Intramural Activities Volume II

October 1, 1991- September 30, 1992

92annual report

Division Of

# Cancer Treatment

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

NATIONAL CANCER INSTITUTE

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ANNUAL REPORT

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CONTENTS FOR VOLUME II

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1991 - September 30, 1992

## 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 (RO1 and PO1) 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 for 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) 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 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 RO1 and PO1 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 agent, 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 reivew 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

1. Dr. David Parkinson was appointed Chief, Investigational Drug Branch. Dr. Parkinson formerly served as Head, Biologics Evaluation Section, Investigational Drug Branch.

2. Dr. Micheal Hawkins resigned as Chief of the Investigational Drug Branch to accept a position at the Georgetown University Lombardi Cancer Research Center.

3. Dr. Richard Kaplan was recruited as a Senior Investigator in the Medicine Section, Clinical Investigations Branch; he was formerly with the University of Maryland Cancer Center, University of Maryland School of Medicine.

4. Dr. Ellen Fiegal joined the Medicine Section, Clinical Investigations Branch, as a Medical Officer; she was formerly with the University of California, San Diego.

5. Dr. Dorothy Macfarlane resigned as Head, Quality Assurance and Compliance Section, Regulatory Affairs Branch, to join the NIH Office of Scientific Integrity.

6. Ms. Joan Mauer accepted the appointment as Acting Head, Quality Assurance and Compliance Section, Regulatory Affairs Branch.

7. Dr. Dennis Cain was appointed Head, Protocol and Information Office, Office of the Associate Director, CTEP.

8. Ms. Patricia Schettino transferred from the NCI-Navy Medical Oncology Branch to the Drug Management and Authorization Section, Investigational Drug Branch, as a Clinical Research Pharmacist.

9. Dr. Malcolm Smith has been appointed Head of the Pediatric Section, Clinical Investigations Branch.

10. Dr. Timothy Moore will join the Developmental Chemotherapy Section, Investigational Drug Branch as a Medical Officer; he was formerly with Medical Oncology Associates in Pittsburgh.

11. Ms. Julie Baltz was appointed Clinical Research Pharmacist, Drug Management and Authorization Section, Investigational Drug Branch; she was formerly with the NCI-Navy Medical Oncology Branch.

12. Dr. F. Andrew Dorr resigned from the Clinical Investigations Branch to accept a position at the Eli Lilly Pharmaceutical Company.

13. Ms. Janet Morgan transferred from the Pharmaceutical Resources Branch, Developmental Therapeutics Program, to the Drug Management and Authorization Section, Investigational Drug Branch, as Clinical Research Pharmacist.

14. Mr. Carl Huntley joined the Drug Management and Authorization Section, Investigational Drug Branch, as a Clinical Research Pharmacist; he was formerly with the NIH Clinical Center Pharmacy Department.

15. Mr. Ray Greene transferred from the Pharmaceutical Resources Branch, Developmental Therapeutics Program, to the Drug Management and Authorization Section, Investigational Drug Branch, as Clinical Research Pharmacist.

## HIGHLIGHTS IN PROGRAM DEVELOPMENT.

### 1. TREATMENT REFERRAL CENTER

In order to meet the challenge of providing patients with refractory ovarian cancer access to the most promising new agents, especially taxol, the Treatment Referral Center (TRC) was initiated in September 1991. An integrated network of NCI-designated cancer centers is responsible for administering taxol through a selective Group C mechanism. Since September 1991, more than 1500 ovarian cancer patients have been treated. This distribution has been relatively equitable, efficient, and efficacious. So successful has this effort been that a similar program of taxol in breast cancer is being initiated. All this is taking place at a time of intense formal research.

### 2. INTRODUCTION OF NEW AGENTS

In an effort to more rapidly develop effective new agents, CTEP successfully introduced several promising drugs. These include CPT-11, Taxotere™, and 9-amino camptothecin. Moreover, development plans were formalized with a number of pharmaceutical companies including Yakult-Dialchi and Rhone-Poulenc. Additionally, Cooperative Research and Development Agreements (CRADAs) are negotiated for three important agents -- 9-amino camptothecin, suramin, and IUDR/BUDR. It would not be an exaggeration to state that there are more active new agents in clinical trials (e.g., taxol, all-trans-retinoic acid, etc.) than ever before.

3. INNOVATIVE GRANT-SUPPORTED RESEARCH

Several crucial new initiatives were started in the last fiscal year. One of the most important initiatives was an RFA for Gene Therapy Clinical Trials. In order to translate the crucial preclinical insights to clinical medicine, this RFA will support the scientific, administrative, and regulatory infrastructure for this research. However, there were several other major research efforts approved or initiated during this year. These included RFAs for Innovative Clinical Studies in Prostate Cancer, in Upper Gastrointestinal Cancer, and Laboratory Correlates of Clinical Trials. Overall, these and other efforts are devoted to increasing the meaningful linkage of laboratory and clinic.

4. COOPERATIVE GROUP PROGRAM

After nearly a decade of relative loss of funding support, a substantial infusion of grant support was made to the cooperative agreement line in this fiscal year. In order to properly utilize this nearly $17 million increment, the CTEP coordinated input from peer review, the Groups themselves, the DCT Board of Scientific Counselors, national strategy meetings, and CTEP staff. Although these funds do not fully fund the approved Group needs (as defined by peer-approved applications), important new protocols will be activated, data management/quality assurance mechanisms upgraded, and accrual increased. It is estimated that more than 4000 additional patients will be annually recruited because of these new funds.

5. UNCONVENTIONAL THERAPY EVALUATION

Based upon a Government Accounting Office (GAO) report that focused on the status of unconventional cancer interventions, a specific program to evaluate such proposals was initiated. Formal guidelines for submitting and evaluating data were devised and distributed. An equitable, disciplined, credible system of evaluation has been utilized. To date, more than a dozen proposed interventions have been submitted and three approaches subjected to trials. Hydrazine sulfate, phenylacetate (so-called "antineoplastons") and imagery are being studied.

## 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 andministrative 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 FY92, the CTEP managed 156 investigator-initiated research grants with the total cost awarded over $57 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 92 and a breakdown of the number of research grants in the different programs administered by CTEP. In FY92, a total of 136 R01 grants were reviewed by the Division of Research Grants (DRG) Study Sections, a 56% increase in grant submissions; 27 were awarded for a funding rate of 20%. Nine FIRST (R29) applications were reviewed by DRG study sections with 3 proposals receiving funding for a funding rate of 33%. Twenty-five R01 grants responding to the Upper GI Request for Application (RFA) were reviewed by a NCI special review committee; 7 grants were awarded for a funding rate of 28%. Nineteen R01 grants responding to the Prostate RFA were reviewed by a NCI special review committee; 5 grants were awarded for a funding rate of 26%. Twenty-five P01 grants were reviewed by NCI special review committees; 13 of them received funding for a funding rate of 33%. The funding rates of CTEP grants reviewed by DRG study sections were lower than the average funding rate for all NCI grants. 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.

GRANT EXPENDITURES FOR FY92 (ESTIMATED)

CANCER THERAPY EVALUATION PROGRAM
DIVISION OF CANCER TREATMENT

| TYPE OF GRANT | NUMBER | TOTAL COST AWARDED* |
|---|---|---|
| Clinical Oncology (CL) | | |
| Research Projects (R01) | 74 | $ 13,607 |
| Program Projects (P01) | 31 | 37,852 |
| Small Business Innovative Research (SBIR) | 12 | 594 |
| Small Grants (R03) | 7 | 492 |
| Conference Grants (R13) | 5 | 19 |
| FIRST Award | 4 | 403 |
| Merit Awards (R37) | 3 | 706 |
| Outstanding Investigator Award (R35) | 2 | 642 |
| Subtotal | 138 | $ 54,315 |
| Surgical Oncology (SO) | | |
| Research Projects (R01) | 6 | $ 966 |
| Program Projects (P01) | 1 | 572 |
| Merit Awards (R37) | 1 | 150 |
| FIRST Award (R29) | 2 | 183 |
| Subtotal | 10 | $ 1,871 |
| Nutrition (NT) | | |
| Research Projects (R01) | 4 | $ 706 |
| FIRST Award (R29) | 4 | 342 |
| Subtotal | 8 | $ 1,048 |
| Grand Total | 156 | $ 57,234 |

* $ 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 67% 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 92, the Program Directors attended 25 site visits for the review of program project submissions and performed 1 formal consultation of P01 submissions. During the formal consulting session, 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. The importance of this mechanism for funding translational research is demonstrated by NCI's use of the P01 mechanism to initiate studies in gene therapy (see RFA section of report).

Investigator-initiated Research Grants

CTEP program staff have been trying to overcome a general perception by the clinical research community that funding is difficult to obtain for cancer treatment research. Program staff have been carefully monitoring the number of grants submitted and the number of grants funded. Furthermore, program staff, as in previous fiscal years, have undertaken specific initiatives to encourage clinical treatment research activities. These initiatives include the issuance of Program Announcements and Requests for Application; and sponsoring workshops.

Program Announcements (PA)

A Program Announcement is a type of grant solicitation used by the National Cancer Institute to encourage investigator-initiated research projects in areas of special importance to the National Cancer Institute.

Cancer Therapy Research

This PA (PA-91-42) encourages investigators to submit grant applications to conduct clinical therapeutic studies of neoplastic disease in human subjects. This initiative encompasses a full range of therapeutic studies and clinical trials employing drugs, biologics, radiation, or surgery. The intent of the announcement was to encourage clinical researchers to translate insights in cancer biology and the development of new agents into innovative cancer therapeutic studies. The PA was re-issued in FY92 (PA-92-69). A total of 72 grants were submitted in response to the two issuances of this PA. Nine grants have been funded. This PA helped to increase the number of clinical research grants reviewed in the Division of Research Grants study sections, especially the Experimental Therapeutics 2 study section where the number reviewed doubled.

Surgical Oncology

This PA (PA-91-16) encouraged surgeons to participate in surgical oncology research and to submit research applications that promote and translate new basic and preclinical research into therapeutic advances. This PA was also re-issued in FY92 (PA-92-81). A total of 35 grant applications were submitted in response to PA-91-16. Five grants have been funded. This PA helped to increase the number of clinical research grants reviewed in the Division of Research Grants study sections.

Lung Cancer Research

This PA (PA-91-83) encouraged grant applications to conduct therapeutic studies of lung cancer in human subjects. The intent of the announcement was to encourage clinical researchers to translate insights in lung cancer biology and the development of new agents into innovative cancer therapeutic studies. The response to this program announcement had been poor. Only 5 grants were submitted. NCI has made one award; 2 grants are scheduled to be reviewed in October, 1992.

AIDS-Associated Kaposi's Sarcoma

Adult 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. Kaposi's sarcoma is one of the

malignancies most frequently seen in AIDS patients. NCI and NIAID jointly issued a PA (PA-92-76) soliciting applications that would bring laboratory research efforts to the clinic to attack the problem of management of this disease. The earliest submission date was May, 1992. The number of submissions is unknown at present.

Small Grants for Lung, Breast, and Ovarian Cancer Clinical Trials

This Program Announcement solicits the submission of R03 small grants limited to $50,000 Direct Costs per year for a total of two years funding. The aim of the initiative is to stimulate pilot, phase I, or phase II therapeutic trials to move new treatment strategies more rapidly from the laboratory into the clinic in the areas of breast, lung, and ovarian cancer. Some examples of categorical areas for R03 studies include biochemical modulation studies, immunotherapy, biological response modifiers in combination with chemotherapy, studies of drug resistance, and therapies with novel mechanisms of action. Thirty-three grants were received and are undergoing review for funding in FY92.

Exploratory/Developmental Grants in Cancer Therapy

A new grant initiative was published in April, 1992 announcing the availability of the R21 exploratory/developmental grant (R21) mechanism to encourage the development of new research activities aimed at improving the treatment of cancer. Two research areas were solicited in this Program Announcement: 1) clinical trials for the treatment of breast, lung, prostate, ovarian, or cervical cancer utilizing drugs, biologics, radiation, or surgery; 2)clinical trials directed at investigating specific strategies for overcoming or reversing clinical resistance to cytotoxic and biological anti-cancer agents. These grants have three receipt dates for FY93 and five grants were submitted for the June receipt date.

Request for Applications

A Request for Applications is a formal announcement soliciting for research grant applications in a well defined scientific area for a one-time competition. Unlike the Program Announcements, a specific dollar amount is set aside to fund the successful proposals.

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. This RFA was supported with a set-aside of $1,500,000 in the FY 92 budget. Twenty-five applications were received with twenty-two applications found to be responsive to the RFA. Seven applications were funded with a variety of clinical approaches to these important disease areas. The NCI awardees are listed below:

Dr. Chung K. Chou
City of Hope National Medical Center
1 R01 CA 56116-01
Intracavitary Hyperthermia & Radiation of Esophageal Cancer

This application addresses the development of hyperthermia to be used in conjunction with intracavitary radiation for treatment of esophageal carcinoma. Intracavitary radiation has the advantage of localizing radiation but also causes ulceration due to high radiation dosage. Hyperthermia can potentiate the radiation effects and therefore reduce the required dose.

Dr. Charles A. Coltman
Southwest Oncology Group
1 R01 CA 56138-01
Pharmacodynamic Correlates in Advanced Gastric Cancer

The purpose of study is to describe correlations between pharmacodynamic parameters and clinical antitumor responses in patients receiving FAMTX chemotherapy for gastric cancer. Clinical response of patients will be correlated with: 1) magnetic resonance spectroscopy (MRS) of 5-Fluorouracil (5-FU) trapping and metabolism in tumors; 2) biochemical pharmacology parameters; and 3) measures of tumor proliferation and proliferating cell nuclear antigen (PCNA).

Dr. Howard S. Hochster
NYU Medical Center
1 R01 CA 56129-01
Topoisomerase-1 Inhibitors Novel Therapy of Upper GI Carcinoma

In this application, the investigators plan to develop phase II protocols with inhibitors of topoisomerase I (topo I), camptothecin and its analogs, for the treatment of patients with upper-GI cancers. The rationale for the proposed studies is supported by pre-clinical preliminary data demonstrating the role of inhibitors of topo I as a novel therapeutic strategy. They will investigate the pharmacokinetics of the camptothecin analog in patients. Finally, the investigators plan to use statistical techniques to correlate clinical anti-tumor response with topo 1 and cleavable complex levels to prove the hypothesis that increased levels of topo 1 and cleavable complex underlie the activity of the camptothecin analogs.

Dr. David P. Kelsen
Memorial Hospital for Cancer
1 RO1 CA 56125-01
Multimodality Treatment & Clinical Biological Correlates of Upper

The investigators propose to treat gastric adenocarcinoma with preoperative adjuvant chemotherapy (FAMTX) and postoperative intraperitoneal chemotherapy (cisplatin and fluorouracil). In addition, they will perform molecular genetic studies. The investigators are among the leaders in the field of clinical treatment of gastric cancer and have established the current operative standard in this disease. The use of combined endoscopic ultrasonography and flow cytometry to determine the "high risk" patient represents a new approach at preoperative staging in gastric cancer.

Dr. Benjamin Krevsky
Temple University Hospital
1 RO1 CA 56121-01
Endoscopic Laser Therapy for Esophageal Cancer

Dr. Krevsky proposes to conduct a prospective evaluation of the efficacy of neoadjuvant endoscopic laser therapy in combination with chemotherapy and radiation therapy in treating esophageal cancer. In addition, he will evaluate the potential role of endoscopic ultrasound in staging esophageal cancer. This appears to be the first effort to evaluate this issue.

Dr. Thomas E. Lad
University of Illinois Medical Center
1 RO1 CA 56117-01
Correlates of Drug Resistance in Gastric Carcinoma

Dr. Lad and his collaborators propose to correlate the clinical response of gastric and esophageal adenocarcinoma to combination chemotherapy with the presence or absence of various drug resistance markers in fresh tissue samples taken before and after in vivo chemotherapy exposure. A multi-center phase II trial of a combination of cisplatin, doxorubicin, and etoposide in gastric and esophageal adenocarcinoma will generate clinical data regarding response to chemotherapy.

Dr. Charles J. Yeo
Johns Hopkins University
1 R01 CA 56130-01
Correlates and Treatment of Pancreatic Carcinoma

Dr. Yeo proposes a comprehensive study of patients with resectable and unresectable pancreatic cancer. Patients with resectable pancreatic cancer will be enrolled in a phase II chemotherapy study with 5-Fluorouracil and leucovorin. Patients with unresectable pancreatic cancer will be enrolled in a phase II study using systemic chemotherapy with the new antitumor drug 773U82, which has just completed phase I testing. Survival data, patterns of failure and cause of death, and toxicities of these regimens will be investigated and compared with those of the control group.

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 envisions funding therapeutic clinical studies that test and exploit basic findings concerning cellular targets of treatment or response to drug or hormone therapies. The RFA was issued in May, 1991, with a set aside of $750,000 in the FY 92 budget. Nineteen applications were reviewed and five applications were funded. The NCI awardees are listed below:

Dr. Anton Wellstein
V.T. Lombardi Cancer Center
1 R01 CA 57406-01
Inhibition of Heparin-Binding Growth Factors

Dr. Wellstein proposes to study the efficacy of pentosan polysulfate (PPS), a structural analogue of heparin which blocks heparin-binding growth factors, in a Phase II trial with prostate cancer patients. He will probe for six known heparin binding growth factor genes and determine to what extent endothelial cell proliferation in normal, hypertrophic and cancerous prostate tissue can serve as an indicator for the disease state, prognosis of the patient and responsiveness to therapy.

Dr. William Fair
Sloan-Kettering Institute for Cancer Research
1 R01 CA 57458-01
Combined Modality Approach for Prostate Cancer

Dr. Fair, Chief of Urologic Surgery, Memorial Sloan-Kettering, proposes to assess a therapeutic strategy designed to improve current treatment results for patients having clinically localized prostate cancer. The clinical strategy consists of a combined modality employing primary hormonal manipulation followed by resection of clinically localized prostate cancer. Laboratory analyses include: pathologic assessment of pre- and post-surgical specimens, immunocytochemical characterization of prostate cell function employing a panel of markers, and analysis of serum markers of prostate cell function.

Dr. Kenneth J. Pienta
Wayne State Univ. Sch. of Med.
1 R01 CA 57453-01
The Nuclear Matrix as a Cancer Chemotherapy Target

Dr. Pienta proposes to study the role of the nuclear matrix in the transformation of prostate epithelial cells and to investigate its interaction with cytotoxic agents that cause DNA damage. Simultaneously, a Phase II clinical trial is planned, utilizing oral estramustine and oral VP-16 in combination based on the investigator's preliminary in vitro studies.

Dr. George Wilding
UW Comprehensive Cancer Center
1 R01 CA 57456-01
Suramin: A Study of Energy Balance in Prostate Cancer

Dr. Wilding hypothesizes that suramin's ability to disrupt cellular respiration and energy balance can be exploited by combining suramin with other chemotherapies for hormonally refractory prostate cancer. In vitro studies utilizing human prostate cells are proposed to examine suramin's effect on cellular metabolism and energy balance.

Dr. Gary R. Hudes
Fox Chase Cancer Center
1 R01 CA 57638-01
Antimicrotubule Therapy in Prostate Cancer

Dr. Hudes proposes clinical trials combining estramustine phosphate (EMP) and vinblastine, two antimitotic agents that have different mechanisms of action. The laboratory studies will explore the possibility of using tumor expression of microtubule associated proteins (MAP), the binding to MAP of a photoaffinity analog of EMP, and the expression of tumor estramustine binding protein to predict clinical outcome of therapy.

Clinical Correlative Studies in Solid Tumors

This RFA for cooperative agreements is designed to promote collaborations and interactions between basic researchers and clinical investigators to advance research on clinical correlations that can improve therapeutic approaches in solid tumors. While advances have been made relating biological studies to clinical behavior of some hematologic malignancies, fewer clinical correlations have been explored for solid tumors. Prognostic factors can play a major role in assisting clinicians in the selection of appropriate therapeutic interventions. New technological advances in methodologies such as Polymerase Chain Reaction (PCR), flow cytometry, immunohistochemistry, and in situ hybridization allow laboratory investigators to do numerous analyses on tumor specimens and study tumor heterogeneity in a variety of tumor types. Special consideration will be given to studies with colorectal, breast, ovarian, lung, and prostate tumors since they account for significant cancer incidence, morbidity and mortality. The RFA was issued jointly with the Cancer Diagnosis Program, DCBDC, and a total of $2,000,000 has been set aside for funding these activities in FY 93. Over thirty letters of intent have been received in response to this RFA.

Hypothesis-Driven Clinical Correlations in Hematologic Malignancies

CTEP supports a program of integrated national networks of clinical investigators and institutions (Clinical Trials Cooperative Groups) for the conduct of large-scale, multi-institutional clinical trials. The Cooperative Groups have access to tumor specimens from a large number of patients with hematologic malignancies. This RFA encourages correlative laboratory studies linked to these large-scale clinical trials. 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. The deadline for submission is September 16, 1992. Over 25 letters of intent have been received. Two million dollars per year for 4 years have been set aside for this RFA.

Implementation Grants for Gene Therapy Programs in Cancer Treatment

The purpose of this RFA is to promote the design and implementation of clinical trials of gene therapy, to support the requisite preclinical studies establishing the scientific and technical basis for human studies, and to foster the development of interactions between basic scientists and clinical researchers necessary for bringing gene therapy to patient trials. For patients with cancer, genetically altering the characteristics of tumor cells or host normal cells ex vivo may enhance the effectiveness of certain forms of immunotherapy and chemotherapy; ultimately, genes may be directly introduced into tumor cells in vivo to alter the malignant phenotype and halt tumor progression. The program project grant mechanism supports the establishment of broadly-based, multi-disciplinary and multi-institutional research programs centered around this goal. This major new initiative for NCI was made

possible with the FY92 budget increase. The Board of Scientific Counselors approved $5,000,000 for funding these activities in FY92. Twenty applications were received and are undergoing review.

Interactive RO1's for Clinical Studies of Innovative Systemic Therapies

The concept for this RFA was approved at the June, 1992 meeting of the DCT Board of Scientific Counselors. Four million dollars were set aside to pay for these grants in FY93. This RFA will be re-advertised in FY93 with an additional 2 million dollar set-aside from FY94 funds. The aims of this RFA are two-fold: (1) to provide support for translational research that brings innovative basic research findings into the clinic and (2) to foster the development of interactions between basic science laboratories of different disciplines and clinicians performing clinical trials to advance therapeutic clinical research. This RFA solicits applications to perform interactive research projects with the goal of developing new clinical studies involving systemic therapies with a therapeutic intent. The focus of these interactive research project grants will center either on: (1) clinical studies investigating promising therapeutic approaches in a single tumor type or (2) the development of new clinical treatment strategies focused on a single class of novel compounds or mechanism of action.

As a result of initiatives begun in FY91 and undertaken in FY92 the number of RO1 and R29 grants funded in FY92 has increased substantially from the previous fiscal year.

Minority Research Grant Supplements

NCI, through its Comprehensive Minority Biomedical Program, provides support to minority scientists and students in order to influence a greater number of minority individuals to develop their research capabilities and pursue independent careers as cancer research investigators. Three minority research grant supplements were awarded. These awards were given to a black, female neurosurgeon at UCSF for performing clinical research on brain tumors, to a female Hispanic medical student to do a clinical project on autoimmune disease in bone marrow recipients at the Fred Hutchinson Cancer Center, and to a black female clinical fellow at the Dana Farber Cancer Center interested in epidemiology and basic research with emphasis on improving the survival of woman with potentially curable cancers.

Small Business Innovative Research Grants

SBIR grants continue to be an important component of the CTEP program. Currently we have 12 phase I grants. These grants can be divided into several generalized categories: (1) computer software for clinical data acquisition, (2) specific chemical/mechanical products, and (3) specific assays for prognostic markers in human tumors. The NCI awardees are the following:

Dr. Robert Tinkelman, Cambridge Computer Assoc, Inc.
R43 CA56252
Computerized Clinical Trial Data Acquisition & Analysis

The applications proposes to develop software for integrating the general administration of the clinical trial process and the gathering and processing of clinical data into one system for tracking and reporting data consistently.

Dr. Glenn Pearson, Civilized Software, Inc.
R43 CA57115
X-Windows MLAB Server for Clinical/Scientific Research

The goal of this project is to develop a separate server module of MLAB, Civilized Software's mathematical modeling system, designed for biomedical and scientific applications.

Dr. Whe Yong Lo, Copley Pharmaceutical, Inc.
R43 CA56261
Buccal Delivery of Prochlorperazine

This proposal is for the development and in vitro testing of an adhesive oral patch for the transmucosal delivery of the anti-emetic drug prochlorperazine.

Dr. Clyde Goodheart, Fibrogenex, Inc.
R43 CA56213
Can Leptomeningeal Fibronectin Modulate Glioma In Vivo

The objectives of the proposed research are to determine the efficacy of solubilized components of the leptomeningeal extracellular matrix in treating malignant brain tumors.

Dr. Myung Chun, MAULWURF, Inc.
R43 CA56206
Method for an Improved Delivery of Anticancer Agents

Dr. Chun will test the feasibility of using anti-tumor antibodies conjugated with inflammatory mediators to initiate a local inflammation and thus increase the accessibility of solid tumors to other therapeutic agents.

John G. Baust
R43 CA58052
Cryoprobes for Ultrasonically Guided Cancer Treatment

Dr. Baust is developing ultrasound probes and other equipment for use in cryosurgery on cancers of the prostate, liver, brain and pancreas.

Daniel T. Casto
R43CA57071
Intraosseous Administration of Chemotherapy: Osteoport

Dr. Casto is testing an implanted septum and reservoir device, the Osteoport TM, for the delivery of anticancer drugs to systemic circulation via intraosseous injection.

Gary L. Griffiths
R43CA54636
The Production of 188Re-labeled Monoclonal Antibodies

Dr. Griffiths is producing rhenium labeled antibodies for diagnostic and therapeutic uses.

Debra J. Trantolo R43CA57063
Slow Release LHRH Analog to Reduce Serum Testosterone

Dr. Trantolo is developing an injectable ninety-day sustained-release polylactic acid polyglycolic acid copolymer matrix formulation of luteinizing hormone-releasing hormone to suppress serum testosterone levels without resorting to castration for the treatment of prostate and breast cancers, precocious puberty in children and endometriosis.

Dr. Sam Barranco, Prism Diagnostics/Development Corporation
R43 CA57062
Rapid Detection of resistance in Human Cancer Biopsies

This proposal is to develop an in vitro screening method to determine the sensitivity of individual patients' tumors to generally used chemotherapeutic agents.

Dr. Ron Zeheb, Oncogene Science, Inc
R43 CA57083
Mutant p53 Proteins in Cancer Patient and Normal Sera

The objective of this study is to measure levels of mutant p53, a regulator of cell growth, in sera from normal human subjects and cancer patients.

## Highlights of Investigator-initiated Grants

Several significant discoveries/leads with potentially important clinical applications/implications were made in FY 92 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) recipient of the 1990 Nobel Prize, was awarded the Kober Medal by the American Association of Physicians. Dr. Thomas continues as a project leader after relinquishing the position of Principal Investigator of the P01 grant. The overall goal of his project is to determine the usefulness of the PCR technique in predicting relapse in CML and ALL. Data developed by Dr. Stanley Riddle and Dr. Philip Greenberg in Project IIId of the P01 while Dr. Thomas was the PI has tremendous implications of the utility of adoptive immunotherapy as a technique in the treatment of cancer and AIDS. Their data on the expansion and infusion of CD8+ CMV-specific Cytotoxic T cells was published in the July 10, 1992 issue of Science (257: 238-41) and received major attention in the press (Washington Post, July 13, 1992).

**Dr. Lawrence Einhorn**, Indiana University School of Medicine (5R35CA39844-08) received the Charles F. Kettering Prize from General Motors for pioneering successful uses of cisplatin in cancer therapy. Currently, he is developing two new regimens, one for bladder cancer and another for germ cell tumors.

