density lipoproteins (LDL) to transport and microinject boron compounds into cancer cells. Uptake and biological efficacy studies in vitro are consistent with receptor-mediated endocytosis of the boronated LDL, and boron remains firmly bound in (cell culture) despite repeated washing and suspension in boron-free medium. The boron distribution is intracellular, with a biological efficacy indicative of a cytoplasmic location. At Brookhaven National Laboratory, investigations continue into the use of the boron-containing amino acid analog, p-boronophenylalanine (BPA). Though originally envisioned as a melanoma-specific boron delivery agent, BPA has been shown to selectively deliver therapeutically useful amounts of boron to tumors other than melanoma, specifically a murine mammary tumor and rat glioma. Thus BPA may have broad utility as a boron delivery agent for BNCT.

SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

In FY 1991, the RDB funded 2 SBIR contract (Phase II) and 16 SBIR grants (6 Phase I, and 10 Phase II). Funded research areas included photodynamic therapy, boron neutron capture therapy, photon therapy, electromagnetic and ultrasonic hyperthermia, expert systems for Radiation Oncology, real time portal scanning, variable collimation, microdosimetry, laser interstitial therapy, dynamic electron arc collimation and optimization of complex multi-field radiation therapy.

INTERNATIONAL ACTIVITIES

US/USSR

The Radiation Research Program initiated a scientific exchange program during the last year between US and USSR scientists in the field of proton radiation oncology. A US-based team traveled to Moscow and Leningrad in September 1990 to establish specific goals for collaboration with the Institute of Theoretical and Experimental Physics, Moscow, and the Institute of Roentgenology, Leningrad. Preliminary goals of an on-going scientific exchange that was outlined at the time of the exchange visit are as follows:

1) Improve access to the achievements and results of USSR investigations through the publishing of research results in US-based scientific journals. To facilitate this process, the US investigators are committed to assisting the Soviet scientists in the writing of manuscripts in English. Achievement of this goal, however, depends on improved communications between the two countries. Telephone lines are inadequate in the USSR, preventing either voice or FAX communication on a regular basis. The implementation of computer networks in the Soviet Union, such as BITNET, is seen as a vital first step.

2) Common definitions and reporting methods for clinical, physical and radiobiological research results are necessary to enable both countries to benefit from the research carried out. Standard data analysis methods that are common to both countries are necessary for sharing data. It was suggested that the methods of the Radiation Therapy Oncology Group (RTOG), Philadelphia, PA, which manages the experimental radiation therapy studies of most US clinical trials, be used for these collaborations. USSR data analysts/statisticians need to work with RTOG personnel in the US for some weeks/months with regard to specific protocols to obtain the necessary background and expertise.

3) Improve treatment planning capabilities in the USSR through mutual exploration of new optimization techniques and the acquisition by the USSR of modern computer technology. USSR investigators must acquire state-of-the-art 3-dimensional treatment planning systems with sophisticated graphics capabilities. Moreover, it is essential that USSR clinicians and physicists and/or computer specialists work with such systems in the US to gain experience and develop expertise with today's modern sophisticated treatment planning systems. This will require that the USSR scientists work in the US for some months, possibly up to one year.

4) Identify human cancers that are of common interest to both countries which will benefit from proton therapy research and pursue the development of common treatment and research protocols. Examples of such tumors include: intracranial malignant and benign tumors and arterial-venous malformations (AVM); ocular melanomas; tumors of the pituitary gland; arteriorsinusal fistulas in the region of the cavernous sinus; prostate; lung and esophagus tumors. Close cooperation and understanding are needed of individual institutional studies of particular interest to both countries. USSR clinicians and physicists need to work for some months, perhaps one year, in US institutions that are participating in protocols of common interest. Ideally, US/USSR teams of clinicians and physicists would exchange work places for a limited time to share experience and methods of data analysis and reporting and work towards the development of mutually agreed-upon protocols.

5) Improve cooperation on technology and methods for the development of proton therapy facilities and future projects. With the development of the new hospital-based proton therapy facility in Moscow, the US scientists have the opportunity to benefit from sharing of technical expertise and ideas not previously available to the West. Exchange of scientists for some weeks or months in the early development of the project in the Soviet Union and similar projects in the US is seen as a crucial first step.

6) Because of the world-wide distribution of proton therapy research it is essential that young USSR/US investigators regularly attend meetings of the Proton Therapy Cooperative Oncology Group (PTCOG) and other international meetings to give papers and hear other reports. The PTCOG group meets twice each year, and selects a European location for its meeting place once every two years. At these PTCOG meetings, it is possible for investigators from both countries to exchange ideas on proton therapy facilities and beam deliver systems.