**Dr. Jessie L. S. Au**, Ohio State University Research Foundation (2 R01 CA 49816-04) was nominated for and was granted a MERIT award. Dr. Au successfully integrated basic and clinical studies to address problems in the treatment of bladder cancer. This grant is a prime example of successful translational research. Data from the grant have impacted the clinical management of bladder cancer. The investigators have identified several causes of the variable and incomplete response to one commonly used agent, mitomycin C and made several additional findings of seminal importance. They have shown (a) that large inter- and intra-patient variabilities in the pharmacokinetics at the target site and in the tumor sensitivity to mitomycin C are the likely causes of the variable and incomplete responses; (b) that the lower sensitivity of invasive tumors to mitomycin C and the insufficient drug concentration at the deep tissues are the likely causes of poor response by the deep invasive tumors; and (c) that tumors with a high labeling index are less sensitive to the drug. Their data further identified several treatment parameters for optimization. These include (a) complete bladder emptying prior to drug instillation, (b) urine alkalinization to reduce drug degradation, (c) fluid restriction to reduce urine production, and (d) increase exposure time. The finding of residual urine at the time of drug instillation, which reduces the drug concentration at the target site by several fold, has prompted clinicians to use ultrasound to ascertain complete bladder emptying. A multi-center phase III clinical trial, comparing the empirical standard mitomycin C protocol with the optimized protocol based on the grant-generated pharmacologic data has been initiated. Their important finding providing a pharmacologic basis for the lower response of deep invasive tumors has suggested several approaches to enhance drug efficacy for intravesical therapy and to identify new agents for the more aggressive disease.

**Dr. Edmund Y. S. Chao**, Mayo Clinic, (4 R37 CA 23751-15) was granted an extension of his MERIT award to continue his studies on general reconstructive orthopedic problems in limb sparing surgery after musculoskeletal tumor resection. Preliminary studies were performed to determine the effect of chemotherapy on extracortical bone bridging/growth. Cisplatin administration has a strong negative effect on extracortical bone bridging and graft incorporation. This finding has important implications in the timing and use of chemotherapy in the adjuvant setting. Sixty one patients with custom made segmental total knee replacements as reconstructive procedures after resection of primary bone tumors of the distal femur were analyzed. The patients had either a Walldius hinge prosthesis or a Kinematic rotating hinge prosthesis. The Kinematic rotating hinge prosthesis was demonstrated to be superior. Twenty one patients with porous segmental protheses containing either Ti alloy or Co-Cr-Mo were followed for 43-74 months. Their urine and serum metal ion levels were determined and were found to be within the acceptable normal ranges.

**Dr. Sydney Salmon**, University of Arizona (PO1 CA17094) and co-workers have made a major breakthrough in drug development. They developed an entirely new technology that permits rapid chemical synthesis of vast libraries of random peptides with each unique peptide on a single solid phase bead. They have also developed a method for rapidly screening the library of beads against various acceptor molecules (monoclonal antibodies, receptors, enzymes) for their abilities to bind to one or a few out of a library of millions of different pentapeptides. They forsee initial applications in B-cell lymphoma and breast cancer. These synthetic peptides can be radioactively labeled as they are being synthesized and used directly as therapeutic agents. This technology is a far more viable strategy than generation of anti-idiotypic antibodies. This discovery lead to a NCI sponsored workshop on "Designer Ligands" in March, 1992.

**Dr. John Kersey**, University of Minnesota of Minneapolis-St. Paul (PO1 CA21737) reported major progress on the development of an immunotoxin in Project 4 of the PO1 grant. Dr. Fatih Uckun, the project leader of Project 4 has received an IND from the FDA for the use of a novel immunotoxin B43 pokeweed antiviral protein (PAP) targeting the CD19 B-cell antigen in ALL as a potential therapeutic for ALL.

**Dr. Leonard Augenlicht**, Montefiore Medical Center, Bronx, NY (RO3 CA53446) has developed a very sensitive dot blot protocol to determine levels of c-myc amplification in human colorectal tumors. Furthermore, this assay can be performed retrospectively using DNA isolated from paraffin embedded tumor and normal mucosa. The sensitivity of this assay is at least an order of magnitude lower than the 5+ copies necessary to detect c-myc amplification by conventional Southern analysis. He has also developed PCR techniques to analyze p53 mutations and deletions of DCC genes in clinical samples. Using

preliminary data generated from this R03 grant Dr. Augenlicht has successfully competed for R01 funding (R01 CA57694) to determine the predictive value of 3 genetic alterations associated with progression of colon cancer in predicting the clinical outcome after surgery of resectable Dukes B and C colon cancers. The samples will come from at least 2 major ECOG protocols.

**Dr. William Crist**, St. Jude Children's Research Hospital (P01 CA23099) The long-term goal of this program project is to advance cure rates for children with malignant solid tumors by integrating studies of tumor cell biology, pharmacology, and pharmacokinetics with testing in xenograft models and clinical trials. Dr. A. Thomas Look is examining a cohort of patients with tumor types associated with the Li-Fraumeni syndrome for the presence of germline and tumor-associated p53 gene mutations. Dr. Look has identified p53 germline mutations in six patients. In addition, preliminary data demonstrates amplification of cyclin D3 in osteosarcoma and N-myc in neuroblastoma. These leads will be pursued.

Dr. David Shapiro has been studying the specific translocation (2;13)(q35;q14) that is found in nearly half the cases of pediatric alveolar rhabdomyosarcoma. Dr. Shapiro is now involved in identifying and cloning the t(2;13) breakpoint regions on chromosomes 2 and 13. These studies may provide information on their contribution to transformation and tumor progression. Dr. Peter Houghton has shown that nearly all rhabdomyosarcomas express the myogenic determination gene MyoD. He is now examining whether the level of MyoD1 expression and the associated increased differentiation potential alters chemosensitivity of rhabdomyoblasts.

**Dr. William N. Hait**, Yale University School of Medicine, (P01 CA 08341) is conducting pharmacological studies of drugs for reversal of multi-drug resistance. BIBW22 is a dipyridamole derivative, that not only sensitizes cells to drugs transported by P-glycoprotein (Pgp), but also sensitizes cells to 5-fluorouracil by virtue of its effects on nucleoside transport. Terfenadine (Seldane) also has potential as a reversing agent and has a low toxicity profile. Dr. Hait has completed a series of studies on the effect of phosphorylation on the function of Pgp. These studies demonstrate that Pgp is a substrate for PKC, that activation of PKC increases the phosphorylation and activity of Pgp, and that drugs which interfere with this process interfere with the function of the protein.

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 has established that pretreatment with Brequinar, a potent inhibitor of dihydroorotate, prior to administration of fluorouracil significantly increases incorporation of the fluoropyrimidine into colon tumor RNA, while minimal effects were seen in normal tissues of C57/BL6 mice. These laboratory findings provide the basis for a phase I-II clinical study that is in progress. In addition, a phase I clinical trial is planned with benzylacyclouridine (BAU), a new agent that has shown promise in preclinical animal models as a modulator of fluorouracil.

**Dr. Roberto Ceriani**, John Muir Medical Center (R01 CA39936) is studying the use of breast epithelial mucins as prognostic markers in infiltrating ductal carcinoma (IDC) of the breast. The maturation of the breast epithelial mucin has shown a strong correlation with prognosis for survival and relapse. Monoclonal antibodies against epitopes of the breast epithelial mucin were developed and used in immunohistochemistry studies of the patient's primary breast lesion. Four parameters (CP, cytoplasmic prevalence; CI, cytoplasmic intensity; MP; membrane prevalence; and MI, membrane intensity) quantified stages in the pathway of maturation of the mucin that were then correlated with survival and relapse time. Three significant achievements resulted from this work. First, these four parameters provided new non-redundant information on survival and relapse of IDC, when compared to traditional variables such as tumor size, axillary node, and grade of differentiation. Second, four distinct risk groups could be identified compared to traditional prognostic markers which separate IDC patients into two to three groups. Third, and most significant, three risk groups could be identified in node-free patients using the composite score. Thus, this research has resulted in the development of markers that have a strong correlation with prognosis for IDC and may identify node-negative patients more likely to relapse.
This grant was chosen for co-funding by the Office of Research on Women's Health.

**Dr. Emil Frei**, Dana Farber Cancer Institute (P01 CA38493 and P01 CA19589) is the principal investigator on two program project grants with the Cancer Therapy Evaluations Program. He recently received the Distinguished Service Award for scientific achievement from the American Society of Clinical Oncology. The overall objective of the "Solid Tumor Autologous Marrow Program" (P01 CA38493) is the integration of 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. Frei is also the principal investigator on the program project "Clinical/experimental Pharmacology of Respiratory Cancer" (P01 CA19589). This grant involves pharmacologic and clinical studies of lung cancer and head and neck cancer. In project 2, Dr. Beverly Teicher is studying the influence of scheduling, dose and volume of administration of a perfluorochemical emulsion (PFCE) on tumor response to radiation therapy. PFCE plus carbogen breathing effectively enhances the antitumor effects of both single dose and fractionated radiation in the Lewis lung carcinoma model. A clinical protocol based on these results will begin soon using a new pO2 probe to monitor tumor hypoxia.

## Meetings and Workshops

### AIDS Lymphoma Meeting

NCI and NIAID jointly sponsored a meeting on AIDS-associated lymphoma during the December 1991 AIDS Clinical Trial Group meeting in Wahington D.C. The Grants Program Director served on the organizing committee. The successful applicants of the AIDS Lymphoma Network RFA were major participants of this meeting. The meeting was held to provide the grantees with an assessment of the state of research activities in AIDS associated lymphoma and served as the first annual meeting of the AIDS Lymphoma Network. Topics for presentation included the biology of AIDS-associated lymphoma, surveillance of lymphoma in HIV-infected populations, and therapy of AIDS-associated lymphoma.

### Gene Therapy Programs Meeting

In February, 1992, the Cancer Therapy Evaluations Program held a briefing on the new RFA initiative, Implementation Grants for Gene Therapy Programs. Investigators who were planning on submitting grant applications for the RFA were invited to attend as well as investigators who were interested in the regulatory approval process for gene therapy clinical trials. An overview of the Gene Therapy Implementation Grants Program and regulatory issues was given and staff addressed any questions concerning the program. In addition, speakers gave presentations on safety issues for retroviral vectors, biosafety issues, the Recombinant Advisory Committee (RAC) approval process, and the FDA approval process. The meeting generated considerable interest and was well attended with 75 registrants.

### Generating Designer Ligands for Biological Targets Meeting

The Grants Program Director served as the Chairman of the organizing committee for a Designer Ligands Meeting held in March, 1992. The organizing committee was composed of representatives from the different programs within DCT and arranged the topics and speakers. Dr. Stuart Kauffman and Dr. H. Mario Geysen provided overviews of generating designer ligands for biological targets. Dr. Mark Greene spoke about the mimetics technology. Dr. Jamie Scott, Dr. Carlos Barbas III and Dr. Stanley Brown described their experience of generating epitope libraries using recombinant phage technology. Dr. Kit Lam, Dr. Richard Houghten and Dr. Ronald Zuckerman discussed their efforts using random synthetic peptide technology to make peptide libraries. Dr. John Toole and Dr. Lawrence Loeb discussed the use of oligonucleotide technology. The DCT Director gave the welcome address and the Associate Director, Biological Response Modifiers Program served as the Chairman of the meeting and lead the discussion. Over 125 scientists attended the meeting.

AACR/ASCO Workshop

During the joint annual meeting of the American Association for Cancer Research (AACR) and the American Society of Clinical Oncology (ASCO) in San Diego, CA, a workshop was sponsored by NCI to promote various clinical research initiatives; and 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. The meeting was well attended with over 200 investigators present. Thus CTEP in FY92 has continued to encourage clinical researchers to submit investigator-initiated research grants so this important area of research can continue to make cancer treatment advances.

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 FY91 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 FY92 conferences on ovarian and brain cancers were held.

P01 Committee

The Grants Program Director served as one of the representatives for the Division on a P01 Committee of NCI. The Committee members are analyzing NCI's current usage of the P01 mechanism and are collecting data on the use of the P01 mechanism for funding translational research.

Transplantation Research Coordinating Committee

The Grants Program Director has been serving as the NCI representative to the Transplantation Research Coordinating Committee chaired by Dr. Robert A. Goldstein, Director, Division of Allergy, Immunology and Transplantation, NIAID. This committee serves as a network for information exchange among the different institutes of NIH on transplantation research.

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## BIOMETRIC RESEARCH BRANCH

### 1. STATISTICAL PLANNING AND MONITORING OF CTEP 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, edatrexate, fenretinide, 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 and plays a primary role in the design of national intergroup trials. BRB staff perform interim analyses of contract supported clinical trials and evaluate reports of promising or unconventional therapeutic regimes for the planning of possible future clinical trials. During the past year, BRB has evaluated data on autolymphocyte therapy for metastatic renal cell cancer and for cyproheptadine for cachexia.

The BRB serves as liaison to extramural statistical centers. BRB staff visit centers, review data management and monitoring procedures and interact with peer reviewers to ensure that statistical centers are carefully and fairly evaluated. BRB organizes national meetings in order to improve statistical and data management practices and holds regular meetings of the cooperative group statisticians. The BRB evaluates whether cooperative groups studies can be utilized for specialized studies such as the effects of silicone breast implants. The BRB evaluates statistical center proposals for supplementary funding.

### 2. PRECLINICAL DRUG DISCOVERY AND DEVELOPMENT

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. Two explanatory manuscripts have been published.

The statistical measures of differential cytotoxicity which accompany the current supplier reports have been revised to be more closely based on a generalization of the nonparametric Wilcoxon statistic. Since the scales of the growth inhibition data are arbitrary, it is felt that the analysis of differential cytotoxicity should include scale-invariant nonparametric measures. We have also developed parametric alternatives to these measures, based closely on a generalization of the t-test. It is hoped that these parametric measures, by preserving sensitivity to scale differences in the growth inhibition data, will be a useful complement to the scale-invariant nonparametric measures. Both of these sets of measures have been programmed and are currently being tested by extensive simulations.

b. 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 published. Work is ongoing to test, apply and extend these methods.

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 Drs. L. Wu, R. Shoemaker and others of DTP, an analysis of the human tumor cell lines was conducted to attempt to correlate the degree of expression of the multidrug 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). A manuscript is in press. Further analyses of the cell lines are planned, to correlate other genetic characteristics with behavior in the in vitro assay.

f. Statistical analyses are being conducted of the factors which effect in vitro cell line response to a drug, in particular, cell line histology and drug mechanism of action. This involves exploration of the degree to which cell lines sharing the same histology behave similarly, as well as a statistical search for redundant cell lines, which might be profitably eliminated from the in vitro screen. It also involves exploration of the degree to which drug mechanism of action may be predicted by the pattern of in vitro responses of the various cell lines, either by standard statistical techniques or by neural

networks (working with Drs. J. Weinstein, K. Paull, and others of DTP). A manuscript concerning prediction of mechanism of action by neural networks has been accepted.

These analyses focus, in particular, on in vitro cell line response to the standard clinical agents, and they are related to further analyses (with Drs. A. Monks and others of DTP) to characterize the cell line responses to 40 of the most commonly used agents, and compare the in vitro profiles with the clinical profiles.

g. Statistical analyses have been conducted correlating in vitro patterns of activity as measured by the cell line screen with in vivo patterns of activity in corresponding cell line xenografts, among drugs chosen for in vivo testing (with Drs. J. Plowman and others of DTP) and have been presented at a DTP conference concerning in vivo testing.

Additional analyses are ongoing (with Dr. M. Alley of DTP and Dr. H. Feibig of Germany) to correlate these in vitro and in vivo patterns of activity with the patterns of activity resulting from a colony-forming assay based on corresponding cell lines.

h. Statistical analyses of the results of the initial two years of compound screening, in particular, analyses of the reproducibility of the data and of the characteristics of the compounds referred for further testing are being conducted (with Drs. K. Paull, J. Weinstein and others of DTP). A computerized algorithm has been developed which appears to successfully discriminate, from in vitro patterns of activity, which compounds are worthy of further testing. The results of the algorithm appear to agree closely with the judgements of expert human reviewers, who have additional information concerning the compounds (including molecular structure and, in some cases, pharmacokinetic data) available to them.

i. Work is ongoing (with Drs. E. Erickson, K. Paull, J. Weinstein and others of DTP) to integrate the results of the Stanford in vitro prostate cell line screen into the over-all DTP in-vitro screening process.

j. Work is ongoing (with Drs. J. Plowman, J. Weinstein and others of DTP) to develop methods for the design and analysis of in vivo drug combination experiments.

### 3. STUDY OF IMAGING-GUIDED STEREOTACTIC TISSUE DIAGNOSIS FOR BREAST CANCER

There is currently a lack of information concerning the appropriate use of stereotactic fine needle aspiration and stereotactic core needle biopsy for the screening and management of patients. It is thought that in some patient subgroups that these techniques might replace open surgical biopsy for which cancer is not found 80% of the time. Two potential advantages would be (1) minimization of tissue damage and (2) cost effectiveness.

BRB staff has been collaborating with Dr. S. Brown of the Radiation Research Program in considering appropriate trial designs, sample sizes and patient populations to answer some major clinical questions: (1) What specific stereotactic technique is most appropriate? (2) Can stereotactic biopsy replace open surgery? If yes, in what specific clinical situations and in what percentage of patients? (3) What gain in patient management and health care costs can be achieved? To address these questions, a request for cooperative agreement (RFA) is being written to stimulate the needed multicenter research.

## 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 published in the *Journal of the National Cancer Institute* and a second manuscript describing the statistical methodology in more depth has been submitted.

## 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. A manuscript describing this work is in revision.

## 6. DATA MONITORING OF COOPERATIVE GROUP CLINICAL TRIALS

The cancer cooperative groups utilize data monitoring committees to monitor interim outcome results of their phase III clinical trials. The BRB represents CTEP on these committees in evaluating when there is sufficient evidence to cease accrual and report results. The BRB takes an active role in many of these important and difficult issues and interacts with the cooperative group statistical centers to ensure that necessary analyses are available.

The BRB has, in collaboration with the cooperative group statisticians, established guidelines for data monitoring committees for national intergroup clinical trials. The BRB organized and sponsored an international workshop on Operational Aspects of Data Monitoring Committees to facilitate its review of data monitoring procedures employed in cancer cooperative group studies. The complete proceedings of this workshop will be published in *Statistics in Medicine*.

## 7. DEVELOPMENT AND EVALUATION OF PROGNOSTIC MARKERS

The BRB is collaborating with the Diagnosis Branch of the NCI Division of Cancer Biology, Diagnosis and Centers (DCBDC) in the conduct of definitive confirmatory studies, to evaluate the clinical importance of new molecular markers as prognostic indicators in malignant diseases. Sample size calculations were performed and a master agreement issued for breast cancer prognostic studies of flow cytometry measurements of ploidy and S phase fraction, heat shock proteins, pS2, EGFR, Her-2/new, etc. The BRB will collect and analyze the data from these studies centrally.

The BRB is collaborating with the Diagnosis Branch (DCBDC) in the organization of an international Workshop on Prognostic Markers for Cancer: Clinical Relevance and Study Design for Evaluation. The workshop will address methodologic problems in the development and evaluation of new prognostic markers.

The Chief of the BRB is developing guidelines for the design, analysis and reporting of confirmatory studies of prognostic markers. These guidelines will be published in an invited editorial in the *British Journal of Cancer*. Whereas there are methodologic standards for clinical trials, there are not similar standards for prognostic factor studies and the literature is filled with conflicting claims.

## 8. PHASE II TRIAL DESIGN FOR SMALL CELL LUNG CANCER

The conventional targeted response rate of 20% is not appropriate for previously treated SCLC patients -- known active first-line agents have response rates less in this population. This has lead to various suggestions in the literature including: (a) lowering the target response rate to 10%, (b) using relapsed patients who had complete responses to conventional chemotherapy, (c) using a window of opportunity design with patients with no prior chemotherapy, and (d) limiting the trial to elderly or poor prognosis patients with no prior chemotherapy. These approaches are critqued and recommendations given in a paper (with Dr. T. Moore) that has appeared in *JNCI*. An additional letter to the editor of *JCO* has been accepted for publication.

## 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, Dr. M. Smith of the Clinical Investigations Branch, and others of CTEP are 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. A presentation of preliminary results has been made at a conference concerning late effects of therapy and a manuscript has been submitted.

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

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 baseline QOL variables as prognostic variables? These issues were addressed by the statistical working group of a DCT/DCPC supported QOL conference held in 1990. A paper has been submitted summarizing the reports from all the working groups of the conference. An additional paper describing some statistical issues is under preparation.

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. Two papers have been published on this topic, one in *JNCI* and the other in *Oncology*.

## 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 two national workshops 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 BRB developed an agreement on collaboration for international studies involving the NCI and NCIC (National Cancer Institute of Canada). The BRB is organizing a workshop on debulking the next generation of breast cancer intergroup trials in order to facilitate accrual of thousands of women per trial.

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. The pediatric groups recently agreed to intergroup trials of osteosarcoma, Ewing's sarcoma and medulloblastoma after extensive interactions with the BRB and CTEP. The first national intergroup study of early stage Hodgkin's disease was also recently initiated as well as national breast cancer trials of autologous bone marrow transplant treatment for high risk or metastatic patients and fenretinide with tamoxifen for low risk women. The BRB is very active in the initiation, design and monitoring of national intergroup trials.

## 12. NATIONAL CLINICAL TRIALS OF EARLY OVARIAN CANCER

A series of ancillary papers is in preparation concerning further results from two clinical trials in early ovarian cancer (FIGO stages I and II) with Dr. L. Walton of the University of North Carolina. Efficacy and toxicity of P32 treatment has been analyzed and a manuscript published. Analyses of survival restricted to stage II patients have also been published. Analyses restricted to the outcome for low malignant potential patients are also completed and the manuscript is in preparation.

## 13. COLLABORATIVE RESEARCH WITH THE LUNG CANCER STUDY GROUP

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

a. 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 with Dr. R. Feld of the University of Toronto and others has been accepted for publication.

b. 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 of Memorial Sloan-Kettering Cancer Center.

c. 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 of the University of Illinois. Additional analyses based on further follow-up have been conducted and a second manuscript has been submitted.

## 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, multidrug 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 antitumor 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. Two manuscripts describing this work has been published in *JNCI* and the *Journal of Biopharmaceutical Statistics*. A third manuscript, applying this model to metastatic breast cancer, has been published in *Breast Cancer Research and Treatment*. A fourth manuscript has been submitted for publication showing how this model, and the Tolerable Dose Diagram, can be used for the design of phase I clinical trials of drug combinations.

## 15. PHASE I TRIALS FOR COMBINATIONS OF AGENTS

Choosing maximum doses of agents to be given in combination is more complicated than choosing a MTD for a single agent. This is because there are many pairs of doses that is tolerated but with an increase in either dose, not tolerated. In a paper recently submitted for publication, we offer guidance in targeting specific MTD combinations, and suggest trial designs to achieve these targets . A graphical Tolerable Dose Diagram is introduced to facilitate the design and conduct of such trials.

## 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 have been submitted for publication.

## 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. A workshop focusing on the operational aspect of data monitoring of clinical trials was organized and sponsored by the Biometric Research Branch NCI in collaboration with the AIDS Program, NIAID and the NHLBI. There was participation by NIH staff, NIH extramural investigators, representatives of the pharmaceutical industry and trial organizers outside of the United States.

The workshop reviewed 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, EROTC, 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 were considered. The complete proceedings including discussion will be published in *Statistics in Medicine*.

## 19. INVESTIGATING A SEQUENCE OF RANDOMIZED PHASE II TRIALS TO DISCOVER IMPROVED TREATMENTS FOR A RARE DISEASE.

Most of the theory of experimental design addresses the design of a single experiment. In may areas of science and technology, however, developments are taking place rapidly and promising treatments become available sequentially in time. Consequently, considerations of how to plan a sequence of experiments become

important. This consideration is magnified when dealing with a rare disease where conventional comparative clinical trials may require such duration in order to accrue the required number of patients that the treatments compared are no longer of interest.

The usual approach to evaluating treatments for rare diseases involves use of historical controls. The difficulties with the reliability of historical controls have been repeatedly described, however. These difficulties may be exacerbated for rare diseases where new patients and controls may be separated substantially in time and location.

We have investigated the use of a sequence of randomized two-arm phase II trials as a framework for therapeutic research in a rare disease with rapidly evolving technology. We consider the case in which the outcome for a specific patient is binary, success or failure. The treatment with the higher observed success probability in a given phase II trial is carried over to the next trial. It is compared there to a new treatment. This process is repeated. If there are N patients available for treatment in a reasonable period of time (e.g. 5 years) and each arm of each trial contains n patients, then there will be approximately N/2n trials in the sequence. We compute the expected value of the success probability for the treatment selected at the end of the sequence. We also compute the expected number of successes obtained in the N patients treated. For a fixed N we study how these expected value vary with n. We also study how the results vary with N. Two models for generating success probabilities for new treatments are studied. In one model, a fixed distribution is used for generating success probabilities for new treatments. In the second model, we assume that new treatments are designed based on treatments previously found to be effective. We assume that the success probability for a new treatment is selected from a distribution with mean equal to the success probability for the current control treatment at that stage of investigation.

The results indicate that a sequence of two-arm randomized trials can improve the expected success probability of the treatment selected based on a limited horizon of patients compared to the conduct of a single two-arm clinical trial. When the patient horizon is 200 and the distribution generating success probabilities for new treatments is fixed, however, the improvement is rather limited unless the distribution is broad. For a patient horizon of 1000, however, the potential improvement from running 10 sequential trials of 50 patients per arm each is greater. When new treatments are designed based on the current control and the mean of the distribution generating the success probability for a new treatment can be assumed equal to the success probability of the current control, then even greater improvements are possible from this sequential approach. The criterion of expected number of successes among the N patients also favors a sequence of trials

These results may have important applications in oncology and AIDS clinical research.

## 20. EVALUATION OF SURAMIN FOR THE TREATMENT OF STAGE D2 CARCINOMA OF THE PROSTATE

BRB has collaborated with Dr. Michaele Christian to organize extramural clinical trials to confirm the promising results reported 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. We have finished a phase II study with measurable disease patients. A randomized study for patients with non-measurable 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. Meyers on evaluation assay calibration at the participating institutions. BRB has analyzed several phase II trials of suramin in prostate cancer and correlated baseline variables with response. We have collected all suramin administration and blood level data from all CTEP sponsored phase I trials. We are acquiring a computer program from Abbott Lab and will use it to develop a population pharmacokinetic model and perform pharmacodynamic analyses such as attempting to predict serious neurotoxicity. We will also evaluate the utility of several adaptive dosing schemes based on the observed blood levels and recommend schedules for broader development of suramin.

## 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 approach being evaluated involves 100% dose level increases until reproducible grade 2 toxicity is encountered. Within patient dose-escalations are also permitted during the first stage. A retrospective analysis of the phase I database is being conducted to evaluate the new design.

In addition, a new design called the continual reassessment method has been reported in the literature to be an improvement over the standard design. In a paper in preparation, we report our comparisons of this new method to the standard design.

## 22. A BAYESIAN SEQUENTIAL DESIGN FOR PHASE II TRIALS OF COMBINATION REGIMENS

Phase II trials of combinations are single arm studies aimed at deciding whether a new treatment E is sufficiently promising, relative to a standard therapy S, to include in a large-scale randomized trial. Thus, phase IIB trials are inherently comparative even though a standard therapy arm usually is not included. Uncertainty regarding the response rate $\theta_s$ of S is rarely made explicit, however, either in planning the trial or interpreting its results. We propose practical Bayesian guidelines for deciding whether E is promising relative to S in settings where patient response is binary and the data are monitored continuously. The design requires a specification of an informative prior for $\theta_s$, a targeted improvement of E and bounds on the allowed sample size. No explicit specification of a loss function is required. Sampling continues until E is shown to be either promising or not promising relative to S with high probability, or the maximum sample size is reached. The design provides decision boundaries and a probability distribution for

the sample size at termination. This research is being conducted in collaboration with Dr. Peter Thall of the Department of Biomathematics at M.D. Anderson Cancer Center. One manuscript has been submitted for publication and the design has been used for several M.D. Anderson clinical trials. A second manuscript is in preparation describing improved properties resulting from early termination when the predictive probability of a definitive conclusion by the maximum sample size is small.

## 23. PLANNING OF MULTI-TREATMENT CLINICAL TRIALS

Clinical trials with more than two treatment arms often require a more complex analysis strategy than do two-arms trials. For example, a 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 ordered according to a secondary criteria such as toxicity or cost. Our methods provide high probability for selecting the most appropriate treatments. 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. Theses results have been used for 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 plus IFN$\alpha$. A paper which deals with the situation where all treatments can be ranked in strictly prior preference is in press. Two other papers which deal with the situations where pairs of treatments have equal prior preference have been submitted for publication.

## 24. A MATHEMATICAL MODEL OF ADJUVANT TREATMENT FOR BREAST CANCER

Bonadonna reported a clinical trial for patients with primary breast cancer that compared three regimens: (i) CMF alternating with Adriamycin, (ii) sequential CMF→A, (iii) sequential A → CMF. Results with regimen (iii) were best. We are developing a mathematical model to better understand the implications of these results. It is assumed that there are four populations of tumor cells categorized by binary resistance or sensitivity to CMF and A. The number of tumor cells remaining after surgery is assumed to vary among patients according to a lognormal distribution. The parameters specify the partition of these cells among the four chemosensitivity types, one parameter for exponential tumor growth in the absence of treatment and two parameters for log cell kill per cycle of each regimen (CMF or A) complete specification of the model. We will attempt to estimate the parameters of the model to best fit Bonadonna's data. This may provide insight to the design of future regimens.

## 25. INTRAMURAL CLINICAL TRIALS

The BRB collaborates with the intramural clinical branches on the design, monitoring and analysis of clinical trials. During the past year these have included clinical trials of acute myelogenous leukemia, CNS lymphoma, pancreatic carcinoma, bioavailability of oral fludarabine, a phase I study of an IL-3/GM-CSF fusion protein (PIXY321) and carboplatin, and a randomized study of pre-chemotherapy GM-CSF for patients receiving topotecan for metastatic melanoma and renal cell carcinoma. A mini-course on clinical trial methodology was also presented for Medicine Branch clinical associates.

## 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 YMP supercomputer was used for this evaluation. A manuscript describing these results has been accepted for publication in the *Journal of Computational Statistics and Graphics* and the results have been presented at several scientific meetings.

## 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 staff 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 a master protocol for TAG-72 localization in colorectal cancer patients with Dr. W. Fogler of the Investigational Drug Branch (IDB) and others of CTEP. 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. THE FUNCTION AND DESIGN OF CONFIRMATORY CLINICAL TRIALS

Of every 100 clinical trials in which the null hypothesis is true, 5 are expected to be statistically significant at the 5% level by chance alone. Since several hundred randomized phase III trials are being conducted by the cooperative groups alone, there is a substantial probability that a statistically significant finding is a false positive. Consequently, it is generally important that positive results be confirmed in an independent clinical trial. Recent experience with confirmatory clinical trials in clinical oncology has been reviewed and a Bayesian statistical model for determining when a confirmatory trial is warranted is presented. The model is based on beliefs about treatment effectiveness prior to the initial trial, clinical trial results, and the degree of effectiveness necessary to justify the expense and toxicity of the new treatment. Methodologic issues in the design of confirmatory trials are addressed. Survey results on the degree of acceptance (and reasons for non-acceptance) of 5Fu + levamisole for the treatment of stage C colon cancer in the U.S. and U.K. are presented. This research is being conducted in collaboration with Dr. Mahesh Parmar of the U.K. Medical Research Council.

## 30. PROGNOSTIC FACTORS FOR CARDIOTOXICITY IN PATIENTS RECEIVING TAXOL

In collaboration with Dr. Susan Arbuck (IDB), the BRB is analyzing risk factors for cardiotoxicity in patients receiving taxol. The data base of over 1000 patients with advanced ovarian cancer treated on Treatment Referral Center protocols is used for this analysis.

## 31. PILOT AND PHASE II STUDIES OF IL-2 IN RENAL CELL CARCINOMA AND MELANOMA

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

Analysis of a randomized phase II study of high-dose IL-2 with or without IFN in advanced renal cell carcinoma has been completed and submitted for publication with Dr. M. Atkins of Tufts University and others. It was found that high-dose IL-2 and high-dose IL-2 plus IFN have comparable and modest response rates.

Analysis of a randomized phase II study of high-dose IL-2 with or without IFN in advanced malignant melanoma has been completed and submitted for publication with Dr. J. Sparano of Montefiore Medical Center and others. It was found that high-dose IL-2 and high-dose IL-2 plus IFN have comparable and modest response rates.

**32. RELATIONSHIP BETWEEN DOSE INTENSITY AND OUTCOME IN TREATMENT OF PATIENTS WITH SMALL NON-CLEAVED CELL LYMPHOMA**

In collaboration with Dr. I. Magrath of the Pediatric Branch, the BRB has analyzed the relationship between delivered dose intensity of individual drugs and outcome for patients treated for small-non-cleaved cell lymphoma. A manuscript describing the results and methodologic issues in dose intensity analyses is in preparation.

**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. Papers have been published in the *Annals of Statistics* and the *American Journal of Public Health*. A third paper has been provisionally accepted for publication by the *Journal of the American Statistical Association*, and another paper has been submitted for publication.

**34. APPLICATIONS OF CRUDE INCIDENCE CURVES**

In the competing risks problem, 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. BRB staff has published a paper with Dr. F. Dorey of UCLA in *Statistics in Medicine* 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 paper written with Dr. D. Roe of the University of Arizona has been provisionally accepted for publication by *Statistics in Medicine*.

## 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 with Dr. S. Baumrind of UCSF has been published in the *Journal of Dental Research*, and another is to be published in the *European Journal of Orthodontics*.

## 37. 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. This design may have application whenever (1) clinicians believe strongly for some patients 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 is ongoing at the University of Pacific Orthodontic Clinic (San Francisco) using this design. A paper describing this design was published in *Lancet*. BRB staff is collaborating with Dr. S. Baumrind of UCSF in conducting this ongoing trial.

## 38. USE OF SEQUENTIAL ANALYSIS IN COOPERATIVE GROUP CLINICAL TRIALS

The BRB participated in collaboration with the U.K. Medical Research Council on evaluation of the use of sequential design methods in phase III clinical trials. The study was conducted in collaboration with Drs. N. Donaldson and J. Whitehead of the University of Reading. It was found that sequential methods are much more extensively utilized in NCI sponsored clinical trial than in MRC sponsored trials. A manuscript is in preparation.

## 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 University of Maryland, 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 University of Maryland, 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. BAYESIAN DESIGN OF FACTORIAL CLINICAL TRIALS

The 2x2 factorial clinical trial involves four treatment arms defined by the selection of level 0 or 1 for each of two factors A and B. When the influence of one factor on patient response is the same for each level of the other factor, the design is particularly efficient and permits the investigator to answer two questions for the "price" (number of patients) of one. Unfortunately, the sample size required to adequately test the hypothesis of independence of the two factors on response is greater than the sample size required to perform the study assuming independence. Consequently, clinical investigators are often in a dilemma about whether to utilize the factorial design. The analysis of a factorial design generally begins with a test of hypothesis of independence of the factors of response. If the hypothesis is accepted, then a standard factorial analysis proceeds. Otherwise the trial is analyzed as a four arm trial, without utilizing the factorial structure. We are investigating a Bayesian approach to the design and analysis of the 2x2 factorial clinical trial. The advantages of this approach are, first, that it permits the investigator to express prior expectation about interactions without having to dichotomously assume either that they do or do not exist. The second advantage is that the analysis also becomes "continuous" rather than dichotomous based on the results of a test of interaction, a test which itself may be lacking in statistical power. The performance of this design and sample size considerations for it are being investigated in collaboration with Dr. Mahesh Parmar of the U.K. Medical Research Council and Lawrence Freedman of the NCI Division of Cancer Prevention and Control.

## 42. 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 studies are analyzed together, the type I error may not have the nominal value. The upper bound type I error in this situation is being investigated. Some simulations have been carried out and theoretical development is under investigation.

## 43. 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 a manuscript is being prepared for submission.

## 44. APPROXIMATIONS TO TYPE II ERROR PROBABILITIES IN SURVIVAL REGRESSION MODELS

We have studied the performance of statistical tests of treatment effects on survival in the presence of nuisance parameters. First and second order asymptotic approximations to type II error probabilities have been determined. It has been shown that there exist M-estimators of the nuisance parameters, i.e., nonlinear least squares estimators, which improve the performance of some statistical tests

over the maximum likelihood estimators of the nuisance parameters for moderate sample sizes. A manuscript has been submitted in collaboration with Dr. E. Slud of the University of Maryland, Department of Mathematics.

## 45. CONTINUAL MONITORING DESIGNS FOR PHASE II CLINICAL TRIALS WITH TIME-TO-EVENT ENDPOINTS

For some phase II clinical trials the most useful endpoint is time-to-progression or survival. This is the case when tumor response is difficult to evaluate or frequently of short duration. This type of endpoint has recently been used for clinical trials of CNS lymphoma, gastric carcinoma, pancreatic cancer and prostate cancer. In many phase II trials in cancer centers or the pharmaceutical industry, results are monitored continuously and hence fully sequential designs are required if misleading results are to be avoided. We have developed frequentist designs (based on the truncated sequential probability ratio test) and Bayesian designs for these applications and are comparing their properties.

## 46. CTEP LOCAL AREA NETWORK

The BRB systems administration for the CTEP local area network Micorsoft's LAN manager and 3-Com Mail have been installed for 50 members of the CTEP staff. Installation on the two MacIntosh computers still awaits the availablility of Eudora software to be provided by the NCI Network Center.

In addition to 3-Com Mail, Harvard Graphics has been installed on the CTEP LAN. Five CTEP users are now experimenting with this package. The LAN version of Harvard Graphics is limited to six simultaneous users.

A public "J:" drive has been set up on the LAN server. Any CTEP user, with the appropriate statements in their autoexec.bat file, can use this drive to store and retrieve their own files and data provided by other CTEP users.

## 47. OTHER ACTIVITIES

The members of BRB consults on major medical investigations with all programs of the Division of Cancer Treatment as well as other NCI divisions, other institutes, and other research organizations. During the past year this has included advising the National Institute of Environmental Health Sciences on the design of a clinical trial of chelating agents, serving on a study section of the National Institute of Dental Research to evaluate grant applications in response to an RFA for "Evaluation of treatments for cleft lip and/or palate," advising Memorial Sloan-Kettering Research Institute on the design of a phase III trial of patients with testicular cancer, advising the University of Chicago on the development of pharmacodynamic models for use in phase I trials, advising the Deputy Director of NCI and the General Accounting Office on the applicability of "cross-design-synthesis" in therapeutic evaluation, advising the International Bone Marrow Donor Program on the establishment of a statistical center and advising NCI and NIH management on issues involving evaluation of the costs of clinical trials and cost-effectiveness of autologous bone marrow transplant in breast cancer. The BRB branch chief has participated in intramural personnel promotion and selection reviews for several institutes and serves as a member of the Time Allocation Committee for the NCI

Scientific Supercomputer Facility. BRB staff serve on the editorial board of JNCI and several other professional journals. The BRB chief played a leading role in determining the effect of detected data problems on the results of major NCI-sponsored clinical trials and organizing a meeting at which these results were presented to NCI and NIH management. BRB staff member gave a presentation on statistical issues in vaginal cancer at a NCI conference on long term DES. The Chief of BRB interacted with Dr. R. Herman, director of the U.S. Joint Mathematics Council on enhancing the contribution of the mathematical sciences to medical research at NIH. The BRB participates in the training of biostatisticians and physicians in clinical trial methodology. The BRB organized and conducted a workshop on Survival Analysis attended by 75 statisticians and physicians at the 1991 Society for Clinical Trials Meeting. The BRB continued to conduct a mini-course on clinical trials methodology for Medicine Branch fellows. During the past year, the BRB has hosted 5 international visitors for training and collaborative research of short to medium duration. The BRB chief is also co-organizer and editor of the NIH Conference on Current Topics in Biostatistics. This national conference, sponsored by the Office of the Director, NIH, will be held in January 1993 with the proceedings published in *Statistics in Medicine*. The conference will highlight NIH research in biostatistics.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06308-21BRB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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 D. Koutsoukos, Ph.D., Visiting Fellow, BRB, CTEP, DCT, NCI
Nurith Strauss, M.B., General Fellow, BRB, CTEP, DCT, NCI

COOPERATING UNITS *(if any)*

Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Biological Response Modifier Program, DCT, NCI; Division of Cancer Prevention and Control, NCI; Diagnosis Branch, DCBDC, NCI; M.D. Anderson Tumor Institute, U.C.S.F., U.K. Medical Research Council., U.C.L.A., University of Maryland.

LAB/BRANCH

Biometric Research Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7 | 6 | 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 and conducts collaborative research with the intramural components.

The Biometric Research Branch performs statistical planning, monitoring and evaluation of all Division of Cancer Treatment supported therapeutic clinical 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 methods 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 scientific and administrative 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 conducting extramural clinical trials, providing a mechanism for the continuous generation of therapeutic and ancillary studies with the intent of improving 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, ensuring proper protocol design, 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)<br>F. Andrew Dorr, M.D.<br>Ellen G. Feigal, M.D.<br>Richard S. Kaplan, M.D. |
| Pediatrics | Malcolm A. Smith, M.D., Ph.D. (Head) |
| Surgery | Richard S. Ungerleider, M.D. (Acting Head)<br>Edward L. Trimble, M.D. |

J. Michael Hamilton, M.D. resigned in January, 1992 to join the Navy Oncology Branch, NCI; F. Andrew Dorr, M.D. resigned as of July 24, 1992 to join Eli Lilly & Company in Indianapolis, Indiana.

Secretarial support is provided by Mindy Kaufman and Bernadette Greenfield, with the assistance of Steven Sudler (stay-in-school program).

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 | Kaplan |
| Breast | Dorr |
| Gastrointestinal | Kaplan |
| Genitourinary | Dorr |
| Gynecologic | Trimble |
| Head and Neck | Feigal |
| HIV-associated cancer | Feigal |
| Leukemia (adult) | Cheson |
| Leukemia (child) | Smith |
| Lung | Feigal |
| Lymphoma (adult) | Cheson |
| Lymphoma (child) | Smith |
| Melanoma | Dorr |
| Myeloma | Cheson |
| Sarcoma (adult) | Trimble |
| Sarcoma (child) | Smith |

| MODALITY | STAFF |
|---|---|
| Bone marrow transplant | Cheson |
| Infectious disease | Cheson |
| Quality of life | Trimble |
| Radiation | Kaplan |
| Surgery | Trimble |
| Minority/Women's Issues | Trimble |

## 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 as well as the pilot and developmental studies which precede them. Approximately $76 million will be devoted by NCI to these activities in FY92, an increase of roughly $16 million over FY91.

Despite this increase in funding, the Cooperative Groups are currently supported (in constant dollars) at a level 18% below that of FY80, while devoting increasing attention to logistically complex intergroup trials as well as studies with expensive laboratory-clinical correlations. The CIB advises and directs the Groups in allocating relatively limited financial, investigator and patient resources. During the past year, Group-related administrative activities have included: assignment of additional funds to the Groups for the purposes of conducting clinical research in breast, lung, and female reproductive malignancies and novel therapeutic approaches, as suggested by the Congress; management of the NCI-designated High Priority Trials initiative and the Minority Accrual Initiative; the development of a funding plan for the successful applicants for the RFA, "Phase I Trials of New Cytotoxic and Biologic Agents in Children"; the development of a Request for Applications for the conduct of clinical trials in Brain tumors, to be

supported by the U-01 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; development of a proposal for the systematic banking of tumor specimens obtained from clinical trials patients; meeting with the Cooperative Group Chairmen on a semiannual basis to discuss matters of mutual interest; 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; redistributing funds within non-competing Groups; 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 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 (7-1-91 to 6-30-92) 44 concepts for Phase III trials were reviewed of which 14 have become active studies.

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 (7-1-91 to 6-30-92), 349 protocols were reviewed by the Protocol Review Committtee, of which 114 achieved active status within the Groups. Finally, but with ever-increasing emphasis, the CIB promotes relevant laboratory-clinical correlative investigations which might prove scientifically productive; currently 101 protocols include laboratory correlative studies. Information concerning the best correlative studies comes not only from Group pilot activities, but also from information gained from 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 FY92 and the CIB staff member responsible for scientific liaison with that organization.

| Cooperative Group | CIB Liaison |
|---|---|
| Brain Tumor Cooperative Group (BTCG) | Kaplan |
| 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) | 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) | Kaplan |
| Pediatric Oncology Group (POG) | Smith |
| Quality Assurance Review Center (QARC) | Feigal |
| Radiation Therapy Oncology Group (RTOG) | Feigal |
| 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 1991, these Groups accrued a total of over 3700 patients to their clinical trials through this Program.

## III. SCOPE OF GROUP ACTIVITIES

**ACCRUAL**

In 1991, the Clinical Trials Cooperative Group Program involved over 4600 investigators in roughly 1300 institutions, hospitals or practices, and accrued 23,181 patients to 493 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 9600 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 1991

| | PATIENT ENTRIES | OPEN STUDIES |
|---|---|---|
| PHASE I | 628 | 64 |
| PHASE II | 4,429 | 246 |
| PHASE III | 18,124 | 183 |
| NON-THERAPEUTIC/CORREL. | 9,608 | 91 |
| EORTC (1991) (EST) | 6,625 | 215 |

**FIGURE A**

COOPERATIVE GROUP TREATMENT TRIALS
PATIENT ACCRUAL BY DISEASE/SITE – 1991
(PHASE II & III STUDIES)

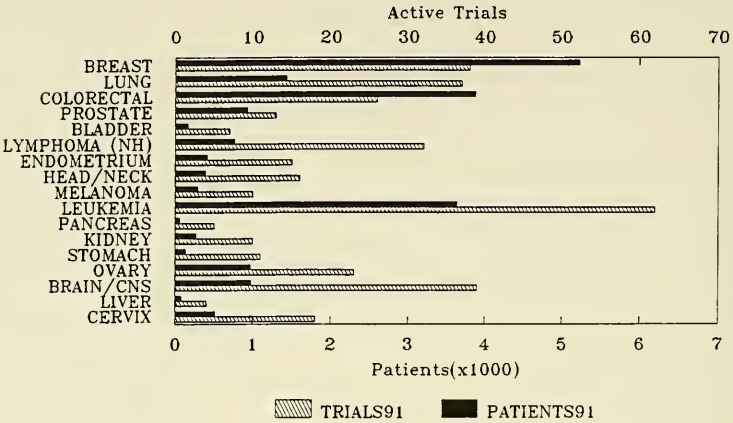
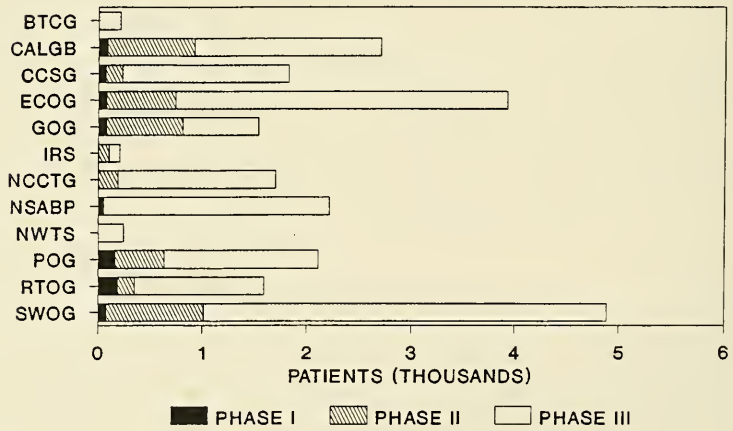



**FIGURE B**

ACCRUAL BY COOPERATIVE GROUPS ONTO
THERAPEUTIC TRIALS - 1991



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 1991 ACCRUAL TO PHASE II AND PHASE III STUDIES

| ORGAN | NEW CASES IN 1991 (ACS DATA) | DEATHS IN 1991 | RATIO DEATHS TO NEW CASES | STUDIES OPEN TO ACCRUAL | TOTAL ACCRUAL 1991 | % ONTO GROUP STUDIES |
|---|---|---|---|---|---|---|
| Lung | 161,000 | 143,000 | 0.89 | 37 | 1,432 | 0.9 |
| Colon/Rectum | 157,000 | 60,500 | 0.38 | 26 | 3,875 | 2.5 |
| Breast | 175,900 | 44,800 | 0.25 | 38 | 5,224 | 3.0 |
| Prostate | 122,000 | 32,000 | 0.26 | 13 | 926 | 0.7 |
| Bladder | 50,200 | 9,500 | 0.19 | 7 | 159 | 0.3 |
| Lymphoma, Non-H | 37,200 | 18,700 | 0.50 | 32 | 762 | 2.0 |
| Endometrium | 33,000 | 5,500 | 0.17 | 15 | 402 | 1.2 |
| Oral (HN) | 30,800 | 8,150 | 0.26 | 16 | 385 | 1.2 |
| Leukemia | 28,000 | 18,100 | 0.65 | 62 | 3,630 | 13.0 |
| Melanoma | 32,000 | 6,500 | 0.20 | 10 | 289 | 0.9 |
| Kidney | 25,300 | 10,600 | 0.42 | 10 | 270 | 1.1 |
| Pancreas | 28,200 | 25,200 | 0.89 | 5 | 54 | 0.2 |
| Stomach | 23,800 | 13,400 | 0.56 | 11 | 120 | 0.5 |
| Ovary | 20,700 | 12,500 | 0.60 | 23 | 971 | 4.7 |
| Brain/CNS | 16,700 | 11,500 | 0.69 | 39 | 980 | 5.9 |
| Liver | 15,000 | 12,100 | 0.81 | 4 | 80 | 0.5 |
| Cervix | 13,000 | 4,500 | 0.35 | 18 | 511 | 3.9 |
| Myeloma | 12,300 | 9,100 | 0.74 | 8 | 183 | 1.5 |
| Esophagus | 10,900 | 9,800 | 0.90 | 8 | 121 | 1.1 |
| Lymphoma, Hodgkin's | 7,400 | 1,600 | 0.22 | 13 | 284 | 3.8 |
| TOTAL | 1,000,200 | 457,050 | | 395 | 20,667 | |

**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. Trials are selected for this program based on their potential to increase the survival rate for common cancers or for their ability to answer questions of special biological significance. The accelerated patient accrual is intended to speed the resolution of underlying medical questions and to bring successful new cancer treatments the cancer patient more quickly.

As of 1992, High Priority Trials represent approximately ten percent of active Cooperative Group Phase III studies. They accrue, however, one third of the total Phase III patients annually. Although the majority of the 21 trials designated appear to have had their accrual enhanced by High Priority status, three of the trials continue to accrue slowly, reflecting the difficulty of conducting randomized clinical trials in certain clinical situations. Overall, however, 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.

Thus far, four series (1988, 1989, 1990, and 1991) of High Priority Trials have been designated by NCI. The titles of these studies (below) provide a sample of the Clinical Trials Cooperative Group Program's content:

Series I High Priority Trials

- Adjuvant Chemotherapy and Radiation Therapy for Rectal Cancer (NCCTG-864751). ACCRUAL COMPLETED
- 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). ACCRUAL COMPLETED
- Adjuvant Chemotherapy Following Surgery for Colon Cancer (NSABP-C-03). ACCRUAL COMPLETED

Initially, five Phase III studies were designated High Priority Trials; two of these remain open. NSABP-C-03 closed in April, 1989 having accrued 1100 patients in roughly half the time originally anticipated. Intergroup rectal study NCCTG-864751, which opened in 1987, closed in September, 1990 having accrued 680 patients in half the time projected. The non-Hodgkin's lymphoma intergroup trial (INT-0067) closed in June, 1991 with 1100 patients accrued in the time frame originally planned. NSABP rectal study (R-02) is currently entering patients at the rate originally planned, and should close on schedule in late 1992. The bladder cancer intergroup study (INT-0080) is an unprecedented effort involving genitourinary surgeons, and accrual is progressing at a reduced rate; an extended accrual period will be required.

Series II High Priority Trials

- 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)

- o Adjuvant Chemotherapy for Colon Cancer (INT-0089)

- o Radiation Therapy and Chemotherapy for Non-Small Cell Lung Cancer (RTOG-8808). ACCRUAL COMPLETED

- o Comparison of Treatments for Early-Stage Breast Cancer (NSABP-B-21)

Six additional trials received High Priority designation in June, 1989. Although they are large studies, their planned accrual periods are only 3.3 years or less. INT-0089, a study of levamisole in colon cancer, was recently revised and expanded; accruing 800 patients annually, it should close by late 1992. The small-cell lung cancer (INT-0096) and node-negative breast cancer (INT-0102) intergroup studies are accruing patients above their projected rates and should close nearly two years early. The NSABP study of occult stage I breast cancer (B-21), dealing with a unique patient population, continues to enter patients slowly; at the current rate, the patient accrual period will be roughly eight years. The non-small cell lung cancer study (RTOG-8808) evaluating radiation therapy with and without chemotherapy closed in January, 1992 after three years of accrual. NSABP B-18, comparing short intensive pre-operative chemotherapy with similar therapy adminstered in conventional post-operative fashion, is accruing well and should reach its accrual goals in late 1992. The overall average accrual currently for the five open Series II High Priority Trials is about 610 patients per study per year.

Series III High Priority Trials

- o Post Remission Treatment of Adult Acute Non Lymphocytic Leukemia comparing Autologous Bone Marrow Transplantation with intensive Chemotherapy (EST-3489)

- o Adjuvant Therapy of Rectal Cancer (INT-0114)

- o Adjuvant Chemotherapy Following Surgery for Colon Cancer (NCCTG-894651). ACCRUAL COMPLETED

These three additional High Priority Trials were designated in June, 1990. The NCCTG study of levamisole as adjuvant treatment for resectable colon cancer accrued over 900 patients in thiry months and was closed in October, 1991. The intergroup study (EST-3489) in adult myeloid leukemia is progressing at the rate originally projected. The intergroup study of levamisole as adjuvant treatment for rectal carcinoma has entered patients at twice the rate proposed and at the current rate will have accrued the 1400 patients required in less than two years.

Series IV High Priority Trials

- 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-012I)

- 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)

The seven Phase III clinical trials listed above were selected for High Priority designation in June, 1991. CALGB-9082, a study of high dose chemotherapy with bone marrow transplantation for Stages II and III breast cancer, is accruing well beyond the projected rate. The trial using alpha-2 interferon in metastatic melanoma (EST-1690) is accruing at twice the expected rate. NSABP B-24, evaluating tamoxifen with lumpectomy and radiation, is also accruing at twice the projected rate. The three Intergroup studies in this series are in the early stages of accrual, but are progressing as projected. The trial assessing hormonal therapy in prostate cancer (EST-3886/SWOG-8973) is accruing very slowly, however, despite High Priority designation.