7) Intercomparisons of dosimetry data, both physical and biological, must be made according to mutually agreed upon protocols. A protocol for making intercomparisons of physical dose has been developed and a new Faraday cup dosimeter is now under development in the US for sharing with the Moscow-based group.

8) Radiobiological experiments carried out in the Soviet Union will enhance and enlarge the knowledge of normal tissue response to radiation and provide new data on partial organ irradiation tolerance (e.g., brain, spinal cord, rectum, liver and lung). A preliminary protocol for carrying out radiobiological intercomparisons was developed.

Three Soviet scientists were brought to the US in November to tour the US proton and heavy ion facilities at Boston, MA; Berkeley, CA; and Loma Linda, CA. In addition, 10 USSR papers were given at the spring meeting of the PTCOG group, which took place in Boston, May 1991. The continued dialogue and exchange of ideas between the two countries will yield increased understanding and sharing of expertise and technological advances in charged particle radiation therapy.

US/UNITED KINGDOM

Clatterbridge Hospital, Liverpool, England, participated in the Phase III neutron therapy clinical trials from 1984 to the close of the trials this year and was a major contributor of patients to the head-and-neck study. Preliminary analyses of the Phase III study currently indicates no significant difference in survival between fast neutrons and conventional therapy, although tumors in advanced head-and-neck disease show a dramatic response when treated with neutrons. Final evaluation of the results of the recently completed study must await long-term follow-up.

US/FINLAND

The Radiation Research Program is currently funding research for the development of sophisticated radiotherapy treatment planning tools that will aid the physician and physicist in the complicated task of planning a patient for radiation therapy. As part of that multi-institutional collaborative effort, results of research in knowledge-based systems at the Technical Research Centre of Finland, Tampere, are being incorporated into new tools for radiation therapy. The program CARTES (Computer Aided Radiation Therapy Expert System) was developed at Tampere to support decision-making in radiotherapy through knowledge-based techniques and has been installed at the University of Washington, Seattle. The first prototype of a treatment selection support system has been constructed with a doman in non-small cell lung carcinoma. The CARTES program is now being installed at the University of Washington, Seattle, and evaluated for its clinical utility.

WORKSHOPS

Radiation Resistance - September 1990

This workshop convened a panel of experts to address the simple yet profound question of why there is such a variation in response to radiation at the cellular, sub-cellular, and molecular biological level. It is expected that recent advances in molecular biology can shed considerable light on this area. It is anticipated that an RFA will be issued on this subject in FY 1992.

Radiation Induced Normal Tissue Complications - September 1990.

Recent and ongoing advances in dose calculation and delivery technologies have lead to the design and implementation of complex radiation treatment techniques known as 3-D conformal radiation therapy. Using advanced software to design treatments that focus more radiation on the tumor, lower doses to adjacent normal tissues can be achieved, resulting in fewer complications. The purpose of this Workshop was to explore methods by which better models of normal tissue complication probability could be developed and what experimental data is available. The Workshop resulted in a number of recommendations to pursue research in normal tissue complications, including animal studies; retrospective clinical investigations of patients who have inadvertedly received doses resulting in severe complications; prospective trials in which doses would be gradually escalated; and data collection on a number of patients.

FUTURE DIRECTIONS (RDB)

The Radiotherapy Development Branch will continue to stimulate, develop and administer clinical research and basic science research in radiation biology, chemistry and physics and support the development of advanced computer-based tools that improve the treatment planning and delivery of radiation therapy. The particle radiation therapy program, using both charged and uncharged particles, will continue to be a high priority research area for the near future.

The use of Hyperthermia as a treatment modality continues to interest the medical community. However, further research needs to be performed in the development of deep heating units and of non-invasive thermometry. Hyperthermia as an effective adjunct to radiation and chemotherapy needs to be confirmed by standardized, randomized clinical trials for specific disease processes and anatomic sites.

Photodynamic therapy (PDT) as a treatment modality is less well developed than the traditional discipline of radiation oncology but because of promise in this area further research efforts will be stimulated. The present chemical compounds used for light-stimulated radiation treatments probably are not the optimal drugs for PDT. Newer photosensitizing drugs will require clinical testing. Further development of light producing lasers and light delivery systems will be encouraged.

Further research and development of radiolabeled immunoconjugates and cell specific receptors, a form of systemic radiation therapy (SRT), is necessary to explore the possibility of cellular radiotherapy. The dosimetry of these radionuclide tagged compounds is an important research area of this rapidly developing therapeutic approach and requires further support. Radiolabeled immunoconjugates for therapy and diagnosis will continue to be a high priority research area of the Radiation Research Program.