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 | ACTIVA-TION DATE | ACCRUAL TARGET | 1/88 | 1/89 | 1/90 | 1/91 | 1/92 |
|---|---|---|---|---|---|---|---|
| Lymphoma (INT 0067) | 04/86 | 1,000 | 219 | 481 | 755 | 1027 (CLOSED) | |
| Bladder (INT 0080) | 08/87 | 298 | 5 | 35 | 96 | 130 | 167 |
| 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 | 667 |

**ACCRUAL TO HIGH PRIORITY TRIALS**
**SERIES II**

| STUDY | ACTIVA-TION DATE | ACCRUAL TARGET | 1/89 | 1/90 | 1/91 | 1/92 |
|---|---|---|---|---|---|---|
| Colon Cancer INT-0089 | 08/88 | 2,700 | 113 | 659 | 1765 | 3064 |
| Lung (small cell) INT-0096 | 04/89 | 250 | - | 34 | 186 | 316 |
| Breast (node-) INT-0102 | 07/89 | 2,600 | - | 233 | 1766 | 3450 |
| Breast NSABP-B18 | 11/88 | 1,275 | 40 | 390 | 763 | 1112 |
| Breast NSABP-B21 | 06/89 | 1,350 | - | 41 | 162 | 307 |
| Lung (non-small cell) RTOG-88-08 | 01/89 | 360 | - | 90 | 263 | 479 (CLOSED) |

ACCRUAL TO HIGH PRIORITY TRIALS
SERIES III

| STUDY | ACTIVATION DATE | ACCRUAL TARGET | 1/90 | 1/91 | 1/92 |
|---|---|---|---|---|---|
| Leukemia (EST-3489) | 2/90 | 560 | - | 61 | 206 |
| Rectal Cancer | 8/90 | 1378 | - | 106 | 969 |
| Colon Cancer | 9/89 | 800 | 63 | 451 | 915 (CLOSED) |

**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, African-Americans have the highest incidence rate for all cancers combined, followed by Native Hawaiians and then Caucasians. African-Americans also experience the highest overall cancer mortality rates, followed by Native Hawaiians and Caucasians (U.S. mortality data classify Hispanics as whites). African-Americans 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 patients from racial/ethnic groups which historically have been medically disadvantaged. Minority patients are defined as African-Americans; Hispanic Americans of Mexican, Puerto Rican, Cuban or Central American origins; Native Americans; and Asian/Pacific Islanders. 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 | TOTAL # OF PATIENTS 4TH QTR 91 | % RACE UNKNOWN | % MINORITY |
|---|---|---|---|
| BTCG | 44 | 2% | 6% |
| CALGB | 1335 | 15% | 14% |
| CCSG | 1096 | 3% | 25% |
| ECOG | 1461 | 16% | 12% |
| GOG | 466 | 5% | 23% |
| NCCTG | 1001 | 3% | 4% |
| NSABP | 556 | 50% | 9% |
| POG | 1576 | 34% | 25% |
| RTOG | 456 | 4% | 15% |
| SWOG | 1377 | 1% | 19% |

**STRATEGY MEETINGS**

Strategy meetings help to 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 (generally at the American Society of Clinical Oncology Spring meeting) to review ongoing clinical experiments and identify short-term priorities for research. The status of current studies is reviewed, follow-up trials are planned, and drug development or toxicity issues are discussed.

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 during the past year:

## 1. ACUTE MYELOID LEUKEMIA

Topic: Multi Drug Resistance in AML

Date: March 30, 1992

Coordinator: Bruce D. Cheson, M.D.

The purpose of this meeting was to assess acute myeloid leukemia (AML) as a model disease for clinical trials evaluating attempts at reversing multi-drug resistance (mdr). Participants included representatives from the pediatric and adult cooperative groups and major cancer centers. Presentations included data on assays of mdr, their limitations and feasibility in large scale trials; preliminary clinical data from adult and pediatric clinical trials were also presented. Of particular interest were data from SWOG correlating response to chemotherapy with the CD34+ phenotype. The session also included a discussion of the mdr-reversing drugs available for clinical trials and how they might best be evaluated and combined with other drugs. New agents worth considering were also mentioned and IDB staff were to evaluate the possibility of acquiring these agents for clinical trials.

## 2. HEAD AND NECK CANCER

Topic: Head and Neck Cancer

Date: March 31, 1992

Coordinator: Ellen G. Feigal, M.D.

Approximately 25 investigators from the major Clinical Trials Cooperative Groups, cancer centers, and the NCI convened to discuss the status of current trials and to identify and prioritize future trials activities that would require a collaborative effort. Discussion of the reasons for low accrual to a phase III trial of radiotherapy with or without concurrent chemotherapy in advanced nasopharyngeal cancer (INT-0099) resulted in several suggestions to improve patient enrollment on the trial. Future phase III studies in resectable head and neck cancer were also discussed (INT-0034, a phase III study to determine the effect of combining chemotherapy with surgery and radiotherapy for resectable head and neck cancer had closed on 1/1/90), and the participants recommended that further analyses of the results from INT-0034 (along with biologic/basic studies of prognostic factors), and soon-to-be-completed phase II trials were needed before a definitive design could be proposed. A proposed Intergroup study of laryngeal preservation (RTOG 9111) was considered and the protocol was given general support to proceed. Additional issues that received attention included the use of prognostic parameters in trials; the control of treatment side effects; Intergroup chemoprevention trials, and the need for future strategy meetings in this disease.

## 3. CENTRAL NERVOUS SYSTEM MALIGNANCIES

Topic: CNS Clinical Trials

Date: May 18, 1992

Coordinator: Richard S. Kaplan, M.D.

The status of brain tumor protocols, and the "state-of-the-art" was discussed at a preliminary Strategy Meeting held in San Diego in May 1992 and attended by investigators from each of the Groups with CNS tumor trials. Issues addressed were:

1) a potential Intergroup effort in anaplastic astrocytomas (AA)
2) new pilot studies in glioblastomas
3) the proposed Intergroup (CCG/POG) medulloblastoma trial
4) a potential CNS germ cell tumor Intergroup protocol
5) how to design trials of stereotactic radiosurgery
6) possibilities of protocols for leptomeningeal metastases
7) issues of measuring CNS tumor response objectively and/or development of clinical common response criteria

A consensus was reached that an appropriate plan for national coordination of glioma clinical trials would include a sequence of cooperative or Intergroup protocols for anaplastic astrocytomas and oligodendrogliomas, with continued institutional or single-group Phase II studies in glioblastoma patients (items 1 and 2 above). Item 3 was resolved in a special strategy meeting involving CCG and POG (see below).

A day-long Strategy meeting with a somewhat larger group of major participants in CNS clinical trials is scheduled for Bethesda in September, 1992 and will attempt to formulate the details of an AA/oligo trial, as well as possible plans for items 4 and 5 above.

## 4. EWING'S SARCOMA

Topic: Planning for Phase III Ewing's Sarcoma Clinical Trial

Date: March 19, 1992

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

The primary objective of the meeting was to discuss plans for a successor study for the current Intergroup Phase III Ewing's sarcoma study. Representatives of the POG and CCG Bone Tumor Committees were present, in addition to CTEP staff. The major terapeutic question of interest to both Groups is whether increasing the dose intensity of therapy will improve outcome for patients with Ewing's sarcoma. The two Groups differ, however, in their preferred strategies for achieving increases in dose intensity. POG investigators prefer to increase the dose of cytotoxic agents while maintaining the current interval between treatment courses, while CCG investigators are pursuing the strategy of increasing dose intensity by decreasing the interval between courses while maintaining the current doses of

cytotoxic agents. A conclusion of the meeting was that additional pilot data is needed to fully evaluate the feasibility of the two approaches, and a plan was developed for obtaining this pilot data.

**5. WILMS' TUMOR**

Topic: Stage III/IV Wilms' Tumor: Therapeutic Options for Clinical Investigation

Date: May 17, 1992

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

The primary objective of the meeting was to discuss possible questions of therapy for patients with Stage III and IV Wilms' tumor, in anticipation of a future Wilms' tumor clinical trial following NWTS-4. Representatives of POG, CCG, NWTS, and CTEP were in attendance. The activity and toxicity of new agents for Wilms' tumor were discussed, with particular emphasis on the possible use of ifosfamide (either alone or combination with other agents). Data presented at the meeting indicated a relatively high incidence of nephrotoxicity associated with ifosfamide therapy, particularly for younger patients and for patients with pre-existing renal abnormalities. The consensus was that it would be difficult to use ifosfamide as a frontline agent for a population with an 80% survival rate, but that ifosfamide regimens could be considered for patients in relapse. The preferred emphasis for a future study by those in attendance was towards attempting to identify biologic factors associated with poor outcome.

**6. CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

Topic: Planning for ALL Risk Classification Workshop

Date: May 18, 1992

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

The primary objective of this meeting with investigators conducting large pediatric Acute Lymphoblastic Leukemia (ALL) clinical trials was to discuss the need for more uniformity in the assignment of risk classification for children with ALL. Those in attendance (from POG, CCG, St. Jude, Dana Farber, and the Pediatric Branch, NCI) agreed with the need for a more uniform risk classification scheme and initial plans were made for a CTEP-Sponsored Workshop in early 1993 to address this issue. The Workshop will make use of a "Consensus Panel" composed of several representatives from POG and CCG, as well as a representative from the Dana Farber and from St. Jude Childrens Research Hospital and the Pediatric Branch, NCI. The recommendations of the "Consensus Panel" will then be considered by the respective ALL Committees of each Group.

## 7. MEDULLOBLASTOMA

Topic: Development of a Phase III Clinical Trial for Patients with Low-Risk Medulloblastoma

Date: June 17, 1992

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

The primary objective of this meeting was to discuss plans for an Intergroup collaboration to study the role of chemotherapy for children with low-risk medulloblastoma. Representatives of the POG and CCG Brain Tumor Committees, as well as CTEP staff, attended the meeting. Both POG and CCG had submitted Concept Proposals for this patient population. The CCG proposal was to evaluate whether a reduction in the dose of craniospinal radiotherapy could be accomplished, without increasing the incidence of isolated neuroaxis relapse (with all patients receiving chemotherapy). The POG proposal was to evaluate the ability of chemotherapy to improve outcome when given with standard dose radiotherapy. The compromise question of therapy accepted at the meeting was to compare standard-dose radiotherapy to reduced-dose radiotherapy plus chemotherapy. Thus, the question is essentially whether the addition of chemotherapy will permit a reduction in the dose (and result in a reduction of the toxicities) of craniospinal radiotherapy.

## 8. GASTROINTESTINAL MALIGNANCIES

Topic: Colon Adjuvant Trials

Date: December 6, 1991; May 19, 1992

Coordinator: J. Michael Hamilton, M.D.; Richard S. Kaplan, M.D.

Pending results of the last generation of colon adjuvant trials (NSABP C-05, NCCTG-894651, and INT-0089), two GI Tumor Strategy meetings were held, a day long meeting in Bethesda in 12/91 and a morning meeting in San Diego in 5/92. Plans were developed for three new Intergroup colon adjuvant trials which will be discussed below (see gastrointestinal malignancies, plans).

## 9. CANCER SURGERY

Topic: Surgical Issues in Cancer Clinical Trials

Date: December 13, 1991

Coordinators: J. Michael Hamilton, M.D.
Edward L. Trimble, M.D., M.P.H.

Historically, surgeons have played a leading role in cancer treatment, often making the initial diagnosis of cancer, initiating primary therapy, and making referrals for adjuvant therapy. Although there have been significant exceptions in the fields of pediatric, gynecologic, and breast surgery, in recent years surgeons have not participated in clinical trial design and

conduct to the fullest extent possible. This meeting brought together surgical representatives from the Cooperative Groups, the American College of Surgeons, the Society of Surgical Oncology, and various surgical subspecialty organizations to determine ways to enhance surgical participation in clinical cancer research. Follow-up has included closer attention to surgical issues in protocol review at CTEP, a discussion with the CCIRC regarding the need for effective surgical quality control and guidance in Cooperative Group research, and an effort to put together standard guidelines for surgical procedures.

**10. OVARIAN CANCER**

Topic: Phase III Trials of Taxol

Date: October 18, 1991

Coordinator: Edward L. Trimble, M.D., M.P.H.

Phase II studies documented a high response rate to taxol in patients with persistent or recurrent ovarian cancer. As yet, the role of taxol in the primary treatment of patients with epithelial ovarian cancer has not been defined. Accordingly, CTEP brought together representatives of the GOG, SWOG, ECOG, NCCTG, and NCIC to discuss potential phase III trials incorporating taxol in patients with optimally and suboptimally debulked ovarian cancer. These discussions resulted in two Phase III trials, GOG 114 and GOG 132, which are currently open to patient enrollment.

**11. PROSTATE CANCER**

Topic: Early Stage Prostate Cancer Trials

Date: March 3, 1992

Coordinator: F. Andrew Dorr, M.D.

The purpose of this meeting was to discuss designs for randomized studies evaluating hormonal therapies in patients with A1-C prostate cancer and those with elevated PSA following surgery or radiation for these early stages. Trials discussed included a proposed Mayo Clinic study of post-prostatectomy patients who develop an elevated PSA with randomization to hormonal therapy either initially or at the time of symptomatic progression; ECOG may participate in this study. ECOG is also planning a trial in patients with resected Stage B disease in which the randomization will be to antiandrogen alone (flutamide or Casodex) or to no adjuvant therapy. SWOG discussed a possible broad study of combined androgen blockade in patients with A-D disease. The RTOG participated in the meeting but already have ongoing studies for this group of patients.

## FUTURE STRATEGY MEETINGS

1. Non-Hodgkin's Lymphoma
2. Non-small Cell Lung Cancer
3. Wilms' Tumor
4. ALL Risk Classification Workshop
5. Pediatric Phase I Studies
6. Hepatoblastoma
7. Anaplastic Astrocytoma/Oligodendroglioma
8. Rectal Cancer
9. Cancer Surgery

## 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 407 active protocols in the CIB Hypothesis Database as of July 14, 1992; 134 of these (33% of all studies, 40% of treatment studies) have laboratory correlates. Of this 101, 38 (28%) are phase II, 1 (1%) phase II/III and 38 (28%) phase III. The remaining 57 (43%) are non-therapeutic protocols. Some examples are:

1. SWOG is evaluating multipdrug resistance (mdr)-reversing agents in the treatment of patients with refractory AML, CML in blast crisis, and non-Hodgkin's lymphomas. Laboratory studies are correlating response with in vitro assays of mdr.

2. SWOG is evaluating the importance of the CD34+ phenotype in adult AML.

3. CALGB and SWOG continue their evaluation of cytogenetics and immunophenotyping in acute leukemias. CALGB is now selecting various risk populations for more specifically directed therapeutic approaches.

4. ECOG is evaluating a variety of in vitro characteristics of myeloma cells for their prognostic importance.

5. Both ECOG and CALGB are assessing the frequency and prognostic significance of the bcl-2 oncogene in follicular NHL.

6. SWOG and ECOG are performing flow cytometric analysis and HER-2NEU expression on large numbers of women with breast cancer to determine prognostic importance in prospective trials. NSABP is also evaluating DNA histograms by flow cytometry in their large trials.

7. SWOG is establishing a solid tumor tissue bank as a repository for future molecular and biologic studies on patients treated on SWOG protocols.

8. The CCG is acquiring samples for cytogenetic and molecular biologic studies on pediatric astrocytomas.

9. Both CALGB and ECOG are evaluating a variety of biologic characteristics of colorectal cancers, including cytogenetics, oncogene expression, and laminin binding proteins.

10. GOG is studying oncogene expression in ovarian cancer.

11. SWOG investigators have data to suggest the importance of flow cytometry as a prognostic factor in head and neck cancer. This is now being studied prospectively in current and planned clinical trials.

## IV. SPECIFIC PROGRAM ACCOMPLISHMENTS

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

### PEDIATRIC MALIGNANCIES

#### Accomplishments

The Pediatric Oncology Group reported major advances in the treatment of children with mature B-cell malignancies. The POG-8616 study was a randomized trial for Stage III diffuse, small non-cleaved cell lymphoma in which patients were randomized to either the "Total B" regimen or to a less intensive regimen predominantly using cyclophosphamide and high-dose methotrexate infused over one hour (Regimen A). Initial analysis shows that outcome for Stage III patients has been very good, with 2-yr EFS of 78% for 57 patients receiving the "Total B" regimen, compared to 64% for patients receiving Regimen A. Thus, the "Total B" regimen represents a new standard of care for this patient population. The POG-8617 study is an on-going single-arm clinical trial for Stage IV/B-ALL patients that uses a regimen very similar to the "Total B" regimen of POG-8616. Initial analysis shows very good outcome for Stage IV patients (75% 2-yr EFS) and less good for B-ALL patients (60% 2-yr EFS), but with poorest outcome for patients presenting with CNS disease (only 10 of 21 patients in continuous CR). These results represent a dramatic improvement for patients with B-ALL, who in previous studies had EFS of < 30%.

The Pediatric Oncology Group reported results of a study for infants with unresectable, disseminated neuroblastoma in which therapy was based on the DNA index of the tumors. Comparing those infants with hyperdiploid tumors to those with diploid tumors, the CR/PR rate to induction therapy was significantly higher for the hyperdiploid group (96% versus 77%), as was the 3-yr RFS (91% versus 59%). The important findings of the study were that: (1) infants with hyperdiploid tumors do well with limited therapy; (2) DNA content and N-myc oncogene expression are independent predictors of outcome for these infants; and (3) clinicopathologic staging is not as important as biologic staging for this patient population.

The Children's Cancer Group reported significantly improved outcome for children with ALL presenting with lymphomatous features (a cohort predominantly composed of children with T-cell ALL). While previous CCG studies for this patient population had a 4-year EFS of 36%, the recently analyzed CCG-123 study had an overall EFS of 60% at 72 months. Of the 4 regimens tested, the two more intensive regimens had significantly better outcome (approximately 70% 4-year EFS) compared to the two less intensive regimens (approximately 50% 4-year EFS). The improved outcome with the more intensive regimens served as the basis for the current study, which seeks to further improve outcome by additional increases in the intensity of therapy.

A preliminary report of the Intergroup Rhabdomyosarcoma Study (IRS) III noted overall improvement in outcome compared to IRS-II (75% versus 67% 3-year survival and 67% versus 58% 3-year PFS). The major improvement in IRS-III was for Group III patients (i.e., patients with residual, localized disease following primary resection). Patients with specific pelvic primary tumors (i.e., bladder/prostate) had the greatest improvement in comparison to IRS-II (79% versus 58% 3-year PFS and 59% versus 25% bladder salvage rate). Other Group III primary sites had improved PFS to 65-70% on IRS-III, compared to approximately 55% on IRS-II. This improvement may reflect the more intensive chemotherapy received by IRS-III patients. An additional important finding on the IRS-III was that patients having parameningeal primary tumors without intracranial extension do well with radiotherapy to the tumor volume and do not require whole brain radiotherapy.

The National Wilms' Tumor Study reported additional detailed findings and analysis from the NWTS-3 study. For patients with Stage II and III favorable histology (FH), no significant differences in outcome were noted for the abdominal radiotherapy randomizations (0 versus 2000 cGy for Stage II patients and 1000 versus 2000 cGy for Stage III patients), supporting the NWTS-4 study which uses no radiotherapy for Stage II-FH patients and 1000 cGy for Stage III-FH patients. The most significant prognostic factors derived from the NWTS-3 study for Stage II and III patients were age (young age favorable), lymph node involvement, and tumor at the margin of resection. For patients with Stage I disease, tumor size was the best predictor of relapse.

Important studies better defining the late effects of therapy for children with cancer were reported in the past year. The Children's Cancer Group described second neoplasms developing from the nearly 10,000 children treated for ALL between 1972 and 1988 (median follow-up 4.7 years). Although the overall incidence of second malignancies was low (estimated cumulative proportion was 2.5% at 15 years), a relatively high incidence of central nervous system (CNS) tumors developed in the group of children who had received CNS radiotherapy (representing 24 of the total 43 second neoplasms). Children less than 5 years of age receiving radiotherapy were at highest risk for development of CNS tumors. The Intergroup Rhabdomyosarcoma Study Committee reported on the late effects of therapy for children with paratesticular rhabdomyosarcoma. A variety of sequelae were reported (including bowel obstruction, loss of ejaculatory function, and gonadal dysfunction), and based on these data specific changes in standard therapy were incorporated into the current IRS study.

A number of important pediatric Phase I investigations have been initiated and/or completed during the past year. Particularly promising results were reported by CCG investigators for the combination of fludarabine followed by cytarabine (both given by continuous infusion). Although the primary objective of the study was to determine the maximum tolerated dose and dose limiting toxicity of the combination, a significant number of responses were seen: 10 CR in AML (16 evaluable patients) and 5 CR in ALL (10 evaluable patients). The CCG, in collaboration with the Pediatric Branch, NCI, completed a Phase I evaluation of topotecan given as a 24 hour infusion, and the POG is beginning a Phase I study of topotecan given on a daily X 5 schedule. POG is nearing completion of its Phase I evaluation of taxol for children with solid tumors, and the CCG leukemia study of taxol is on-going. Phase I evaluations of toxin-conjugated monoclonal antibodies for patients with leukemia (B4-blocked ricin and XomaZyme-H65) are proceeding.

Plans

An Intergroup collaboration to evaluate the role of chemotherapy and reduced dose radiotherapy for children with low-risk medulloblastoma is to open in the coming year. Patients will be randomized between standard therapy (5400 cGy to the posterior fossa and 3600 cGy craniospinal) and an experimental regimen combining chemotherapy with reduced-dose radiotherapy (only 2400 cGy craniospinal). Extensive toxicity monitoring will be performed to test the hypothesis that reduction in craniospinal dose will reduce the CNS sequelae associated with standard dose radiotherapy.

A second major Intergroup collaboration to be initiated in the coming year is a Phase III trial for children with osteosarcoma. Using a factorial design, the benefit of adding ifosfamide (given with doxorubicin) and of using the macrophage activator MTP-PE will be investigated in the context of standard therapy employing cisplatin, doxorubicin, and high-dose methotrexate.

The Pediatric Oncology Group will initiate a follow-up study to its recent successful studies for children with diffuse, small non-cleaved cell lymphoma and B-ALL. The new study will maintain the intensive therapy of the previous study, and will investigate the benefit of adding ifosfamide/etoposide to this standard therapy, and will also compare the relative efficacies of continuous infusion versus 3-hour infusion high-dose cytarabine. The Pediatric Oncology Group will also initiate a Phase III trial for patients with lymphoblastic lymphoma. The study will use a factorial design to compare standard-dose cytarabine to high-dose cytarabine (both given by continuous infusion), and to compare the relative efficacies of the etoposide/cytarabine combination to the intermediate dose methotrexate/intravenous 6-mercaptopurine combination.

An important CCG pilot study that anticipates the next randomized comparison for children with AML combines the fludarabine/cytarabine combination (that showed such high activity during Phase I evaluation) with the anthracycline idarubicin. Assuming that toxicity and preliminary response data are acceptable, this three drug combination would be compared with a standard two drug combination of cytarabine and idarubicin.

Several investigational agents will enter Phase II evaluation for specific pediatric tumors in the coming year. The activity of taxol will be evaluated for a variety of pediatric malignancies. Phase II evaluation of topotecan (particularly for CNS tumors and rhabdomyosarcoma) will be also begin. The evaluation of the iron chelator, desferrioxamine (DFO), for neuroblastoma is also anticipated, and will follow-up on very interesting preclinical data showing a DFO effect on tumor cell growth and n-Myc expression.

**Pediatric Research Program Initiatives**

During the past year, an RFA for "Phase I Trials of New Cytotoxic and Biologic Agents in Children" (RFA CA-91-22) was issued, two applications (from POG and CCG) responding to the RFA were peer-reviewed, and funding for the applicants was approved. A funding plan for each awardee was developed, and the cooperative agreements will be activated in August, 1992.

The Pediatric Section, CIB has also worked on a number of initiatives relating to the use of investigational agents for pediatric clinical trials, including:

A. Collaboration with Investigational Drug Branch (IDB) staff in the development of all-trans retinoic acid as an oncologic agent, with specific emphasis on the problems of altered pharmacokinetics associated with chronic dosing.

B. Collaboration with IDB and Biometrics Research Branch staff in development of a monitoring plan for estimating the incidence of secondary AML following treatment with epipodophyllotoxins.

C. Collaboration with IDB and Pediatric Branch (COP) staff and with representatives of ENZON to assure availability of PEG-asparaginase for pediatric ALL clinical trials.

D. Collaboration with IDB staff and with representatives of Burroughs-Wellcome to assure availability of an intravenous formulation of 6-mercaptopurine for pediatric leukemia trials.

E. Collaboration with IDB and other NCI staff in pre-clinical development of the alkyltransferase inhibitor, $O^6$-benzylguanine.

**GENITOURINARY CANCERS**

Testis Cancer

**Accomplishments**

Accrual has been completed to a randomized study comparing VIP versus BEP chemotherapies in the treatment of patients with poor risk germ cell tumors. This ECOG study should be ready for analysis by mid-1993.

Memorial Sloan-Kettering (MSKCC), together with SWOG, completed accrual to a study comparing etoposide + cisplatin (EP) versus etoposide + carboplatin (EC). This study was being done to evaluate whether EC, a less toxic regimen,

had efficacy equivalent to EP. Unfortunately, patients treated with EC were found to have a higher relapse rate than patients treated with EP.

Plans

A subsequent study is planned comparing EP vs EC which will utilize more frequent dosing of EC and administer carboplatin using a pharmacokinetically guided dosage (AUC target of 5). This has been associated with a very impressive response rate in a study at the Royal Marsden Hospital. This study comparing EC vs EP will likewise be done at MSKCC with SWOG and will continue to examine the rate of marker decline as a predictor of response for this good risk population. In addition other tumor markers will be evaluated including i(12p) and p53.

Taxol will be evaluated in the treatment of patients who have failed initial and salvage chemotherapy for metastatic germ cell tumors.

A replacement study is being developed by ECOG for poor risk patients pending the analysis of BEP vs VIP. The specific design for this study is not finalized.

Retinoids are being evaluated by Indiana University and by MSKCC in patients with the growing benign teratoma syndrome following treatment for germ cell cancer.

High-dose chemotherapy continues to be evaluated in non-randomized studies for patients failing primary and salvage chemotherapy.

## Prostate Cancer

Accomplishments

A Phase I study of Suramin has been completed which employs a more feasible schedule of administration than that used previously, and which perhaps achieves better efficacy in patients with hormone-refractory prostate cancer.

Accrual continues to be brisk on the large 1250 patient study of orchiectomy + flutamide vs orchiectomy + placebo in patients with newly diagnosed D1 or D2 prostate cancer. Accrual also continues on early stage adjuvant radiation and adjuvant hormonal therapy studies in ECOG, RTOG and SWOG.

The RTOG has completed two adjuvant hormonal therapy studies. One study compared 2 months of complete androgen blockade (CAB) before radiation therapy and 2 months of CAB during radiation versus radiation alone in patients with bulky primary lesions. Thus far few results are availabe from the study as it is early in follow up but it appears that time to recurrence is delayed in patients receiving this short course of hormonal therapy. The other RTOG adjuvant trial has examined adjuvant hormonal therapy following radiation to radiation alone. No results are available from that study.

Studies at several institutions recently have suggested that the combination of estramustine phosphate + vinblastine may be reasonably effective in patients with hormone refractory prostate cancer.

Taxol was not found to be effective in patients with hormone refractory disease

**Plans**

The Mayo Clinic is developing a trial of early versus delayed hormonal therapy for patients who develop an elevated PSA following radical prostatectomy. ECOG is planning to evaluate the efficacy of adjuvant anti-androgen (Casodex) versus placebo in patients with resected Stage B prostate cancer.

A randomized study comparing suramin to some other therapy or therapies for hormone refractory prostate cancer will be started this year.

ECOG is planning a comparison of the combination of estramustine plus vinblastine versus vinblastine alone, as well as further exploring the utility of PSA as a marker of tumor response.

New agents, including all-trans retinoic acid, will continue to be evaluated. The initial patient treated with this differentiating agent responded.

## Bladder Cancer

**Accomplishments**

SWOG has completed two studies of superficial bladder cancer. One compared BCG induction alone versus BCG induction followed by maintenance BCG. Patients receiving maintenance BCG had far fewer recurrences than those who did not. SWOG also compared BCG vs mitomycin given intravesically and found BCG, in an interim analysis, to be much more effective in the prevention of recurrences.

In locally advanced bladder cancer, approximately 170 patients have been accrued to the intergroup neoadjuvant MVAC + cystectomy vs cystectomy trial. The accrual goal is 299 patients. The RTOG is also accruing to an adjuvant chemotherapy study in which all patients receive radiation therapy as the primary treatment of their bladder tumor.

Ifosfamide has been shown to be an active agent in the treatment of bladder cancer.

**Plans**

Gallium nitrate has shown some promise in a small trial and will be explored as a single agent and in combination with ifosfamide.

A pilot trial by Don Lamm has suggested that patients treated with several vitamins in addition to BCG for superficial bladder cancer have a lower risk of recurrence. ECOG plans a study to evaluate this hypothesis.

## Renal Cell Cancer

### Accomplishments

IL-2 was approved by the FDA this year for the treatment of renal cell cancer.

ECOG and SWOG completed an intergroup study comparing nephrectomy alone with nephrectomy followed by adjuvant interferon. Results of that trial should become available in the coming year.

### Plans

ECOG is planning a trial of autolymphocyte therapy versus interferon alone versus interferon + IL-2. A three-arm trial will be undertaken only if additional participants can be recruited. SWOG is planning an adjuvant therapy trial in nephrectomized patients of IL-2 + interferon versus no adjuvant therapy. The no treatment control arm will depend on the analysis of the ECOG trial of adjuvant interferon alone.

## Melanoma

### Accomplishments

ECOG is nearing a final (publishable) analysis of their adjuvant interferon study in which patients were randomized to one year of high-dose interferon versus no adjuvant therapy. In their current trial, patients with resected melanoma are randomized to either no adjuvant therapy, one year of high-dose therapy or to two years of lower dose interferon. This study has accrued well but is unlikely to complete accrual before the final results of the previous trial are available. If interferon is shown to be dramatically effective, then the no treatment control arm of the current study will likely be closed.

The intergroup study comparing 2 vs 4 cm margins of excision and node dissection versus no node dissection in patients with intermediate thickness melanoma (1-4 mm) was closed to accrual this year. The first public analysis of this trial should become available in the next year.

In metastatic melanoma, ECOG has undertaken a trial of DTIC alone, DTIC + tamoxifen, DTIC + interferon, or DTIC + interferon + tamoxifen.

### Plans

SWOG is planning a trial to evaluate a melanoma vaccine (Melacine) as an adjuvant in patients with thin primary melanomas (<1cm). New agents being studied include new cytotoxic therapies, monoclonal antibodies, and biologic response modifiers.

## BREAST CANCER

### Accomplishments

Ongoing trials in the adjuvant setting include evaluation of dose intensification, chemoendocrine therapy, different combinations of chemotherapy, treatment sequencing, the role of adjuvant tamoxifen in DCIS patients, and the value of combined endocrine therapy in the elderly. Two of the dose intensification studies are evaluating doses which require autologous bone marrow rescue.

Accrual has completed to several adjuvant and metastatic breast cancer trials over the past year. One ECOG trial comparing CAF vs CAF alternating with Thiotepa-Adriamycin-Vinblastine-Halotestin (TsAVbH) in metastatic breast cancer will be analyzed and published in the coming year. In addition, patients responding on this trial and others were randomized to maintenance chemotherapy or no maintenance chemotherapy to help answer the question whether continued chemotherapy is of use in women with metastatic breast cancer who achieve a response to induction chemotherapy.

SWOG and ECOG and NCCTG have nearly completed accrual to their study of node negative breast cancer in which patients are randomized to receive CAF, CMF, CAF + tamoxifen or CMF + tamoxifen. Over 4000 patients have been registered on this study with over 2000 having been randomized (many were not eligible for randomization).

NSABP has completed accrual to its study of preoperative versus postoperative chemotherapy. A replacement study will be developed in the next several weeks (a planning meeting is scheduled for July 28, 1992). NSABP also began its new dose intensification of adriamycin + cyclophosphamide as adjuvant therapy in women with node positive breast cancer. Another new NSABP study has begun for women with ductal carcinoma in situ in which patients undergo lumpectomy and radiation and are then randomized to tamoxifen or placebo.

CALGB has reported initial results of its dose intensification trial in which patients receiving the higher dose chemotherapy have a superior disease-free survival and survival. The high-dose therapy in this study, however, was far lower than more recent studies of dose intensification; although this study demonstrates that there is a dose-response relationship in early stage breast cancer, it does not fully define that relationship.

Taxol has been shown to be a very effective drug in the treatment of metastatic breast cancer, with two trials showing there to be a response rate in the range of 60%. Two trials combining adriamycin with taxol have both reported modestly higher response rates with the combination.

The Illinois Cancer Council has completed a Phase I study of tamoxifen plus fenretinide demonstrating that they can be combined reasonably safely up to a dose of fenretinide of 400 mg/day.

Other new drugs which have been shown to be active in breast cancer include edatrexate and piroxantrone. Edatrexate in particular is of interest as it had an approximate 40% response rate in its initial Phase II study.

Plans

Several trials will be done in the coming year in which taxol will be combined with other cytotoxics effective in the treatment of breast cancer. These include cytoxan, cisplatin, and 5-FU + leucovorin. A phase III comparison of taxol versus adriamycin versus taxol combined with adriamycin will be done by ECOG. SWOG is planning a comparison of taxol vs vinblastine in patients who have progressed on front-line chemotherapy for metastatic disease. CALGB will begin a comparison of bolus taxol vs taxol infusions (3 hour versus 24 hour) and of lower versus higher doses of taxol. The NSABP will conduct a pilot of taxol in combination with some other cytotoxic drug or drugs with the possibility of using this combination in their next adjuvant trial.

There is intense interest in evaluating fenretinide in low-risk node negative breast cancer and in women with ductal carcinoma in situ. This is based on in vitro and in vivo animal data suggesting synergy between tamoxifen and fenretinide. The design of both trials would be tamoxifen + placebo vs tamoxifen + fenretinide. A pilot trial of the combination will be done at the NCI Clinical Center to further evaluate the toxicity of the two drugs together and to explore potential synergy in patients with advanced disease. Fenretinide is not currently available for the large randomized trials and will probably not be available for at least one year.

ECOG and SWOG are considering an evaluation of chemotherapy versus zoladex $\pm$ tamoxifen in premenopausal women with node negative receptor positive breast cancer. The rationale for this study is based on the finding of the adjuvant therapy overview that ovarian ablation produced a reduction in risk of recurrence and death of a magnitude similar to chemotherapy. An ongoing trial is comparing chemotherapy alone versus chemotherapy + zoladex versus chemotherapy + zoladex + tamoxifen.

Additional trials exploring other aspects of dose intensity considerations are under development by SWOG/ECOG and by CALGB.

SWOG will continue to collect tumor samples from patients with node negative breast cancer to evaluate tumor markers so that patients' prognoses can be more accurately defined.

CIB staff will coordinate a conference on breast cancer in premenopausal women, focusing on reproductive, screening, and treatment issues. This is planned for November, 1992.

**GYNECOLOGIC MALIGNANCIES**

Ovarian Cancer

Accomplishments

As noted elesewhere, CIB hosted a strategy planning meeting to determine the evaluation of taxol in the primary treatment of ovarian cancer, resulting in two Phase III trials evaluating taxol as a component of therapy in patientw with optimally and suboptimally debulked disease. In addition, CTEP has initiated a compassionate-use taxol protocol for patients with ovarian cancer. To date, over 1000 patients have been enrolled on this protocol. CTEP

representatives participated in a workshop on early ovarian cancer sponsored by the Organs Systems Coordinating Branch of the Division of Cancer Biology, Diagnosis and Centers. CIB representatives helped plan a workshop on ovarian cancer in older women, jointly sponsored by the American Cancer Society, the National Institute on Aging, and the National Cancer Institute.

Plans

CTEP has worked with the Cooperative Groups to design a trial of taxol dose-intensity in patients with refractory ovarian cancer. This trial, which will be coordinated by the GOG, will open in summer, 1992. CTEP is working closely with the GOG and SWOG to evaluate the role of high-dose chemotherapy with autologous bone marrow support in patients with ovarian cancer. CTEP representatives are working with the GOG and the NIA to design a protocol which will examine the impact of age on initial surgical treatment, entry onto clinical trials, and intentions for adjuvant chemotherapy in patients with newly diagnosed ovarian cancer.

## Endometrial Cancer

Accomplishments

Phase II trials of both whole abdominal radiotherapy and circadian timed chemotherapy have demonstrated signficant response in patients with advanced endometrial carcinoma.

Plans

CIB is working with the GOG to evaluate whole abdominal radiation versus circadian-timed chemotherapy in patients with advanced, persistent, or recurrent endometrial cancer. In addition, the GOG also plans a prospective study of estrogen replacement therapy with patients with early stage endometrial cancer.

## Cervical Cancer

Accomplishments

Several trials of chemosensitizing agents used as adjuvants to radiotherapy in the treatment of advanced cervical cancer are expected to mature within the next 12 months. In addition, investigators at M.D. Anderson and Guadalajara have demonstrated significant response to oral retinoic acid and subcutaneous interferon in patients with advanced cervical cancer.

Plans

Both SWOG and the GOG plan to open larger trials evaluating the interferon/retinoic acid regimen. Based on these results, as well as those of radiosensitizers, CIB plans to work with the GOG, SWOG, and RTOG in designing the next generation of trials for patients with advanced cervical cancer.

Vaginal Cancer

Accomplishments

CTEP representatives helped plan, and participated in, an NIH workshop on long-term effects of exposure to diethylstilbestrol (DES), held April 22-24, 1992.

Plans

To implement some of the recommendations arising from this conference, CTEP has worked to set up phase II trials of chemotherapy in patients with recurrent vaginal clear cell carcinoma, to ascertain epidemiologic evidence of DES carcinogenesis through the Cooperative Groups, and to draft recommendations for health-care practitioners on appropriate screening guidelines for women exposed to DES.

Vulvar Cancer

Accomplishments

The GOG has completed a prospective study of modified radical vulvectomy in the treatment of vulvar melanoma. As the results of this study mature, it should be possible to draft recommmendations on the optimal surgical treatment of this entity.

Plans

CTEP is working with the GOG on the design of a large study to evaluate the role of adjuvant radiotherapy and chemotherapy with patients with "poor prognosis" primary vulvar carcinoma, as well as those with metastatic disease in the inguinal lymph nodes. In addition, CTEP is encouraging prospective evaluation of the role of the HPV virus in a companion study to this trial.

ADULT MALIGNANT LYMPHOMA

Accomplishments

SWOG completed its 1300 patient comparison of CHOP vs MACOP-B vs m-BACOD vs ProMACE/CytaBOM demonstrating no difference among the arms except for less expense and toxicity with CHOP.

Plans

CALGB is about to compare the drug combination CHOPE at maximal doses with CHOPE at higher doses with growth factor support in patients with intermediate grade NHL. ECOG is planning a similar study using ProMACE/CytaBOM.

CALGB is planning a study of 2-chlorodeoxyadenosine in patients with follicular lymphomas to confirm preliminary data from Scripps.

SWOG is planning the first large scale evaluation of the therapy and biology of Waldenstrom's macroglobulinemia.

Both ECOG and SWOG are preparing to activate studies to evaluate the role of ABMT in multiple myeloma.

**ADULT LEUKEMIA**

**Accomplishments**

An international intergroup study demonstrated the superiority of DCF over alpha-IFN in patients with previously untreated hairy cell leukemia.

An international study is comparing fludarabine with chlorambucil with the combination of the two agents in previously untreated CLL.

CALGB demonstrated the improved efficacy of dose intensity during consolidation in patients with AML.

In contrast, SWOG demonstrated that high dose ara-C as an induction regimen is more toxic and no more effective than the drug given at conventional doses.

The comparison of allogeneic BMT, ABMT and consolidation therapy in adults with AML is accruing well.

**Plans**

An intergroup study has been activated comparing standard chemotherapy with all-trans retinoic acid for remission induction in newly diagnosed patients with APL.

CALGB is evaluating the role of hematopoietic growth factors in ameliorating treatment-related toxicities in elderly patients with AML.

CALGB is planning to evaluate the cross-resistance between CDA and fludarabine in patients with CLL.

CALGB is evaluating whether the use of G-CSF during induction therapy of ALL reduces myelotoxicity and, therefore, permits a higher CR rate.

SWOG is preparing to activate a front-line study in AML in which cyclosporine will be used to prevent the development of mdr.

**MALIGNANT BRAIN TUMORS**

**Accomplishments**

Cooperative Groups are investigating several fundamental questions in treatment of high-grade malignant brain tumors:

The Brain Tumor Cooperative Group has accrued 235 of a planned 350 patients to an important randomized controlled trial (8701) that will define the role of $^{125}I$ interstitial brachytherapy boost, a promising technology for delivering higher doses of radiation to address the high rate of recurrence of malignant astrocytomas at their original sites. Another trial (8901), which should

complete accrual in the next year, is investigating the role of intra-arterial cisplatin to augment the results of initial therapy with external radiation and conventional IV BCNU chemotherapy.

At the same time, the RTOG (9006) has been studying techniques of hyper-fractionated radiation in high-grade gliomas and has accrued approximately half of a planned 564 patients; SWOG (9016) has piloted the combination of IV BCNU + cisplatin simultaneously with radiation; NCCTG (88-72-52) has entered about 2/3 of the 290 patients required for their randomized trial of the addition of $\alpha$-interferon to radiation and BCNU, and is also assessing the importance of 9p deletions in these tumors.

Other major large scale initiatives in high-grade gliomas are two NCOG randomized trials, one (6G-90-1) that evaluates the use of the polyamine inhibitor DFMO with hyperfractionated radiation for glioblastomas, and the other (6G-90-3) looking at the radiosensitizers BUdR and IUdR along with multiagent chemotherapy for anaplastic astrocytomas. The MDAH (DM88-113) team is evaluating the combination of carboplatin and hyperfractionated radiotherapy. Numerous Phase II trials of new agents in recurrent gliomas are ongoing in the Groups, as well as in large Centers.

In low grade gliomas, NCCTG and ECOG are more than half through with a randomized comparison (86-72-51 and E2389) of higher versus lower doses of cranial radiation, but an Intergroup trial (BTCG 8730) of early versus delayed radiation has not been able to accrue sufficiently (seemingly because of widely polar clinical biases on this question) and will be discontinued.

For brain metastases, major randomized trials of radiosensitization by BUdR (RTOG 8905), hyperfractionation versus conventional fractionation (RTOG 9104), and radiation versus no radiation after resection of single metastases (INT-0124/SWOG 9021) are all accruing well.

A double-blind placebo controlled trial of the antiprogestational agent Ru486 in unresectable/recurrent meningiomas is now opening (SWOG 9005). Several Groups have ongoing pilot studies for primary CNS lymphomas.

**CNS Research Program Initiative**

Finally, a concept was developed for a major new cooperative clinical research effort in malignant gliomas that would serve two goals: a) to complete Phase II trials of high-priority new agents in rapid succession and b) to share brain tumor specimens for the collection of molecular and cellular biologic data that may ultimately be used for response prediction and to elucidate mechanisms of tumor response or progression. The structure would be a consortium of major brain tumor research programs, funded by a U10 mechanism. This concept was approved by the DCT Board of Scientific Counsellors in June and is being prepared as an RFA for FY93.

**Plans**

The most important goals for FY93 will certainly be related to organization of the two major initiatives described above: a network of centers for large Phase III trials in the intermediate grade gliomas (AA and oligodendroglioma), and a consortium for biologic studies and Phase II trials in glioblastomas.

This will give CTEP a matrix of complementary mechanisms for brain tumor studies ranging from innovative pilots to comparative trials of complex new technologies which will be accompanied by an expansion of the Strategic Planning efforts in CNS tumors.

Within these new structures it should also be possible to develop plans for appropriate trials in CNS lymphomas, germ cell tumors, meningiomas, and parenchymal and leptomeningeal metastases.

## GASTROINTESTINAL CANCERS

### Accomplishments

In colorectal cancers, two major colon adjuvant trials have been completed with Intergroup participation: NCCTG 89-46-51 evaluated 6 vs 12 months of 5FU/levamisole with or without leucovorin and had been designated a High-Priority Trial in 1990. 915 patients were accrued in just over 2 years. Initial analysis will be done after sufficient time has passed for followup.

Intergroup 0089 was activated in August 1988 and designated a High-Priority Trial in 1989. Its goal of 3475 patients will be reached in July or August of 1992. This study randomized patients to conventional 5FU/levamisole vs 5FU + leucovorin in either high- or low-dose regimens or the combination of 5FU/levamisole + leucovorin.

The other large Group effort in colon adjuvant therapy is the NSABP C-05 trial of 5FU + leucovorin ± $\alpha$-interferon which was activated in FY92 and should be completed next year. Pending results of this last generation of trials, two GI Tumor Strategy meetings were held, a day long meeting in Bethesda in 12/91 and a morning meeting in San Diego in 5/92. Plans were developed for three new Intergroup colon adjuvant trials which will be discussed below.

In the area of rectal adjuvant studies, the two major trials, NSABP R-02 and INT-0114, have continued to accrue well and should both complete accrual early in FY93. The former was designated a High Priority Trial in the first series and evaluates 5FU + leucovorin vs the MethylCCNU/vincristine/5FU combination, with or without regional radiation. The Intergroup study, a series III High Priority Trial, randomizes among 5FU ± levamisole ± leucovorin, all with 5FU/radiation starting in the ninth week. Planning for the next generation of studies is underway in parallel with the strategic planning for other GI sites.

Other GI tumors for which major trials have reached landmarks in the last year are:

Esophagus - INT-0113, a trial of neoadjuvant cisplatin + 5FU versus surgery alone in locoregional disease, which began recruitment from five Cooperative Groups, and INT-0123 for which the planning is being completed; this will evaluate various potential timings for the combined modality use of radiation and chemotherapy.

Gastric - INT-0116, a comparison of adjuvant chemoradiation to surgery alone in resected adenocarcinomas, was activated in 8/91.

Metastatic and Stage IV-NED Colorectal - INT-0103 and NCCTG 90-46-52, trials respectively of chemotherapy vs control and of two chemotherapy regimens after gross total resection of metastatic disease, began accrual. Additionally, three major Phase III randomized Group trials of various chemotherapy regimens for advanced metastatic disease have had rapid accrual and are approaching completion.

Plans

The three new Intergroup protocols developed at the GI Strategy meetings of 12/91 and 5/92 are:

INT-0130, a Phase III evaluation of the effect of regional radiation added to 5FU/levamisole adjuvant for patients with $T_{4b}$ (extension to adjacent structures) and poor prognosis $T_3$ colon tumors penetrating into the peritoneum

NCCTG-91-46-53, colon adjuvant trial of 5FU ± leucovorin + either conventional or high-dose levamisole in a new administration schedule developed under CTEP Phase II contract

Colon Intergroup (currently called E1292 or CR92-026) which will evaluate systemic perioperative chemotherapy (beginning within 24 hours of surgery) as a followup to a previous NSABP trial (C-02) that demonstrated that perioperative portal vein chemotherapy improved survival even though it did not reduce the number of liver metastases.

As these studies are getting underway, data should be forthcoming from adjuvant and other trials that have completed accrual more than a year ago. This information will be utilized in the planned GI strategy meetings in 12/92 and 5/93 to design the next iteration of rectal cancer and other protocols.

## HIV-ASSOCIATED MALIGNANCIES

The Clinical Investigations Branch sent a letter on 5/4/92 to all Pediatric and Adult Clinical Trials Cooperative Group Chairs encouraging the development of concepts and protocols in the area of HIV-associated malignancies. The areas of interest include non-Hodgkin's lymphoma (both peripheral and primary central nervous system), Hodgkin's Disease, Kaposi's sarcoma, and cervical and anal dysplasia and cancer. The purpose of the letter was to increase the menu of clinical trials in the NCI-supported Cooperative Groups involving the climbing numbers of individuals with HIV-associated malignancies.

## Kaposi's Sarcoma

### Accomplishments

The NCI as primary sponsor and NIAID recently (April, 1992) co-sponsored a program announcement (PA) in Kaposi's Sarcoma (KS). The PA was to encourage applications (R29, R01 and interactive R01s) from interested investigators to study innovative correlative laboratory studies of relevance to new or on-going AIDS-KS clinical trials, or to develop new therapies for the treatment of AIDS-KS with laboratory correlations. The PA was designed to promote collaborations and interactions among researchers from a variety of basic and clinical disciplines to facilitate better treatment and management of AIDS-KS patients.

## Angioinhibins

### Accomplishments

Recent discoveries suggested that neovascularization and angiogenesis were important in the pathogenesis of KS. Angioinhibins, a new class of drugs of which AGM-1470, an analog of fumigellin, is one, are agents that inhibit angiogenesis and new vessel growth, and thus may be useful in the treatment of KS.

### Plans

CTEP approved protocol T92-0041, from the intramural branch of the NCI, on 2/20/92, pending completion of additional preclinical studies. It is a pilot phase I study of the toxicity, pharmacokinetics, and activity of AGM-1470 in patients with HIV infection and Kaposi's sarcoma. Preclinical studies are in the final stages to determine a safe first dose in humans.

## Retinoids

### Accomplishments

Retinol (vitamin A) is a naturally occurring vitamin that is responsible for the normal growth, maturation, and maintenance of epithelium throughout the body. All-trans-retinoic acid (RA) is a retinol metabolite formed by intestinal oxidation of beta-carotene and from tissue metabolism of retinol and retinaldehyde. Clinically, retinoids have been used for many years to treat a variety of skin disorders. Due to their effects on epithelium, chemopreventive and therapeutic trials of retinoids against various epithelial tumors are being explored. Recent anecdotal reports indicate that RA may have activity against HIV-associated KS. L. Bonhomme and associates reported that topical application of RA to KS lesions in 8 HIV-infected patients resulted in clinical and histological improvement, and 7 patients obtained a PR (Ann. Oncol. 1991; 2:234-35). Gill and colleagues reported responses in HIV-associated KS upon oral administration (personal communication). There is conflicting data on the effects of RA on HIV replication in vitro. Drs. Poli, Fauci, and colleagues have noted that RA can decrease HIV replication in chronically infected monocytoid-like cell lines (VII Int. Conf. on AIDS,

Florence, Italy. 1991 abstract:159), but Turpin and Meltzer noted the stimulation of HIV expression in monocytes by RA with M-CSF (VII Int. Conf. on AIDS, Florence, Italy. 1991 abstract:106). Drs. Foli, Saville, and Yarchoan (unpublished observation) have also found that RA markedly increases HIV replication in monocytes exposed to M-CSF, but it does not have such an effect in the absence of M-CSF. They also found that the effectiveness of AZT in inhibiting HIV replication in these monocytes was not affected. There is both in vitro and in vivo evidence that the combination of RA and interferon-alpha may result in better responses than either agent alone. Lippman and associates recently reported a 68% response rate in 28 patients with cutaneous squamous cell cancer, 7 of whom had a CR (JNCI 84:235-241, 1992), and a 50% major response rate ($\geq$ 50% tumor regression) in 26 patients with locally advanced squamous cell cancer of the cervix (JNCI:84241-245, 1992).

**Plans**

CTEP reviewed and approved protocol T92-0037 on 2/20/92 from the intramural branch of the NCI to conduct a pilot/phase II study of the activity of all-trans-retinoic acid, both alone and in combination with interferon-alpha, administered to patients with HIV infection and Kaposi's sarcoma.

CTEP reviewed and approved protocol T92-0032 on 2/20/92 from New York University. It is a phase II study of interferon-alpha with all-trans retinoic acid in the treatment of AIDS-associated Kaposi's sarcoma.

CTEP reviewed on 2/13/92 Group C protocol I92-0001, a study of all-trans retinoic acid for therapy of refractory AIDS-related Kaposi's sarcoma.

In October 1991, CTEP approved two studies of single agent all-trans retinoic acid for the treatment of Kaposi's sarcoma, one at University of Southern California, and one from the Illinois Cancer Council. Both studies are actively accruing patients, and preliminary data should be available in the near future.

## AIDS Lymphomas

**Accomplishments**

In response to an NCI-issued RFA in 1991 to fund investigators pursuing innovative therapies with laboratory correlates in AIDS-associated lymphomas, or pursuing innovative laboratory studies for on-going clinical trials, 12 investigators were funded for a total of 3 million dollars per year over 3 years. The purpose of funding these investigators, who comprise the AIDS Lymphoma Network, was to facilitate phase I/II clinical studies with laboratory correlation including molecular and immunological parameters. The first meeting of this group was held in December, 1991 in Washington, D.C. The investigators presented their proposed plans, and discussed potential collaboration between the Network members. Grant recipients attending ASCO met on May 17, 1992. Participants discussed interim progress, and potential collaborative laboratory endeavors. The annual meeting of the AIDS Lymphoma Network has been scheduled to take place at the American Society of Hematology meetings in December, 1992 in Anaheim, CA.

The standard treatment for HIV associated NHL has not been established. A combination of chemotherapy, AZT, GM-CSF and pentamidine was studied at the NCI in patients with AIDS-associated NHL and resulted in complete responses in 4/10 patients, with a duration of response between 7-19 months. 8 of the 10 patients had grade 3-4 hematologic toxicity that may in part have been related to the AZT.

**Plans**

CTEP reviewed and approved protocol T92-0039, from the intramural branch of the NCI, on 2/20/92, a phase I/II study investigating infusional chemotherapy, DDI, and G-CSF for the treatment of AIDS-associated NHL. The study will determine the tolerability and efficacy of this regimen as well as the efficacy of CNS prophylaxis. Markers of HIV infection and immune competence, cytogenetics and cytokine production by lymphoma cells and circulating lymphocytes will be monitored.

CTEP reviewed and approved protocol T92-0036, from the Univ. of Texas at San Antonio, on 4/30/92, to do a phase II trial of MGBG in AIDS-associated NHL patients whose lymphoma is refractory to or relapsing on conventional chemotherapy.

CTEP reviewed and approved protocol EST-P-A491 on 1/31/91, from ECOG, a phase I trial of rIL-3 + AZT in HIV positive patients with neutropenia, and a phase I trial of rIL-3 + AZT + standard dose CHOP chemotherapy in patients with AIDS-associated NHL.

CTEP reviewed and approved protocol T91-0045 from NYU and USC, on 9/30/91, a phase I study of anti-B4 blocked ricin immunoconjugate in patients with AIDS-associated NHL.

CTEP reviewed and approved LOI L90-0364 from UCSD, a phase I/II study of CHOPE + G-CSF + EPO with or without AZT in AIDS-associated NHL. The protocol was submitted by CALGB in 7/92 and is currently in CTEP review.

CTEP reviewed and approved protocol POG-9182, on 11/15/91, a pediatric oncology group AIDS NHL Network study on pediatric AIDS NHL.

<u>Cervical Cancer</u>

**Plans**

CTEP approved an epidemiology study, protocol NCIEPI 90-C-223 on 10/10/90, of cervical cancer and HIV in Zambia.

The Clinical Investigations Branch sent out a letter to all Clinical Trials Cooperative Group Chairs in 5/92 encouraging the development of concepts and protocols in the area of HIV associated cervical dysplasia and cancer.

## HEAD AND NECK CANCER

### Organ Preservation

#### Accomplishments

In 1985 the VA Cooperative Studies Program initiated a prospective, randomized, multi-institutional study to determine if induction chemotherapy with CDDP + 5FU combined with definitive XRT and surgery salvage was an effective organ preservation treatment strategy compared to conventional surgery and postoperative XRT in patients with stage III/IV laryngeal squamous cancer. Tumor response was assessed after 2 cycles of chemotherapy; responders (CR + PR) received a third cycle followed by XRT. Non-responders underwent salvage laryngectomy. Interim results were reported previously (ASCO 8:167, 1989; NEJM 324:1685, 1991). Final analysis of 332 patients (166 Chemo + XRT; 166 Surgery + XRT) indicated similar 3 year survival rates of 53.3% (95% CI 45-61%) for chemo + XRT and 55.9% (95% CI 48-64%) for Surgery + XRT (p=.79). Median follow-up was 43 months. Overall, 50% of the patients have died (87 Chemo + XRT; 79 Surgery + XRT). Survival was similar for CR, PR, NR and surgery groups. Patterns of failure differed by treatment with more frequent local failures and fewer distant metastases in the Chemo + XRT group. Of the 166 Chemo + XRT patients, 63 (38%) required salvage laryngectomy, with ultimate larynx preservation in 52 of 79 (66%) surviving patients.