As research advances unfold in the chemistry and biology of boron containing compounds which preferentially concentrate in tumors, the interest in boron neutron capture therapy (BNCT) should increase. Irradiation of a boron compound with low energy neutrons causes emission of a short range alpha particle, which deposits an intense radiation dose at the cellular level. This is an additional example of possible cellular radiotherapy. The RDB anticipates an increasing role in this research area.

Radiosensitizers have demonstrated an ability to increase the sensitivity of neoplastic tissue to radiation and attempts will continue to improve the efficacy of these agents and to decrease their toxicity. Many of these radiosensitizers also have chemosensitizing activity. New compounds need to be developed. This development is dependent on the capability to screen a large number of compounds for radiosensitizing activity. A more rapid screening system with greater capacity is needed.

The rapidly emerging research area of medical informatics, which encompasses a broad spectrum of information management and technology, is beginning to impact the fields of diagnostic radiology and radiation oncology. Digitally-derived diagnostic images are slowly taking over the role of x-rays and films. With the development of high-performance computer workstations, high-speed transmission of imaging data and the advances of image processing software, the prospect of a filmless diagnostic radiology department is gaining momentum. The volume of data that is involved, however, is so large that it requires sophisticated software management tools that have emerged from the "artificial-intelligence" research community in which symbolic logic provides a means of developing management software able to function at a higher level of complexity. With the greater complexity of medical practice, new tests, questions of reimbursement for unnecessary tests, new drugs, new methods of treatment, etc., the physician of the '90s needs computer-based tools that organize, catalogue and retrieve patient data quickly, efficiently

and reliably and offer the physician intelligent databases that can assist in the development of an optimal therapy.

Research supported by NCI over the last decade has shown that the development of sophisticated computer-based treatment planning tools is essential to the routine use of three-dimensional planning and treatment in the clinic. New computer tools are needed that 1) support the management of medical images through computer networks, 2) develop decision-support aids for the radiotherapist in the therapy selection, tumor definition and treatment planning processes; and 3) interface the computerized medical record with intelligent databases. Radiotherapy is the most computer-intensive discipline in medicine, primarily because of the anatomic information required to define the tumor and treatment volume, and the calculations that are needed to characterize the radiation dose to the tumor and the normal tissues at risk. Development of new computer-based systems that support the physician in all aspects of radiation therapy planning and delivery will result in better care and management for the cancer patient.

Dynamic conformal radiotherapy using conventional photon and/or electron beam accelerators is a new and exciting research area in which complex treatment plans are developed and implemented that conform precisely to the tumor treatment region, resulting in greater sparing of normal tissues. These developments will require advances in three-dimensional treatment planning, robotic vision techniques, expert knowledge systems and digital imaging verification systems. Technology transfer from the artificial intelligence community and medical informatics will greatly assist in this effort.

A number of scientific concepts were approved in the past year by the Board of Scientific Counselors for development and funding. These include:

Medical Informatics Research Training Fellowships. Up to four post-doctoral fellowships which address medical informatics approaches to the problems of diagnosis and treatment of cancer will be funded in conjunction with the National Library of Medicine Medical Informatics Training Program.

Program Announcement: Individual Postdoctoral National Research Service Award Fellowships in Radiological Sciences Related to Cancer. Purpose of the Announcement is to stimulate qualified candidates to apply for postdoctoral fellowships in diagnostic radiology and radiation oncology that address the diagnosis and treatment of cancer.

Proton Monte Carlo Calculations. This two-year study, to be funded as an Interagency Agreement with the National Institute of Standards and Technology (NIST) would carry out therapy-simulated Monte Carlo calculations for proton particles in the energy range of 60-250 MeV. The data will provide a foundation for biological models that explain the approximately 10 to 15 percent difference in biological effectiveness that is observed between proton and photon irradiations.

National Collaborative Radiation Therapy Trials: 3-D Dose Escalation Study for Prostate Cancer. This three-year study, to be funded as a Cooperative Group effort, will support a Phase III trial to compare conventional standard therapy with the advanced methods of 3-D conformal

radiation therapy in the treatment of prostate cancer. The 3-D technology is emerging at a few leading institutions as a new advance in the delivery of radiation therapy by focusing higher radiation doses on the tumor target with no increased risk of complications to the normal tissues.

Gene Regulation of Radiation Resistance: Studies directed toward the identification and characterization of the genetic mechanism(s) responsible for the inherent levels of radioresistance frequently observed in some solid human tumors will be solicited through a Request for Applications (RFA). The grant proposals submitted in response to this RFA should be directed toward understanding whether the regulation of genes and their products have relevance to clinical radiotherapy problems. Identification of the mechanism(s) may eventually lead to the ability to modulate these mechanisms to improve the results of radiation therapy as a cancer treatment.


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