#### Plans

At the CTEP sponsored Head and Neck Cancer Strategy meeting on 3/31/92, a significant item on the agenda was an organ preservation study of the larynx. Surgeons, radiation oncologists, and medical oncologists representing the major Clinical Trials Cooperative Groups and Cancer Centers discussed a potential intergroup protocol for organ preservation of the larynx. After much discussion, it was decided that an organ preservation study was important based on the mutilating and debilitating side effects of conventional radical surgery, and the promising response and overall survival results of an organ preservation approach from the VA Cooperative Study. CTEP approved NCI protocol # RTOG 9111 in 4/92. The study is a three arm randomized study comparing CDDP + 5FU x 2 cycles, with CR/PR receiving 1 more cycle of chemo and then proceeding to XRT vs definitive XRT + concomitant CDDP vs standard XRT, with salvage laryngectomy reserved for all three arms. Concurrent studies are ongoing to determine the optimum sequencing of chemotherapy and XRT, and the optimum dosing interval of XRT, i.e., hyperfraction vs continuous hyperaccelerated XRT vs standard XRT.

Further studies are being planned for organ preservation in non-laryngeal head and neck cancer sites, randomizing patients to initial surgery vs induction chemotherapy/XRT with salvage surgery to compare overall survival and quality of life issues.

## Retinoids

### Accomplishments

Lippman presented results of the first long-term and largest phase III trial in oral premalignancy (PASCO March 1992, abstract 781). After establishing the efficacy of the retinoid 13cRA in oral premalignancy (NEJM 315:1501,1986), a study was designed to solve the two major problems with high dose 13cRA, that of significant toxicity, and a high relapse rate after stopping therapy. After a 3 month induction phase with high-dose 13cRA, patients were randomized to 9 months of maintenance with low dose 13cRA or beta carotene. Of 65 patients who completed induction therapy, 40 (62%) had a PR or CR, 18 (28%) were stable and 7 (11%) progressed. The 58 patients who did not progress were randomized to maintenance therapy. The progression rate was significantly lower (8% vs 52%) and the response rate significantly higher (50% vs 16%) in the 13cRA arm than in the beta carotene arm.

### Plans

The 13cRA dose of 0.5 mg/kg/day used in this study is the current low-dose standard for phase II cancer chemoprevention trials in the aerodigestive tract, including those with early stage head and neck cancer, and in lung cancer, to prevent second primary tumors.

## Resectable Head and Neck Cancer

### Accomplishments

Four studies have shown a decrease in distant metastases but no improvement in overall survival in patients with locally advanced operable squamous cell cancer of the head and neck treated with post-operative chemotherapy and radiotherapy vs post-operative radiotherapy alone. Results from the Intergroup 0034 study of 442 patients, however, showed a strong tendency of adjuvant chemotherapy to improve local-regional control and overall survival in the patients with "high-risk" treatment volumes, i.e., those with surgical margins < 5 mm or extracapsular nodal extension. This same tendency for benefit was not seen in the "low-risk" group.

### Plans

Studies are being developed for adjuvant chemotherapy and radiotherapy in the "high-risk" and positive margin patients, in an effort to target those patients who might benefit from such an approach.

## Radiosensitizers

### Accomplishments

RTOG 8527, a phase III study of standard RT vs SR2508 + standard RT in locally advanced head and neck cancer, closed to accrual in 10/91 with 521 patients. The purpose of the study is to determine whether the addition of the hypoxic cell sensitizer SR2508 to conventional RT improved local-regional control and tumor-free survival, and will be analyzed in 9/92.

Other agents being considered as potential radiation sensitizers for clinical use are Taxol, Topotecan, and CPT-11.

Plans

Further use of the radiation sensitizer SR2508 await the planned analysis of the study in 9/92. Investigational agents in combination with radiation therapy are planned.

## LUNG CANCER

Accomplishments

Recent studies in Non-small Cell Lung Cancer (NSCLC) have focused on developing new agents with higher response rates in numerous phase II trials. Two recent trials of Taxol in previously untreated patients with stage IV NSCLC have revealed 21%-24% response rates. Two abstracts (PASCO March 1992, abstracts 308, 978) from Japan discussed in vitro effects of CPT-11, a new camptothecin derivative , in human NSCLC cell lines. A trial of CPT-11 in combination with CDDP revealed a 57% response rate in advanced NSCLC patients.

Several trials, two with an intergroup effort (RTOG 8901 with recent activation by ECOG, and INT 0115) are investigating the respective roles of multimodality treatment with chemotherapy, surgery, and radiotherapy vs combinations of chemotherapy and radiotherapy in improving local response, decreasing distant metastases, decreasing local relapse and improving overall survival in stage IIIA NSCLC.

Studies in Small Cell Lung Cancer (SCLC) are investigating intensive radiotherapy regimens in combination with chemotherapy in an attempt to improve local control, decrease distant metastases, and improve overall survival. Studies of new agents, particularly Taxol, are being investigated in extensive stage disease, where the current long term survival is only 0-2%.

Two of the series II High Priority Trials in Lung Cancer completed accrual this year. INT 0096, an intergroup phase III randomized study of CDDP + Etoposide + standard thoracic RT vs CDDP + Etoposide with hyperfractionated thoracic RT in limited stage, SCLC, completed accrual in 6/92. The purpose of the study is to compare the median and long-term survivals of limited stage SCLC patients; to compare intrathoracic, within radiation portal and distant failure rates, and to compare the toxicities. The second intergroup study (RTOG 8808, EST 4588, SWOG 8992), a phase III randomized study of standard RT vs hyperfractionated RT vs Velban + CDDP followed by standard RT, in locally advanced NSCLC, closed to accrual in 1/92 and will be analyzed in 1/93. The purpose of the study is to compare the overall survivals, and toxicities.

Plans

All the adult Clinical Trials Cooperative Groups have active trials in progress or soon to be activated trials answering questions of the importance of pre-operative surgical staging and multi-modality therapy in NSCLC. Additional plans include combining active new agents with agents traditionally shown to have response in lung cancer, or with RT as potential radiation sensitizers (based on in vitro data).

A High Priority Trial, RTOG 88-08 (EST 4588/SWOG 8992), closed in 1/92 and will be analyzed to assess the therapeutic efficacy of chemotherapy and standard RT, or hyperfractionated RT, compared to the standard therapy of conventional RT in unresectable NSCLC.

CTEP is sponsoring a one-day strategy meeting on clinical trials in NSCLC in 9/92. The purpose of the meeting is to exchange information on clinical trials and laboratory correlates between the major cancer cooperative groups and cancer centers; to focus on the important questions relevant to the different stages of NSCLC; to coordinate efforts to implement trials and correlative studies, in order to answer such questions in a timely manner, and to discuss trials requiring intergroup participation. Agenda items include the role of staging, clinicopathologic correlation, and multimodality therapy in stage IIIA NSCLC; to discuss the multitude of phase III trials encompassing almost identical patient populations, and the setting of priorities for studies in stages I-IV NSCLC (i.e., adjuvant studies to reduce the incidence of second primaries in stage I; studies of prognostic parameters to select for patients with a poorer prognosis as a target population for more aggressive therapy in stage II; and trials of new investigational agents with or without radiation therapy, altered fractionation, or other new modalities for stages IIIb and IV).

Hyperfractionation and continuous accelerated hyperfractionation are being studied in SCLC in an effort to improve local control. Analysis of one of the High Priority Trials recently completed in SCLC will be performed, to assess the contribution of hyperfractionation vs standard RT to chemotherapy. Preliminary data of Taxol in extensive stage SCLC is still pending, but if promising could be combined with other active agents in an effort to improve survival.

**CONTRACTS**

Extramural Clinical Trials Office--EMMES

The EMMES Coporation is currently funded for two independent contracts for CTEP; Dr. Bruce Cheson, CIB is project officer for these contracts. The first is to provide information support for strategy meeting planning and coordination, to maintain a clinical trials hypothesis data base, and to assist in the preparation and writing of scientific publications. In the second contract EMMES serves the operations and data management functions for the CTEP-sponsored Group C protocols including 2-chlorodeoxyadenosine for hairy cell leukemia, and taxol for ovarian cancer and, in the near future, breast cancer. Over 2000 women have been entered onto the ovarian study thus far.

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## INVESTIGATIONAL DRUG BRANCH (IDB)

### 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.

### CHALLENGES AND OPPORTUNITIES:

A wide range of new investigational agents, many with novel modes of action, have recently been brought into DCT-sponsored clinical trials by the IDB. New cytotoxic agents of great importance include the taxanes (taxol and taxotere) and the camptothecin analogs (topotecan, CPT-11, 9-aminocamptothecin) New strategies for therapy attracting increased interest include biochemical modulation and reversal of multi-drug resistance. The availability of strategies to decrease myelosuppression, including recombinant novel hematoregulatory molecules and the use of peripheral blood stem cells, is allowing the establishment of new MTD's for cytotoxic agents whose dose-limiting toxicities have been myelosuppression. Identification of differentiation-induction as an active anti-cancer mechanism has led to considerable interest in the strategies as discussed below.

The purpose of IDB-sponsored drug development remains the identification of tumor activity for new investigational agents. Increasingly, the clinical trials which are approved include laboratory correlates to yield information into the pharmacokinetics, metabolism, and biological effects of the agents. As new targets, including cell surface receptors, signal transduction mechanisms, and specific RNA and DNA sequences are identified for anti-cancer drug development, these types of laboratory correlates will become increasingly necessary.

The interaction of biologic and cytotoxic agents has been another important trend in drug development. Colony-stimulating factors have allowed new strategies in dose-intensification. Other cytokines, including the interferons and tumor necrosis factor, are being used in strategies designed to increase the effectiveness of cytotoxic chemotherapy.

The current high level of interest in new cancer drug development has translated into increased activity by the IDB. Clinical trials are underway under more that 200 IND's. In the past year new IND's involving a range of treatment strategies from natural products development through gene therapy have been initiated. Well over 100 letters of intent for Taxol-related studies have been reviewed this year, and numerous studies placed to investigate this active new agent. Similarly, requests for letters of intent for the topoisomerase I inhibitor CPT-11 resulted in 120 responses from institutions interested in evaluating this drug. Requests for letters of intent for taxotere, Interleukin-6, and 9-aminocamptothecin also met with enthusiastic responses. As a result, the IDB in dealing with an unprecedental level of new activity, which while preventing significant challenges, also offer real opportunities in the development of more active cancer treatment.

The success of IDB's results will ultimately be reflected in a greater number of anti-cancer agents available to patients with malignancy. Within the last year Interleukin-2, fludarabine, 2-chlorodeoxyadenosine, and deoxycoformycin have all been approved often, with data obtained from NCI-sponsored clinical trials.

The status of some of the interesting new investigational agents currently in or coming into clinical trial is discussed below, along with new strategies implemented during the past year to distribute the drugs widely in the period between the recognition of drug activity, and final FDA approval. As examples of the success of these latter efforts, over 500 patients with acute promyelocytic leukemia have now received all-trans retinoic acid under a compassionate mechanism, and over 1500 women with chemotherapy-refractory ovarian cancer have received taxol, first through a Treatment Referral Center protocol, and more recently under a Group C mechanism.

## THE INVESTIGATIONAL DRUG BRANCH ROLE IN EVALUATING UNCONVENTIONAL THERAPIES

The status of various Unconventional Cancer Treatments (UCT's) in the United States has been recently reviewed by the Office of Technology Assessment (OTA). In the published OTA report, options were presented to broaden the information and investigative base of UCT's. To address the recommendations in this report, the National Cancer Institute has developed a system of review and guidance to assist individuals who wish to have data evaluated or who wish to develop data which would support further research with their approach. The role of the Investigational Drug Branch in this process is to conduct retrospectively a "best case series" review which identify a particular patients who may have benefitted from UCT. Technical advice is also available to assist individuals in the design of prospective UCT studies which will generate interpretable clinical data.

## IMMUNOREGULATORY CYTOKINES

### INTERLEUKIN-2 (IL-2)

In May, 1992, the FDA approved the Cetus Oncology application to market IL-2 for the indication of advanced or metastatic renal cell carcinoma. Approval was based primarily on data gathered in NCI intramural and NCI-sponsored extramural clinical trials. Cetus Oncology, Division of Chiron and the DCT/NCI have reached an agreement that will allow the NCI to continue to sponsor clinical trials with this agent.

IL-2 remains the major component of clinical studies exploring immunotherapeutic approaches to treating cancer. Clinical trials are planned or are in progress which combine IL-2 with other biologic agents or chemotherapy to improve anti-tumor activity. High response rates have been seen in studies using IL-2 in combination with chemotherapy or monoclonal antibodies in metastatic melanoma, but the data are preliminary and will require confirmation. Similarly, there is evidence that IL-2 administered after marrow transplantation for hematologic malignancies may decrease relapse rates, and Phase III trials are planned. Earlier Phase I studies will explore combinations of IL-2 with GM-CSF, M-CSF, IL-1, and IL-6 to enhance immunologic effector mechanisms *in vivo* which in turn may lead to more effective tumor therapy.

### INTERLEUKIN-4

Interleukin-4 displayed antitumor activity against B-cell lymphoma and Hodgkin's disease in early trials. Phase II trials had been solicited, but final approval and initiation of the studies was delayed to resolve regulatory issues regarding toxicity in animal studies. A drug supply has been secured, regulatory issues have been resolved, and a series of clinical studies have been initiated together with Schering-Plough.

### 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 antitumor, radiolabelled 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 antitumor effects of IL-1 when used together with a number of chemotherapeutic agents (carboplatin, methotrexate, and etoposide).

## 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 antitumor 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 granulometaus 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; a U.S. trial has confirmed this result; pre-licensing trials of IFN-gamma for this indication are in design. Other trials to evaluate low-dose gamma interferon in other cancers and to determine potential mechanisms of action are in design. Interferon-gamma has also been combined with other biologicals, including IL-2. A large Phase I evaluation of this ed 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 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.

TUMOR NECROSIS FACTOR (TNF)

Phase II trials designed to define the antitumor 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. Humans tolerate only very much lower doses of systemic TNF; the very high doses which have significant antitumor activity in rodents are not possible because of the development of hypotension. Very high doses of TNF can be given by isolated regional limb perfusion. Particularly exciting are data demonstrating synergy between TNF and melphalan in the therapy of bulky primary limb tumors; including recurrent and soft tissue sarcomas randomized trails to define the extent of benefit provided by this combination have recently been activated. Recent data suggesting synergy between TNF and the topoisomerase I inhibitor, topotecan, may also warrant clinical evaluation. 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. Because TNF is a negative regulator of blood cell production, a new trial has recently been activated to examine the possibility that it may have activity against acute leukemia.

COLONY STIMULATING FACTORS

GRANULOCYTE (G-CSF), GRANULOCYTE MACROPHAGE (GM-CSF) COLONY AND MONOCYTE/MACROPHAGE (M-CSF) STIMULATING FACTORS

A great deal of information regarding the toxicity and effectiveness of G- and GM-CSF 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. New schedule of administration and combined use of G-CSF and GM-CSF are also being explored as a means to prevent chemotherapy induced toxicity; priming the bone marrow with these agents before chemotherapy may prove especially effective and a means to prevent hematologic toxicity. Preliminary information has also implied that the G- and GM-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 G- and GM-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 G- and GM-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 G- and GM-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 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 three CSF's 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 G- and GM-CSF 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 M-CSF and GM-CSF, may augment the anticancer effects of macrophages and neutrophils. The CTEP has approved three trials to address this possibility; certain trials will assess the antitumor activity of M-CSF or 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 are underway. 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 have begun are about to begin patient enrollment.

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. Cancer patients often experience anemia and many require blood transfusions during treatment of their cancers; as a result 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.

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.

INTERLEUKIN-6 (IL-6)

IL-6 is a promising agent that has both antitumor and hematopoietic stimulatory activity in preclinical animal models. An IND for interleukin-6 was filed in the past year. A comprehensive Phase I program coordinated with the Sandoz Cytokine Development Unit was initiated to explore different schedules and routes of administration. There is clear evidence from these studies that IL-6 enhances production of platelets, and thus may be a useful agent in thrombocytopenic states and recovery from thrombocytopenia following chemotherapy. Studies have been solicited to explore the effects of IL-6 in combination with other colony-stimulating factors and cytokines such as G-CSF, GM-CSF, IL-3, and IL-2, and to determine its antitumor activity in both solid tumors and hematologic malignancies. IL-6 will also be used as part of a cocktail of growth factors used to increase the transfection rate of stem cells in gene-therapy studies.

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.

## ACTIVATED CELLS

### ADOPTIVE IMMUNOTHERAPY

Studies of adoptive immunotherapy continue to move away from the non-specific effector cells (i.e., LAK cells which kill most tumors non-specifically and spare normal tissue) to 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. Clinical trials are exploring means to increase the potency of the CTL to enhance the generation of CTL from multiple malignancies and to test their therapeutic efficacy in patients. The Surgery Branch of the NCI is continuing a study of tumor-infiltrating lymphocytes (TIL) transfected with the gene for tumor necrosis factor (TNF). The transfected TIL are expected to deliver large amounts of cytotoxic TNF directly to the tumor. Methods of improving the generation of TIL in vitro, such as addition of TNF or IL-4, are under investigation. Studies will also examine vaccination with autologous tumor or systemic treatment with IL-2 or other cytokines prior to the tumor/TIL harvest to produce 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, colorectal, and breast cancer. For 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. A second study will examine the effects of the CD8+ subset of TIL cells, and a third study will use gene-transfected TIL cells to determine their distribution in vivo. Gene-transfection will also be used to determine the distribution of selected TIL subsets in a study of patients with renal cell carcinoma.

The NCI will sponsor a randomized Phase III confirmatory study of autolymphocyte therapy, a form of adoptive immunotherapy which was reported to increase survival in a small randomized trial of patients with metastatic renal cell cancer.

### GENE THERAPY

A program announcement for gene therapy of cancer was released by CTEP in the last year, and peer review of several program project proposals is scheduled for the near future. It is expected that three basic strategies will be examined: transfection of cytokines into tumor cells to increase their immunogenicity for vaccine applications, transfection of genes into lymphocytes to increase their potency against tumor targets, and transfection of genes into hematopoietic stem cells to increase their resistance to chemotherapy agents (thus allowing more dose intense chemotherapy).

## 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 or humanized, and these newer generation antibodies, with their promise of longer circulating half-lives, reduced immunogenicity, and increased immunobiological activity, are now being introduced into NCI-sponsored trials. 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.

Clinical trials using monoclonal antibodies against the transferrin receptor are being initiated; in preclinical studies a combination of two of these antibodies was effective in causing tumor regression.

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.

A limitation in the development of monoclonal antibodies for therapy over the past year has been difficulties in obtaining sufficient suppliers of clinical grade material. The IDB has been actively involved in strategies to increase availability of such antibodies. In addition, the cost of testing monoclonal antibodies for the good manufacturing practice (GMP) standards necessary for clinical trial has limited the numbers of antibodies entering trial. In January, 1992, a conference on the requirements for testing for Phase I trials of monoclonal antibodies was jointly sponsored with the FDA, to address these issues; the result may be increased numbers of monoclonal antibodies entering early clinical trials in the future.

## 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 non-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. The radioistype Lutechium is being used in early Phase I trials now initiated in the clinical center, in preclinical studies this isotope has yielded superior therapeutic results.

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 DCT-sponsored Phase II trials in chronic lymphocytic leukemia, in pediatric T-cell leukemia and lymphoma, and in the prevention of acute GVH disease. A series of clinical trials with B4-blocked ricin is being studied in B cell non-Hodgkin lymphomas, where Phase I trials have demonstrated activity.

## DIFFERENTIATING AGENTS

### TRANS-RETINOIC ACID

The oral Vitamin A analog all trans Retinoic Acid (TRA) has reproducible activity in the induction of adults with acute promyelocytic leukemia. 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 2 years 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 and over 50 clinical trials of tRA alone or in combination with other differentiation-inducing agents or cytotoxic chemotherapeutic agents have been activated 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. Particular attention has been paid to the sponsorship of follow-up pharmacokinetic trials designed to stabilize the long-term plasma levels of TRA including studies of alternative dose and administration schemas as well as combinations with potential tRA metabolism inhibitors. National clinical trials involving the major cooperative groups have been developed for newly-diagnosed patients with APL. The NCI has worked actively with the pharmaceutical company involved (Hoffman LaRoche) 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 progress in the understanding of retinoid biology. A series of nuclear receptors for the retinoids has been described, the genes cloned, and the chromosomal locations 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. In addition, retinoids with discriminating receptor binding activities have been developed. The recent recognition of differential binding of distinct synthetic retinoids to different retinoid receptors, coupled with the developing information that different retinoid receptors are variably expressed different normal and malignant tissues, provide a rationale for the differential 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 additional retinoids into clinical trial; this effort includes the provision 4-hudroxyphenl retinamide for U.S. evaluations of adjuvant activity in combination with tamoxifen in breast cancer patients. 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.

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 an allogeneic membrane lysate from two melanoma cell lines combined with a non-specific adjuvant will be compared to observation alone.

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 and IL-1 are ongoing. Recently, transfection of cytokine genes directly into tumor cells was shown to increase their immunogenicity. A clinical trial using this approach has been initiated by investigators in the Surgery Branch, and several trials have been proposed as part of a CTEP/DCT announcement for program projects in gene therapy of cancer.

A recombinant vaccinia virus containing the gene for CEA has recently been produced and will enter clinical trials in patients with colorectal cancer. It is expected that vaccines containing specific melanoma antigens or antigenic mutated oncogene products will also enter clinical trials within the next year.

## DEVELOPMENTAL CHEMOTHERAPY SECTION (DCS)

In addition to coordinating and monitoring clinical trials for almost 100 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. Important dose intensification trials have been completed with taxol and topotecan and are ongoing with a number of other agents including piroxantrone and hepsulfam.

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. A Blood Level Working Group composed of DCS and other NCI officials and FDA members reviews each new drug entering clinical trials for the appropriateness of expedited dose escalations. Most recent Phase I trials have employed such an approach.

## 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, Lev, Urd, Hydrea)

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, Hydrea 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).

Modulated Fluorouracil regimens have been most active against colorectal adenocarcinoma and form the standard frontline therapies for this disease. The 5-FU/levamisole combination is recommended for patients with resected stage C2 disease. An update of the large Intergroup Phase III trial (>900 patients) that established the effectiveness of this combination in the adjuvant setting was presented at this year's ASCO meeting. These patients have now been followed for a median of 5 years. The superiority of the combination over levamisole or surgery alone persists for patients with resected stage C2 disease (39% reduction in recurrences, 31% reduction in death rate). However, a trend toward benefit for stage B2 patients has not materialized into a statistically significant benefit. Although several attempts have been made to document a biochemical or immunomodulatory interaction, the mechanism of this synergy remains an enigma. Current adjuvant trials are examining combinations of 5-FU/LV with or without levamisole. In advanced disease, several 5-FU based regimens have produced response rates in the region of 40% in phase II trials but only the 5-FU/leucovorin combination has been shown to enhance survival, albeit modestly. A large randomized trial 5 arms, ECOG/CALGB) of the most developed regimens is now underway and should provide good information on the relative effectiveness of different modulating strategies. In the meantime, several investigators are examining novel 5-FU schedules combined with two or more modulators in attempts to squeeze the maximum therapeutic effectiveness of this rudimentary fluoropyrimidine.

## 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 Mar 1990. A few studies have been opened since then and are ongoing.

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.

This agent has shown remarkable activity against the rare disorder known as hairy cell leukemia (HCL). Most patients obtain a complete response after a single 7-day course of 2-CdA with minimal toxicity. This therapy was favorably reviewed by the FDA's Oncology Drug Advisory Committee (ODAC) in June '92 and NDA approval is anticipated before the end of the year. Until then, CTEP is providing 2-CdA for patients with HCL via the Group C Treatment Protocol mechanism. Some Phase II evaluation of this agent has already been performed with the 7-day schedule, showing activity against cutaneous T-cell lymphoma and low grade non-hodgkin's Lymphoma. However, it is unclear at present if the current schedule is optimal. A few alternate schedules (5 day continuous infusion, daily x 5 2-hour infusion) are being tested in Phase I studies in patients with solid tumors as well as leukemias and several Phase II studies will commence this year.

CARBOXYPEPTIDASE(CPDG2)

CPDG2 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. 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. Since last year's report, an IND application has been submitted to the FDA. Clinical trials should begin this year.

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. Since last year's report, CTEP has obtained and IND and the first Phase I trial of this agent has been opened at the NCI Clinical Center.

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). In December 1991, the FDA approved dCF for this indication and CTEP closed its Group C Treatment Protocol.

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.

Two Phase I studies of the same schedule (15 min infusion every 3 weeks) were opened in 1991, the first in Europe and the second at the NCI. The European study was closed after dose-limiting toxicity, primarily gastrointestinal & hematologic, was encountered at 3.5 mg/$m^2$. Surprisingly, no significant toxicity has occurred on the American trial at this or lower doses. This discrepancy may be due to differences in patient selection. The patients on the American trial were more homogeneous; all had gastrointestinal tumors, most had previously been treated with 5-FU/LV regimens, etc. One possible explanation for the higher tolerance in the US trial is the recent exposure to moderate doses of leucovorin with resultant higher intracellular folate levels. This hypothesis is currently being investigated at the NCI. A few responses have been reported on the European trial. It is anticipated that Phase II studies of D1694 will open in the fall of 1992. The isolated site of action of this agent (a pure TS inhibitor) may prove useful in modulating studies and CTEP is planning preclinical evaluation of 5-FU/D1694 combinations.

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 and over two dozen studies have been approved by CTEP. The most interesting activity has been seen in patients with advanced breast cancer (reported response rates of approx. 40%) and there is exciting preclinical evidence for synergy with several other cytotoxic agents. The use of leucovorin to rescue normal tissue

from antifols has become an established component of several regimens with MTX despite the concern that the leucovorin might be compromising the antitumor activity of the antifol. The superiority of the single agent over a rescue combination can only be established in a Phase III trial but it is unlikely that such a trial will be performed for MTX. ETX dramatically increased the activity of ETX in murine studies and two Phase I studies of this combination are underway. CTEP is organizing a large Phase III study to compare ETX alone versus ETX with LV rescue.

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. Both studies have been closed after meeting accrual targets and comparative survival data should be available this year.

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 vivo murine tumor models. Phase I trials of high dose hepsulfam. It produced cumulative myelosuppression at standard doses are currently ongoing in the bone marrow transplant and acute leukemia settings 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.

TEMOZOLOMIDE

Temozolomide is an imidazotetrazine derivative which is similar to dacarbazine but which readily undergoes spontaneous ring opening to the active alkylating metabolite. In limited European trials, promising activity has been reported in high grade gliomas. Responses have also been reported in melanoma and Kaposi's sarcoma. European trials have been delayed because of lack of drug supply. Based on the encouraging European results, DCT has produced as supply of temozolomide and is preparing to submit an IND. A rapid Phase I trial is planned to ensure that the optimal dose is taken to Phase II. A Phase II trial in high grade gliomas is expected to begin immediately thereafter. Broad Phase II screening is also planned. This development will be done in cooperation with European investigators, and with Schering-Plough, which has acquired world-wide development rights.

MITOTIC SPINDLE TOXINS

TAXOL

This unique natural product derived from the bark of Taxus brevifolia has shown promising antitumor activity in several tumor types. Results of 3 Phase II studies indicated that 30% of women with relapsed ovarian cancer who had been treated with multiple regimens (radiation, chemotherapy) responded to taxol. Responses were seen in women who were refractory to conventional therapy. To pursue this activity, the first Phase III comparison of standard therapy (cytoxan + cisplatin) with the combination of taxol + cisplatin was conducted in newly diagnosed patients. Response and survival data await additional follow-up of patients; however, toxicity data indicate that treatment was well tolerated. A new study in this patient population compares higher dose single agent taxol, higher dose single agent cisplatin (which some also consider standard therapy) and the combination of taxol and cisplatin. Another trial in a more favorable prognostic group of women with ovarian cancer compares standard therapy (cytoxan and cisplatin) with taxol and cisplatin and with a third more dose-intensive regimen consisting of carboplatin, intraperitoneal platinum and taxol.

For those women who do not have the opportunity to receive taxol as part of their initial treatment and who have platinum-refractory disease, a study is comparing three different taxol doses. This study will establish whether administration of higher taxol doses improves response rates in ovarian cancer. A wide range of doses has been administered to ovarian cancer patients. Higher doses are associated with more toxicity but it is not yet known whether higher doses result in increased response rate or prolonged survival.

In the past year, important results have also been reported in women with breast cancer. Fifty-six percent of 25 women who had received one prior regimen as adjuvant therapy or for metastatic disease, responded to therapy with taxol. In a second trial, 62% of 25 women who may have had adjuvant therapy but no chemotherapy for metastatic disease, responded. Taxol is an active drug in women who have had minimal prior chemotherapy for breast cancer. Preliminary information in a poorer prognosis group of patients who had received at least two prior chemotherapy regimens (some had received more) and all of whom had received prior doxorubicin (but not all of whom were doxorubicin-resistant) indicates that the response rate is approximately 22%. The follow-up time on this study is short; it is possible that with additional courses of therapy, the number of responding patients might increase. In any case, based on these early data, a compassionate protocol is planned for women with refractory breast cancer.

Combination and pilot trials are underway in preparation for Phase III trials in breast cancer which will begin in the fall of 1992. Taxol has been administered in combination with doxorubicin, the most active standard agent for breast cancer. Evaluation of different schedules and drug sequences has been performed in an effort to identify the optimal way to combine taxol with doxorubicin. Recently, trials have been initiated using leads developed by our contractors who have used preclinical mouse model systems. Preliminary response rates are encouraging but additional investigation of this and other combination chemotherapy regimens, including taxol with cyclophosphamide, cisplatin or other agents, continues.

Taxol has activity in patients with previously untreated non-small cell lung cancer. Two studies have resulted in response rates of 21 and 24%. The Eastern Co-operative Oncology Group, which performed one of these trials, reported that taxol is the most active drug for non-small cell lung cancer that ECOG has studied in the past ten years. Additional combination trials are being developed for evaluation in non-small cell lung cancer. A preliminary report, also from ECOG, suggests that taxol also has activity in extensive small cell lung cancer. Based on this information, two new trials have been activated with a different study design that permits patients to receive additional courses of taxol provided that there is no indication of tumor progression.

A Phase II trial in patients with head and neck cancer who have had no prior chemotherapy is being completed. The preliminary response rate (which could change as additional patients are accrued) is 42%. Future studies will evaluate taxol in combination with cisplatin, the most active standard agent for head and neck cancer.

Studies have not demonstrated activity in prostate, colon, renal or cervix cancer. Response rates of only 12 and 14% have been reported in melanoma but two patients have had prolonged and continuing complete responses. Assessment of efficacy in sarcoma, lymphoma, gastric, esophageal, pancreatic cancer, hepatoma, leukemia, multiple myeloma pediatric and other malignancies is continuing.

Efforts conducted by Bristol Myers Squibb to increase the taxol supply have succeeded so that more than 30 clinical trials have been initiated with more in review and are expected to begin soon. Now broad Phase II screening is being performed in all major cancer types that had not been studied previously. Many trials evaluating combinations of taxol with other standard and promising investigational anticancer drugs are underway.

In addition, important developmental trials are underway to determine the optimal dose and schedule for taxol administration, to determine the optimal dose for patients with impaired liver function, to assess the relationship between response and expression of the multidrug resistance (mdr) phenotype and to combine taxol with mdr reversing agents in an effort to increase response rates and overcome one known mechanism of resistance to taxol.

Taxol frequently causes asymptomatic bradycardia and rarely is associated with other arrhythmias. On very rare occasions these have been symptomatic and required therapy. Based on these early reports, patients with potential cardiac risk factors and those taking medications known to alter cardiac conduction were excluded from most taxol trials. Since cancer is a common disease in elderly patients who frequently fall into these potential risk groups, it is important to carefully assess the toxicity in this population. A study is planned in patients with potential risk factors, for whom taxol is otherwise appropriate therapy. Patients will be evaluated by a cardiologist and undergo special cardiac evaluation and cardiac monitoring to maximize safety. This study should permit us to prospectively identify those patient groups who truly are at risk and those who might benefit and can be treated with reasonable safety.

Based on results with taxol in women with refractory ovarian cancer, taxol has been made available to women with refractory ovarian cancer at 39 National Cancer Institute-designated Comprehensive Cancer Centers through the Treatment Referral Center. More than 1500 patients who had received at least three prior regimens and who were not eligible for clinical trials of higher priority were entered in this trial between September 15, 1991 and July 1992. This number is approximately 15-fold higher than the number of ovarian cancer patients who had been treated prior to the opening of this first Treatment Referral Center study. Until taxol is approved by the Food and Drug Administration for refractory ovarian cancer, this protocol will remain open for patient accrual. Recently, a limited Group C treatment IND was approved. The protocol was recently amended to permit patient entry after only two prior therapies.

TAXOTERE

Taxotere is an analogue of taxol that is prepared by partial synthesis from a precursor obtained from the needles of the European yew. Phase I trials were recently completed. The NCI recently signed a CRADA with Rhone-Poulenc Rorer, the manufacturer of taxotere. Phase I adult and pediatric leukemia trials, a phase I pediatric solid tumor trial and phase II clinical trials in small cell lung cancer, colon, cervix, prostate, gastric cancer and non-Hodgkin's lymphoma are scheduled to begin in the fall of 1992.

RHIZOXIN

Rhizoxin is another natural product isolated from Rhizopus chinensis. Based on responses documented in four of six refractory breast cancer patients treated on a Phase I trial in Europe, clinical trials are planned in the United States. Preclinical data and the short half-life of the drug suggest exploration of a schedule of administration different from that used in the European trial. A Phase I trial of rhizoxin administered by 4 day infusion is planned.

TOPOISOMERASE I INHIBITORS

Camptothecin and its analogs are inhibitors of topoisomerase I, an enzyme necessary in mammalian DNA replication. Three analogs currently being developed by DCT includes topotecan, CPT-11, and 9-aminocamptothecin.

TOPOTECAN

Topotecan is a water soluble camptothecin analog with a wide range of preclinical activity. Phase I trials of several alternative topotecan schedules are underway, in cooperation with the drug sponsor Smith Kline Beecham. Phase II trials of the daily x 5 schedule are currently underway in a spectrum of malignancies.

CPT-11

This water-soluble camptothecin analog has been studied extensively in Japan. In cooperation with Yakult Honshen. DCT has recently approved a series of letters of intent for clinical trials further to explore the range of clinical activity of this agent.

9-AMINOCAMPTOTHECIN

This relatively insoluble topoisomerase I inhibitor has been required considerable formulation development by DCT prior to the initiation of clinical trials. NCI has recently developed a CRADA for the joint development of this agent with Adria Laboratories, and clinical trials will soon be initiated.

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 began recently.

INTERCALATORS

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. Phase I trials are underway at Wayne State University and Johns Hopkins. Neurotoxicity was dose limiting on a short infusion every 21 days, therefore, longer infusions (3 hour) and weekly administration are being attempted to avoid high peak concentrations that may be associated with neurotoxicity.

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 150 $mg/m^2$ 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 agent preclinically.

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 dose 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 continuously 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)

The Drug Management and Authorization Section consists of nine pharmacists, one authorization technician, and a secretary. The section is responsible for the following: 1) authorization, processing and distribution of all agents distributed for NCI sponsored clinical trials, 2) maintaining the registration documents for all NCI clinical investigators, including the required annual reregistration, 3) Group C and Special Exception (compassionate) drug registration and authorization, 4) management and operation of the Treatment Referral Center (TRC), including registration and administration of TRC protocols, 5) management, labelling and distribution of agents for several double blind studies including tamoxifen for the breast cancer prevention trials, 6) acquisition of agents and coordination of drug distribution for industry collaborators, 7) protocol review for pharmaceutical data and provision of pharmaceutical drug information.

GROUP C PROTOCOLS

Group C authorization and distribution for individual patients has continued to expand over the past year. Group C drugs are those investigational agents that have demonstrated reproducible efficacy in a particular tumor type. By definition a Group C drug must alter the pattern of patient care and may be administered by physicians without specialized supportive care facilities.

Group C Guideline Protocols are standardized treatment designs which use Group C drugs to treat a specific tumor or stage of cancer. DMAS participates in the protocol writing. DMAS's primary focus is the pharmaceutical requirements, including the product description, preparation, storage, indications, dosage, precautions, warnings and known adverse effects. In addition, management procedures for registration unique for each protocol are established.

These procedures are used by the section in screening and registering individual patients. The entry criteria are protocol specific, often requiring the development of specialized data entry forms. The Group C program is generally open to all qualified U.S. and international physicians. These physicians register the patient with DMAS. Once registered the physician agrees to obtain patient informed consent and, as necessary, Institutional Review Board approval. Investigators are required to report any adverse drug reactions and, depending on the agent, may be required to submit patient response reports.

The current drugs in Group C status are Amsacrine for refractory acute myelogenous leukemia (AML), 5-Azacytadine for refractory AML, 2-Chlorodeoxyadenosine (2CdA) for Hairy Cell Leukemia, Erwinia Asparaginase for acute lymphocytic leukemia (ALL) for patients sensitive to E. coli Asparaginase, and Teniposide for refractory ALL. Taxol is available on a limited access Group C protocol, due to limitations in supply.

During the first half of this year extensive section resources were directed toward registering physicians and patients for the use of Fludarabine. Prior to marketing availability in January 1992, the average monthly registration for fludarabine was 208 patients to the Group C protocol and an additional 120 patients for Special Exception protocols.

Two Group C drugs attained commercial status this year. Fludarabine for refractory chronic lymphocytic leukemia (CLL), and Pentostatin for refractory Hairy Cell Leukemia. Two new drugs were added to Group C this year. 2-CDA for Hairy Cell Leukemia and Taxol for refractory ovarian cancer. DMAS was instrumental in the development of these protocols.

The following is a summary of fludarabine activity during the two plus years it was under the Group C program (November 20, 1989 through December 5, 1991). A total of 4,009 patients were registered for the Group C protocol and 1,767 patients were approved for Special Exception protocols.

It is worth while to review the steps of the typical Group C registration. Initially an investigator calls DMAS to discuss the patient's case with the clinical research pharmacist. When possible, attempts are made to refer the patient to an existing clinical trial. If the patient is ineligible, unable or unwilling to enter a clinical trial and the patient qualifies for the Group C protocol the request is approved. If the physician is not already registered with DMAS, they must complete an FDA 1572 form (statement of Investigator). The quantity of the initial drug shipment is calculated, (typically a 2 month supply), and then shipped. A patient specific registration letter is generated and shipped with the Group C registration packet which includes the Guideline protocol, informed consent, the agent's clinical brochure, investigational drug accountability forms, and adverse drug reaction reporting forms. Shipment records are then maintained and reorder requests are considered as long as the investigator remains in active status and in good compliance. The typical Group C request may involve a number of telephone (or FAX) conversations as well as DMAS resources to complete.

Over the past year, for all Group C drugs, more than 1,153 new patients were approved to receive treatment and 18,216 drug orders, (original and reorders), were processed by DMAS.

SPECIAL EXCEPTION PROTOCOLS

Special Exception protocols or Compassionate protocols are used to treat patients who are refractory to standard measures. The Special Exception mechanism is the functional equivalent of a compassionate IND but differs from it in that the investigator may obtain drugs directly from DMAS, instead of having to obtain an IND from the FDA. There must be published objective evidence that the investigational drug is active in the disease for which the request is being made. In addition the patient must be ineligible, unable or unwilling to enter a clinical trial. Occasionally the Special Exception mechanism is used in instances when a patient fails to meet the entry criteria for a Group C protocol.

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 contacts DMAS and discusses the case with a clinical research pharmacist. The prior treatment, performance status, blood chemistries and organ functions are reviewed and the proposed treatment is presented. The basis for the proposed treatment must also be presented. This rationale should be based on published or known objective evidence of efficacy of the agent for the patient's diagnosis.

Unlike Group C where the patient is joining an established treatment protocol, Special Exceptions require that the physician complete and return an individualized single patient protocol. Before therapy is initiated the investigator must obtain Institutional Board Approval and patient informed consent. Special exception protocols are filed with the FDA. Physicians must report any Adverse Drug Reactions. The Report of The Independent Investigator, must be submitted to DMAS at the completion of therapy to document patient outcomes.

In the past year DMAS has formalized the Special Exception process for two agents, 2-chlorodeoxyadenosine (2CdA) and All-Trans Retinoic Acid (TRA). A drug/disease specific protocol and registration forms were developed for TRA for acute promyelocytic leukemia and 2Cda for hairy cell leukemia to simplify and standardize the Special Exception mechanism, to assist the investigators, and to facilitate DMAS review and monitoring. A specific informed consent was also developed for TRA to address potential toxicities with this agent. 2CdA was subsequently approved for Group C status for this indication.

DMAS revised the guidelines for approval of Special Exception requests. These guidelines are jointly developed by DMAS and IDB Senior Investigators and are used by the DMAS Clinical Research Pharmacist to approve Special Exception requests. These guidelines were updated to assure that they are current and represent the intentions of the Senior Investigators and CTEP.

To date for FY92, the average number of Special Exception is 122 per month. Through June 30, 1992 1,538 Special Exception requests were made, 1,095 Special Exception requests were approved and 134 patients were referred to ongoing clinical trials.

TREATMENT REFERRAL CENTER (TRC)

The TRC was originally organized to: 1) manage inquiries about specific agents in short supply (e.g. taxol and/or taxotere for breast and ovarian cancer), 2) ensure that appropriate treatment options are considered and, 3) optimize patient referrals to clinical trials. Emphasis is given to directing patients to regional NCI designated cancer centers.

The Treatment Referral Center (TRC) has been active in the Drug Management and Authorization Section of the Investigational Drug Branch since January 1991. In the fiscal year October 1, 1991 to September 30, 1992 the TRC has been very active secondary to increased recognition and the activation of the TRC protocol for taxol in refractory ovarian cancer.

The TRC also is tasked with developing protocols for patient populations that would not be eligible for ongoing clinical trials if available data indicate that treatment with certain investigational agents is reasonable. These protocols are offered to NCI cancer centers. Initially patients with ovarian cancer who had failed multiple prior therapies were recognized as a population that could benefit from treatment with taxol. However there were no clinical trials available to these patients. Therefore the TRC developed the first Treatment Referral Center Protocol, TRC-9108, Taxol (NSC 125973) in Refractory Ovarian Cancer. The Protocol was activated in September 1992 and is currently active at 39 Cancer Centers. Over 1,500 patients have been registered on study as of July 1992 and registrations continue at a rate of approximately 160 patients each month. A protocol to provide taxol for patients with refractory breast cancer after multiple prior therapies is in development and should be active before October 1992. Taxotere for either ovarian or breast cancer may be considered for inclusion in the TRC mechanism if responses seen in Phase I trials are verified in Phase II studies.

The TRC currently is handling 300 to 350 telephone calls a month. These calls can be broken down into three categories. 1) Drug information - approximately 150 calls per month asking for information about obtaining taxol or taxotere to treat various diseases. The most common diseases are breast and ovarian cancer. 2) Protocol registration - approximately 160 calls a month to register patients on the TRC protocol, TRC-9103. 3) Protocol management - approximately 30 calls per month for protocol management. With the activation of the taxol study in breast cancer these numbers are expected to double or quadruple.

NEW COMPUTER SYSTEM DEVELOPMENT

In July 1991, the DMAS completed the implementation of a new relational database Drug Distribution Computer System (DDCS). This system provides the overall management of Investigational Clinical Supplies for studies sponsored by NCI. Additionally, it effectively controls the registration of over 7,137 Clinical Investigators. Expansion of this system from 15 to 27 workstations was a major accomplishment. Flexibility within DDCS allows the DMAS to effectively manage and distribute drugs in limited supply.

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 150 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. It has thus helped to reduce drug costs to NCI. Plans to enhance and update the system are underway.

PROTOCOL REVIEW ACTIVITIES

The DMAS continues to expand its 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 drug information content of specific protocol sections, which include the pharmaceutical data, treatment plan, dose modifications, and drug supply section.

Pharmaceutical data sections are reviewed for adequate product description including available dosage forms, ingredients, and packaging as appropriate. Information regarding the supplier of the agent is also required. The directions for preparation of the drug, including reconstitution and further dilution, are evaluated for completeness and clarity. The stability and storage requirements for the original dosage form, the reconstituted solution, and the final diluted product are examined for quality assurance and control. A detailed description of the method of administration including route, rate of administration, and any precautions that the health care professional should be aware of to ensure patient safety should complete the pharmaceutical data section.

Treatment plans and dose modifications are evaluated for accuracy as well as clarity to help guarantee protocol compliance and proper administration of the investigational agent. In addition to reviewing each protocol for correct and up to date pharmaceutical and treatment information, clinical brochures,

Investigational Agent Acquisition and Quality Control

In October, 1991, due to a programmatic reorganization, responsibility for obtaining investigational drugs for NCI-sponsored studies was assumed by the Drug Management and Authorization Section. Two pharmacists are responsible for maintaining adequate supplies of investigational drug products which are obtained from pharmaceutical manufacturers. This requires extensive negotiation among CTEP personnel, industry scientists, and clinical investigators. In addition, DMAS staff members manage the purchase of commercial antineoplastic agents for several intramural programs, and interact with NCI staff in the Developmental Therapeutics Program in order to assure supplies of drugs which are manufactured by NCI contractors. DMAS pharmacists review and approve analytical data and labeling from each new lot of drugs that is received. DMAS staff monitor current expiration information for each lot of drug which has ever been received, and notify NCI investigators as updated stability information is obtained.

Storage and Distribution of Clinical Drugs

The Drug Management and Authorization Section manages a contract for the NCI Clinical Drug Repository, from which clinical drug products are sent to authorized investigators. Repository staff receive large shipments of drugs and antibodies from a variety of pharmaceutical manufacturers throughout the U.S. and the world. Incoming shipments are carefully inspected and stored at the specified temperature. Approved Clinical Drug Request Forms are forwarded to the Clinical Drug Repository, where dosage forms are repacked into shipping containers and shipped to clinical sites. Dry ice shipments, wet ice shipments, and rush deliveries are often necessary. Computerized recordkeeping accompanies each step of the receipt, storage and distribution of these medications. The contractor also provides pharmacists and support staff who perform patient-specific blinded labeling, distribution to individual clinical sites, and computerized recordkeeping for large, randomized double-blind clinical trials in prevention of breast cancer and other cancers.

DRUG COST EXPENDITURES

The total drug costs for FY91 were $ 2.867 million for cancer and $ 120,000 for AIDS. In FY91, because of budget cuts CTEP provided chemotherapeutic agents to the Clinical Center for approximately the first 5 months with the remainder coming from the NIH management fund. The allocated budget for FY92 is the same as FY91. Effective October 1991 responsibility and administration of the DCT drug budget was transferred to DMAS.

Drug Distribution Data for the Past Year

| Number of Orders (Line Items) | New Group C Orders (Reorders) | New Special Exception Protocol (Reorders) | Total units (Btls, etc.) Distributed |
|---|---|---|---|
| 25,089 (37,077) | 2,157 (5,517) | 1,986 (1,944) | 951,465 |

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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 investigational 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 investigational antiAIDS agents;

4. Coordination of responses to correspondence from FDA regarding INDs and subsequent 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 in developing the preclinical data required for investigational anticancer and antiAIDS agents;

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) to facilitate the negotiation of assurances and to help formulate policy for the protection of human subjects;

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 Division of Scientific Investigations, FDA; and

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

In addition, the Regulatory Affairs Branch is responsible for developing and implementing Clinical Development Agreements and Cooperative Research and Development Agreements with pharmaceutical companies for the codevelopment of investigational anticancer or antiAIDS agents. Secrecy Agreements and Material Transfer Agreements are also established when appropriate.

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 -

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

Linda Alms, M.G.A.

Gary Smith, M.G.A.

A summary of the activities for FY '92 includes:

1. 38 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 13 agents were inactivated.

3. During CY '91 315 adverse drug reactions were reported to FDA. An additional 1247 adverse drug reactions were received, reviewed and held for reporting to FDA through the Annual Reports. In addition, 74 adverse drug reactions for commercial drugs were received and evaluated.

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

5. On-site audits were made to 16 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 130 member institutions, 165 affiliates and 43 CCOPs (or CCOP components).

7. Meetings were held with the 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 was held. The meeting was co-sponsored by the FDA, NCI and NIAID.

10. Developed four Cooperative Research and Development Agreements and eleven Clinical Development Agreements with pharmaceutical companies.

## DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY '92, 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 |
|---|---|
| 9-Amino-camptothecin | NSC 603071 |
| Anthrapyrazole (DUP 937) | NSC 355644 |
| Anthrapyrazole (DUP 941) | NSC 357885 |
| Bryostatin I | NSC 339555 |
| CAI | NSC 609974 |
| CPT-11 | NSC 616348 |
| Cyclopentamyl Cytosine (CPE-C) | NSC 375575 |
| Fenretinide (4-HPR) | NSC 374551 |
| Fostriecin | NSC 339638 |

| Agent | NSC Number |
|---|---|
| Hydrea IV | NSC 32065 |
| Penclomidine | NSC 338720 |
| PSC 833 (Cyclosporin A Analogue) | NSC 648265 |
| Rhizoxin | NSC 332598 |
| Sulofenur | NSC 645012 |
| Taxotere | NSC 628503 |
| Temozolomide | NSC 362856 |
| Thymopentin | NSC 645363 |
| TNP-470 | NSC 642492 |

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

| Agent | NSC Number |
|---|---|
| Adenosine Deaminase Gene Therapy | Not Assigned |
| Anti-Idiotype (Lymphoma) | Not Assigned |
| Carboxypeptidase G2 | NSC 641273 |
| CD3+/CD8 Selected IP TIL | Not Assigned |
| Human Growth Hormone and Insulin-like Growth Factor-1 | NSC 648119<br>NSC 648210 |
| 131-I-Human Serum Albumin | Not Assigned |
| IL-2 Transduced Autologus Cancer Cells/TIL/IL-2 | Not Assigned |
| IL-4 | NSC 618085 |
| IL-6 | NSC 643497 |
| Interferon Beta | Not Assigned |

| Agent | NSC Number |
|---|---|
| Interferon Gamma | NSC 635256 |
| Monoclonal Antibody 14.18 Chimeric | NSC 623408 |
| Monoclonal Antibody 177-LU-CC49 | NSC 647944 |
| NeoR Marked PBSC (AML) | Not Assigned |
| NeoR Marked PBSC (Breast) | Not Assigned |
| NeoR Marked PBSC (Myeloma) | Not Assigned |
| NeoR Marked TIL-PBL | Not Assigned |
| PIXY (GM-CSF/IL-3 Fusion Protein) | NSC 645014 |
| Recombinant CEA-Vaccinia Vaccine | Not Assigned |
| TNF-TIL | Not Assigned |

INDs Inactivated.

The INDs for the following agents were inactivated:

| Agent | IND Number |
|---|---|
| Beta Interferon | IND 2533 |
| Dichloromethotrexate | IND 3168 |
| Dideoxyadenosine | IND 30,971 |
| Dideoxyinosine (Hepatitis) | IND 33,746 |
| Flavone Acetic Acid | IND 26,886 |
| IL-2 + Educated Lymphocytes | IND 2940 |
| IL-2 + Monocyte Depleted and Expanded LAK | IND 2946 |
| Monoclonal Antibody B6 | IND 1833 |
| Monoclonal Antibody HeFi-1 | IND 2400 |
| Monoclonal Antibody 9.2.27 | IND 1793 |

| Agent | IND Number |
|---|---|
| Monoclonal Antibody 45-2D9 | IND 2262 |
| Pibenzimol | IND 23,268 |
| Spiromustine | IND 21,354 |

The Regulatory Affairs Branch currently maintains 185 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 '91 315 adverse drug reactions were reported to FDA. An additional 1247 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 the CTEP-Information System.

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.

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 resulting from the recent FDA/NCI/NIAID Workshop on Preclinical Testing and Evaluation of Monoclonal Antibodies. The Section's staff assisted in the planning and were major participants in this workshop. A monoclonal antibody guideline was also established for monoclonal antibody contractors, companies and grant applicants. The guideline 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 and extramural investigators in obtaining FDA approval for clinical therapies involving retroviral gene insertion. To date 13 INDs have been approved for treatments involving retroviral gene insertion. DRAS staff assisted in the organization of and participated in the National Cancer Institute Meeting on Implementation Grants for Gene Therapy Programs. 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 FDA submissions including protocols and protocol amendments approved by CTEP.

Initiated a revision of the Cancer Therapy Evaluation Program Investigator's Handbook. This Handbook serves as a manual for participants in clinical trials sponsored by the Division of Cancer Treatment, NCI.

The Section's professional staff serves on the 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.

Proposals from pharmaceutical companies were requested for the codevelopment of three orphan investigational anticancer agents (9-Amino-camptothecin, BUDR/IUDR and Suramin). The proposals were reviewed and evaluated and a company selected for each agent. Cooperative Research and Development Agreements were developed and implemented. A Cooperative Research and Development Agreement was also developed for Taxotere. In addition, eleven

Clinical Development Agreements for various investigational anticancer agents were negotiated and implemented.

## 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.

### 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 130 member institutions, 165 affiliates and 43 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, 16 visits to Cancer Centers or other single institutions conducting trials with DCT/NCI-sponsored investigational agents were accomplished.

### Additional Monitoring Activities.

Five special audits were carried out to examine the data and verify response determinations in promising Phase II trials. These included: Antineoplastons in brain cancer, Taxol in non-small cell lung cancer and small cell lung cancer, Taxol in ovarian cancer and Homoharringtonine in CML.

Protocols.

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 also 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 initiated a review of the monitoring procedures of each of the Cooperative Groups to determine if revisions are required.

The Section staff completed quality assurance review of 18 patient cases entered on the Group C protocol for Deoxycoformycin and 19 patient cases entered on the Group C protocol for Fludarabine Phosphate.

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

Three for-cause audits were conducted.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1991 - September 30, 1992

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. This Office also supports a Biostatistics Data Management Section, supervised by Dr. Seth Steinberg.

CLINICAL PHARMACOLOGY BRANCH

**1. Major Personnel Changes:**

None

**2. Major Advances:**

--Suramin: The use of this drug has improved to the point where nearly all patients are treated as outpatients. We have now demonstrated that, in addition to prostate cancer, this drug has significant activity in indolent non-Hodgkin's lymphoma. Parke-Davis has won the rights to a CRADA with NCI to conduct final trials to qualify for an NDA. At NCI, a trial testing the combination of leuprolide, flutamide and suramin in previously untreated patients has yielded a high response rate and modest toxicity.

--Aminoglutethimide: This drug normally has a response rate in prostate cancer of 15%. After suramin, the response rate to this drug is in excess of 50%.

--Lovastatin, an inhibitor of HMG-CoA reductase, has been shown to trigger death of hormone refractory prostate cancer cells at drug levels approximately 20-50 times those attained in the use of this drug to treat prostate cancer. A Phase I trial testing the safety of these lovastatin levels is in progress. To date, we are giving 10 times the normal dose without any toxicity. Only minor tumor responses have been seen to date.

--Phenylacetate, a major component of antineoplastons, shows considerable activity against both prostate cancer and brain tumors in vitro and brain tumors in vivo. A Phase I protocol testing the use of this agent has been approved by NIH and we are awaiting IND approval.

--P2 receptor agonists have been shown to arrest the growth of prostate cancer cells. These agonists appear to trigger an IP3-dependent calcium wave that preceeds these biologic effects. Several analogs have been identified which offer promise as therapeutic agents.

--Several benzoquionone ansamycins show significant activity against prostate cancer, melanoma and multiple myeloma. Two of these compounds, geldanamycin and macbecin, have passed DN2A.

--Pharmacokinetics Laboratory: This past year, we have dramatically expanded the size and scope of our pharmacokinetics group. In the coming year, we plan to see it offer much better support for Phase I-II trials in both the Medicine Branch and the NCI-Navy.

**3. Plans for the Coming Year:**

**Prostate Cancer**

Clinical: Phase II trials testing the combination of suramin and aminoglutethimide in hormone refractory prostate cancer and suramin, leuprolide and flutamide in patients with untreated metastatic prostate cancer will be the major focus. Phase I trials on phenylacetate and lovastatin will also be major effects. Clinical pharmacology support for the AIDS trials, beginning with fumagillin, will also be a high priority.

Laboratory: We will continue to search for agents which trigger physiology death on the part of hormone refractory prostate cancer.

**Glioblastoma**

Clinical: Phenylacetate and suramin show activity against glioblastoma in vitro and will be tested against the disease in Phase II trials during the coming year.

Laboratory: We will continue to search for agents with promise in the treatment of this tumor.

MEDICINE BRANCH

During the past year several significant organizational changes occurred in the Medicine Branch. With the appointment of Dr. Carmen Allegra as Chief of the NCI-Navy Medical Oncology Branch, the Allegra laboratory was transferred to Navy at the end of

1991. In addition, plans were initiated to transfer the clinical trials activity in gastrointestinal cancer to the Navy as soon as feasible; this will probably occur by the end of 1992. With this move we expect that the Navy's patients with cutaneous T-cell lymphoma will move to the Medicine Branch, thus bringing all of COP's medical oncology activities in lymphoma under one roof. Dr. Louis Matis was recruited from the BRMP as head of the Experimental Immunology Section; Dr. Matis is expected to move with his laboratory group sometime in the summer of 1992. Dr. Ron Gress, currently in the Experimental Immunology Branch (DCBDC), was recruited to establish a Transplantation Therapy Section, which will focus chiefly on the antitumor potential of the marrow graft itself. The Medical Breast Cancer Section plans a significant redirection of its activities toward the area of retrovirally mediated gene transfer into hematopoietic stem cells. The initial study examining feasibility with a marker gene (*Neo*) has been approved by the NIH's recombinant DNA advisory committee. After completion of this study, the group plans to attempt transfer of the *mdr1* gene, to see whether bone marrow can be made more resistant to the toxic effects of cytotoxic natural products. If this is successful, other drug-resistance genes may follow.

During the past year clinical activities in the Branch remained largely targeted on five general disease areas: breast cancer, colorectal cancer, lymphoma, ovarian cancer, and AIDS. Approaches were focused on the following themes: (1) the effect of high-dose or dose-intense therapy in a variety of tumor types, including breast cancer, ovarian cancer, and lymphoma; (2) modulation of multidrug resistance; (3) early clinical trials of new agents in cancer and AIDS. Selected findings and activities are described briefly below; although the clinical efforts in GI cancer continue in the Medicine Branch up to the present, highlights of this particular program will be presented in the Annual Report of the NCI-Navy Branch. In collaboration with investigators in the BRMP and the ROB we continued with the implementation of a new generation of lymphoma studies, several of which have been activated.

**NOTEWORTHY CLINICAL RESULTS**

1. Clinical trials in the use of **modulating agents to reverse drug resistance** are an area of expanding 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 antitumor effect in approximately 12 of 44 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. Several additional studies in resistance

reversal will be activated in 1992; these include: (a) A trial of a three-drug combination (cytarabine, etoposide, and idarubicin) in acute myeloblastic leukemia; patients failing one cycle of this regimen will cross over to the same regimen plus R-verapamil. The study will include attempts to document effects of the reversing agent on intracellular idarubicin accumulation. (b) A phase I study of the non-nephrotoxic, non-immunosuppressive cyclosporin analogue PSC 833 in combination with vinblastine in patients with advanced solid tumors; this will be the first trial of this promising reversing agent in cancer patients. (c) A phase I study of R-verapamil in combination with taxol and G-CSF.

2. 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 to minimize the duration and depth of neutropenia. Examples of ongoing studies of this approach include: (a) FLAC + escalating doses of IL3, with and without GM-CSF in advanced breast cancer; the aim is to define the MTD of IL3 in this setting and to determine the extent of marrow sparing with this pair of cytokines, as well as effects on peripheral-blood stem-cell numbers. (b) Doxorubicin + taxol + G-CSF in advanced breast cancer (Phase I) has been completed; surprisingly the dose-limiting toxicity has been typhlitis. (c) Piroxantrone + G-CSF in breast cancer (Phase I)has been completed. Piroxantrone's MTD with G-CSF is roughly twice the MTD without it. Prior treatment with doxorubicin appears to be a strong predisposing factor for the development of cardiac toxicity. The drug showed modest antitumor activity in Phase I. (d) 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; at this dose the response rate is 50% (22/44 patients), which seems higher than that previously reported for lower doses. A randomized comparative trial of high and low taxol doses will be undertaken by the Gynecologic Oncology Group. (e) 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. In view of the impressive degree of activity with EPOCH, however, and the demonstration in a large multicenter trial of the lack of obvious superiority of ProMACE-CytaBOM over CHOP, we shall explore the activity of EPOCH in this patient group in our next trial. (f) ICE (ifosfamide, carboplatin, etoposide) plus interleukin-1 and autologous bone marrow reinfusion in patients with lymphoma and selected carcinomas. A phase I trial of the ICE combination established a safe level for further study; we are currently exploring whether pretreatment of the patient with escalating doses of IL-1 results in significant shortening of time to marrow reconstitution. Future efforts will focus on the addition of other cytokines to the ICE-IL1 combination.

3. **Early clinical trials of new anticancer 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 has been completed; the drug is tolerable but appears to have disappointing antitumor activity, even allowing for the pretreated nature of the patient population. (b) CAI, a signal-transduction inhibitor with broad-spectrum activity in murine models, entered phase I study in early 1992. The antitumor and antimetastatic properties of this compound, as well as its putative mechanism of action, were delineated in the Liotta laboratory. (c) $^{177}$Lu-CC49, a beta-emitting radiolabeled monoclonal antibody from the Schlom laboratory with panadenocarcinoma (TAG-72) specificity. Three patients have been treated thus far in a phase 1 study which also seeks to determine whether metastases can be imaged successfully.

4. **Clinical studies in HIV infection.** This year saw the FDA approval of ddI and ddC for particular indications in the HIV-infected patient population. At this time, therefore, the only three agents known to be safe and effective for HIV infection were discovered and initially developed by Mitsuya, Yarchoan, Broder, and their colleagues in the intramural program. Clinical studies of new antiretroviral agents continue in the Medicine Branch; drugs of current interest include the (-) enantiomer of 3'-thiacytadine (3TC), in collaboration with Glaxo, and an HIV protease inhibitor due to start clinical trials shortly. Active combination studies are examining the advantages of administering 2 or more dideoxynucleosides simultaneously or sequentially, in an attempt to suppress the emergence of resistance. In one such trial (simultaneous versus sequential AZT and ddI) in patients without significant prior therapy, it appears that CD4 rises are greater and more sustained with the simultaneous regimen. A retrospective analysis of patients treated with antiretroviral chemotherapy has shown that patients on AZT or ddI rarely die until their CD4 counts fall below 50. If confirmed in independent studies elsewhere, this may enable the use of CD4 counts as a surrogate for mortality in clinical trials. In long-term follow-up studies of the cohort of patients treated on antiretroviral regimens, of a total of 116 patients treated with either AZT or ddI, 12 have developed high-grade B-cell lymphomas. Thus after 24 months of therapy the estimated risk of developing NHL is 8.4%; this rises to nearly 20% after 36 months of follow-up. Risk is clearly correlated with CD4 count below 50. It also seems that patients who go on to develop lymphoma have a higher initial plasma level of circulating IL-6 than those who do not. Currently patients with B-cell lymphoma in the setting of HIV infection are being treated with infusional chemotherapy.

**LABORATORY STUDIES**

1. **Drug Resistance in Breast Cancer.** Studies directed at clarifying mechanisms of resistance in breast cancer cells have identified several previously undescribed non-P-glycoprotein

(PgP) mechanisms. Specifically, a mitoxantrone-resistant line has decreased levels of drug accumulation and enhanced drug efflux in the absence of detectable PgP or mdr1 gene expression. Membrane proteins have been identified that is overexpressed in this line, suggesting the possibility of a novel drug efflux pump distinct from PgP. Similarly, a line resistant to etoposide has been isolated which appears to have decreased drug accumulation in the absence of PgP and also altered topoisomerase II activity. Further studies in these lines are in progress.

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 progesterone receptor, and preliminary analysis in 60 patients with node-negative breast cancer suggests that expression of GST-p is associated with a poorer prognosis; this result is independent of hormone receptor status, tumor size, patient age, or nuclear grade. A much larger data base is now being analyzed in a collaborative study with the National Surgical Adjuvant Breast and Bowel Project (NSABP). Studies on the regulation of the GST-p gene suggests that control of expression is predominantly post-transcriptional. Studies to identify the responsible DNA sequences are in progress.

2. **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, the occurrence of genetic polymorphism at the mdr1 locus has permitted an analysis of the genetic events that occur during selection for resistance to natural products. Briefly, in the course of selection, overexpression of one or both alleles may occur in association with amplification of an individual allele as resistance evolves. During selection one clone becomes the predominant or exclusive member of the population, as shown by identical patterns of overexpression and/or amplification in selected subclones. How this information applies to clinical samples is not yet clear, inasmuch as in vitro selection procedures are quite different from drug treatment in vivo. Preliminary data suggests that overexpression of one or both alleles can occur.

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. PKC inhibition with a variety of pharmacologic agents results in decreased phosphorylation of PgP; this is associated with impaired transport of vinblastine, actinomycin D, doxorubicin, daunorubicin, azidopine, and cyclosporin, but increased transport of progesterone and verapamil. These effects may be mediated by changes in binding affinities of drug substrates for PgP. In related studies, 8-Cl-cAMP downregulates expression of mdr1 mRNA and PgP expression and

increases drug accumulation; modulators of PKA do not appear to affect *mdr1* expression. Studies with antisense oligos to the subunits of PKA may help clarify the role of this enzyme in the multidrug-resistance phenotype.

3. **Platinum Resistance.** 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 the ERCC1 gene and resistance to platinum-based therapy. Of great interest are data suggesting that, for patients treated with cisplatin or carboplatin alone, the extent of platinum-DNA adduct formation 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 a recently completed collaborative study in testicular cancer patients treated with multidrug combinations, sister chromatid exchanges, rather than DNA adduct formation, appeared to correlate with response to treatment. In either case extent of DNA damage in one normal tissue compartment appears to correlate with antitumor effect.

In separate studies, the selection of about 15 platinum-resistant ovarian, breast, colon, and squamous cell lines and several sensitive revertants has permitted a search for previously unreported mechanisms of resistance. These studies have revealed reduced cisplatin accumulation in all, along with constitutive metallothionine expression and cisplatin resistance. A 55 kD protein appears to be a correlate of the resistant phenotype; this protein is currently being purified and sequenced. An entirely novel observation is the occurrence of changes in certain cytoskeletal proteins in the resistant lines.

4. **Folate Binding Proteins and Methotrexate Resistance.** In nine methotrexate-resistant KB cell clones grown in the presence of physiological concentrations of folate, analysis of resistance mechanisms shows that 9/9 have transport defects and that the extent of reduction in folate transport (Vmax) correlated with reduction in the expression of the FBP. Four of the nine had increases in DHFR activity (1.5-9 fold higher than WT). Stability of this phenotype after 6-9 months in culture suggests a mutational mechanism, a possibility which is being investigated by sequencing studies currently in progress. Transfection of the FBP cDNA into mutant cells improves growth, particularly in folate-depleted medium, and restores methotrexate sensitivity. Studies in these mutant lines have also identified a 50 kD membrane-associated protein that is overexpressed in several of these lines and which cross-reacts with a polyclonal FBP antiserum. In wt cells, folate depletion increases expression and repletion decreases expression. This protein probably co-purifies with the FBP, rather than having shared epitopes with the FBP. Efforts are underway to clone and characterize this protein, which may have a role in normal folate homeostasis.

5. **Transcriptional Regulation of the *c-myc* Oncogene.** Efforts to identify and characterize recognition sequences within Intron I and corresponding trans-acting factors have 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; its negative effect is antagonized by MIF-1. Cells from patients with Burkitt's lymphoma frequently have mutations in the recognition sequences for MIF1-4 and in MIF-3 itself. 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. Studies are also in progress to see whether the intron 1 region in *c-myc* is a mutational hot spot in tumors other than Burkitt's lymphoma; colon, lung, and follicular lymphoma cells are currently being examined. Plans are to clone the MIF-3 protein and to study its interactions more fully. Attempts are also in progress to use oligodeoxynucleotides to inhibit the *c-myc* P2 promoter activity by triplex formation at the ME1 recognition sequence.

Exposure of cells to certain differentiating agents, such as retinoic acid, TPA, and DMSO, results in dramatic downregulation of *c-myc* expression and alteration of complex formation between nuclear proteins and *c-myc* DNA. Undifferentiated HL60 cells synthesize a protein with specificity for the MIF-1 sequence but which is distinct from the MIF-1 protein of HeLa cells. Cross-linking analysis suggests that undifferentiated HL60 contains two proteins that interact with the MIF-1 recognition sequence and that, upon exposure to differentiating agents, MIF-1 is induced and binds to its recognition sequence in the presence of these two proteins. Since it is known that 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.

6. **Antiretroviral Drug Discovery.** This work is resulting in the discovery and development of a number of promising leads. In collaboration with the Sanyo-Kokusako Pulp Company (Japan), a series of lipophilic dideoxynucleosides has been synthesized and tested. These agents appear active in vitro and, in preclinical studies in animals, appear to penetrate into the cerebrospinal fluid. Members of this class may, therefore, be useful in the treatment of HIV infection in the nervous system. Identification of an active HIV protease inhibitor is in progress. In collaboration with investigators at Kyoto Pharmaceutical University, two promising compounds have been identified; these appear to block replication of AZT-sensitive and AZT-insensitive viral isolates in vitro. Preliminary studies appear to show adequate bioavailability for clinical use. In a very important development, the technique of RNA PCR has been adapted for use in

the quantitation of circulating virus in the plasma of infected patients. This method seems to have a high degree of sensitivity and is quite reproducible. This method may be invaluable in the diagnosis of early infection, before immunological tests are positive, and, even more importantly, in permitting the quantitation of viral load as a function of therapy. Additional studies have addressed the question of how easily resistance to dideoxynucleasides develops in the course of therapy with these drugs; the data suggest that HIV-1 develops resistance to AZT more readily than to either ddI or ddC. In addition, an alternating regimen of AZT and ddC does not appear to block emergence of variants with decreased sensitivity to AZT. The clinical significance of these findings is at present unclear; they will have to be interpreted in light of the emerging clinical results from the trials comparing combined therapy to single-agent treatment.

7. **Cytogenetics.** Recent studies in cell lines infected with HTLV 1 and 2 and HIV 1 and 2 in vitro and in vivo revealed multiple chromosomal abnormalities, including breakpoints at 6q11-13 (3/6 HTLV-1 lines), involvement of 21p11 and +20 in both HTLV-2 lines, and abnormalities of 17, 3, and 21 in the HIV-1 lines. In collaboration with the Medical Breast Cancer Section, in-situ hybridization studies of the HGPXI and RHOH12 genes succeeded in localizing these genes to 3p21, confirming molecular studies showing that these genes are within 800 bp of each other. In collaboration with CPB, an HL60 line exhibiting vincristine resistance and myeloperoxidase positivity was shown to contain double minutes, which were absent in the peroxidase-negative cells.

## NCI-NAVY MEDICAL ONCOLOGY BRANCH

### Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

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. The cloning and characterization of chromosomal abnormalities is also 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. 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 five 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.

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 affect and alter the repertoire of immune receptor diversity, suggest that an underlying defect in AT may be chromatin "hyperaccessibility," and 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. 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.

We have localized several genes of interest to specific regions of human chromosomes. Using biotinylated probes we have mapped one putative neurogenic gene NSCL-1 to human chromosome 1q21 and a second, NSCL-2, to human chromosome 1q12. 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.

Pharmacology of Antimetabolite Agents

This project is divided into two broad areas that include the development of strategies for the treatment of solid tumors 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 resistance to the antimetabolite class of agents. These studies have identified that the treatment of patients and malignant cells in in vitro model systems with fluoropyrimidines and antifolate agents results in an acute induction of thymidylate synthase and dihydrofolate reductase respectively. Since the level of intracellular thymidylate synthase is an important determinant of sensitivity to fluoropyrimidine agents, the acute induction of this enzyme following fluoropyrimidine exposure is a central cause of resistance to these agents both in vivo and in vitro. Interferon gamma has been shown to repress the acute thymidylate synthase induction resulting from fluoropyrimidines. This repression results in enhanced sensitivity of malignant cells. While the precise mechanism of interaction between interferon with 5-FU represents an ongoing investigative effort, we have identified an unprecedented mechanism of autoregulatory translational control as a means by which cells regulate the intracellular levels of thymidylate synthase. Given the importance of thymidylate synthase as a chemotherapeutic target, we have developed sensitive assays for the quantitation of this enzyme in cells and human tissues using a monoclonal antibody directed against thymidylate synthase. The use of monoclonal antibodies has resulted in ultrasensitive detection of thymidylate synthase, quantitation of enzyme free and bound by fluoropyrimidines and quantitation on a per cell basis in human tissues and cells. The availability of these sensitive assays will help delineate the role of thymidylate synthase as a prognosticator of survival and response. The investigations of therapies for opportunistic infections is focussed on the interactions of antifolate agents on the metabolic pathways in *T. gondii, P. carinii* and micobacteria (MTb, MAI). We are currently using the tools of molecular biology to clone, sequence and express clinically relevant target enzymes for characterization and as an aid in the search for new therapeutic agents.

Molecular Genetics of Differentiation and Transformation

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 (MEL) cell line. By constructing and expressing mutant c-myb transgenes in MEL cells, we have demonstrated that the DNA binding and transactivation domains are necessary and sufficient for the c-myb mediated block. We have not yet been unable to determine whether these oncogenes block differentiation indirectly - by promoting proliferation - or directly - by disrupting the

differentiation pathway. Our goal is to understand the molecular mechanisms which 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 that are expressed in most murine plasmacytomas but rarely in B lymphomas. We have identified two classes of genes having this property: 1) genes differentially expressed in plasmacytomas and normal plasma cells but not in B cells; and 2) genes differentially expressed in plasmacytomas but not normal plasma cells. Curiously, a number of genes in the former category are expressed in pre-B and plasma cells but not B cells, suggesting shared functional properties of the cells at either end of the B-cell maturation pathway. We have not yet been able to demonstrate that either of two genes in the second category is a primary determinant of the malignant plasmacytoma phenotype. Our goal is to identify genes that not only mark but also determine the phenotypes of plasmacytomas and terminally differentiated normal plasma cells.

Preclinical and Clinical Pharmacology/Experimental Therapeutics

Colorectal cancer has proven refractory to most chemotherapeutic agents; the best available therapy is 5-fluorouracil (5-FU) modulated with leucovorin. A composite analysis of nine randomized trials indicates that the addition of leucovorin to 5-FU approximately doubles the response rate from 13% to 25%. The majority of responses are partial and not durable. Thus, although biochemical modulation of 5-FU with leucovorin has met with some success, innovative strategies are crucially needed to improve the prognosis of patients with colorectal cancer. Our current investigations focus on two areas. The first is the identification of new agents with potential activity against adenocarcinomas of the gastrointestinal tract. Of particular interest are new drugs which display potent in vitro activity (IC50 for a 24 hour exposure $\leq$ 10 mM) and/or in vivo efficacy against human colorectal carcinoma cell lines. Studies designed to elucidate the optimal schedule of administration and mechanism of action of such agents are vital to facilitate their rational clinical use. The second line of investigation includes the interaction of other agents with 5-FU in an attempt to define optimal doses and sequences of drug combinations for potential clinical use. Finally, we are implementing Phase I clinical trials which incorporate biochemical or molecular endpoints in addition to clinical endpoints as a reflection of the biologic activity of the particular agent. The ultimate goal is to develop new agents and drug combinations which may be useful in the treatment of patients with gastrointestinal and breast malignancies.

Molecular Genetic Events in Lung Cancer

A. We reviewed the serum sodium values of 263 lung cancer patients before the initiation of treatment. In contrast to none of the 130 non-small cell lung cancer (NSCLC) patients, 21 of 133 (16%) small cell lung cancer patients had hyponatremia ($p=0.0001$). Eleven of 21 patients with hyponatremia had tumor cell lines available and 9 expressed ANF mRNA, 7 expressed AVP mRNA, and 5 of 11 cell lines produced both ANF and AVP mRNA. All of the 11 cell lines produced ANF mRNA, AVP mRNA, or both. 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.

B. The atrial natriuretic factor A receptor mRNA has been detected on small cell lung cancer cells by PCR analysis of cDNA from the cell lines and RNase protection assay. Binding studies with iodinated ANF showed saturable binding but the number of sites were to low to accurately estimate. The cells respond to exogenously added atrial natriuretic factor with an increase in intracellular cGMP, similar to the normal receptors. Therefore, there appear to be functional ANF receptors on the surface of small cell lung cancer cells.

C. The serum and urine calcitonin values of 86 different individuals were studied by radioimmunoassay which included 20 nonsmokers, 17 smokers, and 49 patients with small cell lung cancer. The urine values were the most different in these three groups of individuals ($p<0.00001$). RNase protection assays (RPA) and RIAs of 11 tumor cell lines from these patients with small cell lung cancer showed some had elevated serum and urine calcitonin values but did not have ectopic calcitonin production in their tumor cell lines. Therefore it appears that elevated calcitonin levels in patients with lung cancer may come from a source other than the tumor cells.

Treatment of Patients with Small Cell Lung Cancer

A. A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. Forty-four patients have been entered onto study and 29 of 41 (71%) patients who have completed therapy have achieved a complete remission. The projected median survival is 24 months with a median potential follow-up more than 3 years. Seventeen patients have undergone biopsy for attempts at in vitro drug sensitivity testing. Seven of the 17 (42%) have been treated with an in vitro determined combination and those patients have survived for a median 39 months

B. The study of dose intensity using cisplatin and etoposide in patients with extensive stage studies continues. Eighty-three patients have been randomized to receive either VP-16 80 mg/m2 on

days 1-3 days with cisplatin 80 mg/m2 administered on day 1 (43) or etoposide 80 mg/m2 and cisplatin 27 mg/m2 on days 1-5 (40) every 3 weeks for the first 2 cycles and receive standard chemotherapy after the first 6 weeks. Although there is increased hematologic toxicity in patients treated with higher dose etoposide cisplatin, there is not difference in complete response rate, overall response rate, or survival between the high and low dose arm . The trial has completed accrual. This prospective study of dose rate intensity of etoposide cisplatin in small cell lung cancer does not show any survival advantage to administering 67% more chemotherapy in the first six weeks of treatment.

C. A monoclonal antibody (2A11) directed against gastrin releasing peptide (bombesin) which functions as an autocrine growth factor in small cell lung cancer has been used to treat patients with small cell lung cancer. Twelve patients have been treated with the phase II dose of 250gm/m2. One of the ten patients (10%) who completed therapy has responded with a clinical complete response.

Mechanisms of Oncogene Action in Tumorigenesis

We are investigating the role of tumor suppressor genes in the pathogenesis of human cancer. Our recent findings are as follows: 1) we have examined 130 lung cancer samples for inactivation of the retinoblastoma (RB) gene and have determined that 90-95% of small cell lung cancer and 15-20% of non-small cell lung cancer have homozygous mutations within this gene. We are completing a correlation of this data with clinical outcome and drug sensitivity testing to determine if RB testing offers prognostic information for patients with lung cancer; 2) using information obtained from our previous analyses of in vivo RB mutant proteins, we have constructed a series of in vitro mutations within the RB protein to define amino acid residues that are critical for RB function; 3) using RB fusion proteins as a reagent to molecularly clone RB-binding proteins, we have identified the interaction of the RB protein with a nuclear matrix product; 4) we have also studied the expression of another RB-binding protein (designated RBP-1) and have determined that it undergoes extensive alternative exon processing that modulates its interactions with the cell-cycle regulator, p34cdc2. We have also raised specific antisera against this 200kDa binding protein and are examining its biologic activity on cell growth. 5) we have successfully expressed RB protein in lung tumors and have observed suppression of tumorigenicity in nude mice. We have also observed that we can reverse the "tumor suppressor" effect with extracts enriched in extracellular matrix (ECM), and we are currently examining how ECM and RB pathways interact. 6) in collaboration with Dr. Crystal (NHLBI), we have constructed a series of retroviral and adenoviral constructs containing the open reading frames of RB and p53. Finally, we plan to use these vectors to determine the feasibility of in vivo tumor suppression in animal models.

Max, a Positive and Negative Regulator of Myc in Cell Growth and 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 recent findings which demonstrate specific interactions between Myc and Max resulting in a transcriptionally active complex, we investigated the regulation, expression, and role of the max gene in cell growth and differentiation of murine erythroleukemia (MEL) cells.

Etiology of Cutaneous T-Cell Lymphomas

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.

Transcriptional Regulatory Factors and Their Role in Malignant Proliferation

The zinc finger transcriptional regulatory factors constitute a family of early response genes. The recent cloning and characterization of four of the genes in this family by this laboratory has stimulated an investigation of the role of these factors in the malignant phenotype. Presumably, the

dysregulation of factors critical to cellular proliferation such as the zinc finger transcriptional factors could lead to unbridled malignant proliferation. Recent investigations from this laboratory have demonstrated that these early response genes are expressed in a constitutive fashion in T-cells transfected with either of the human retroviruses HTLV-1 or HTLV-2. Also of interest are preliminary studies that have demonstrated that the 225 zinc finger gene as well as other members of this gene family are also constitutively expressed in a variety of human malignant cell lines and tissues. The role of this constitutive expression in human malignant cells either in the pathogenic process or as a potential therapeutic target are the topic of ongoing and future investigations.

Major Staff and Administrative Changes in FY 1992

During FY92, the NCI-Navy Medical Oncology Branch has seen a major change in its staffing. The following individuals have been recruited as new members of the NCI-Navy Medical Oncology Branch:

Carmen J. Allegra, M.D.
Chief, NCI-Navy Medical Oncology Branch
Patrick Johnston, M.D.
James Drake
Jean Grem, M.D.
Pedro Politi, M.D.
Lorrin Yee, M.D.
Pamela Daychild
John Wright, M.D.
Bennett Yu, M.D.
Edward Chu, M.D.
Donna Voeller
Chris Takimoto, M.D.
Mike Hamilton, M.D.

Departures that occurred during FY92:

Dr. Peter Aplan who joined the staff at the Roswell-Park Memorial Institute.
Dr. Albert Lin who joined Dr. Tucker's group in the Epidemiology Branch.
Joseph Fedordo who retired.
Mercedes Gilliom, research nurse.
Rudy Phelps who joined DCPC.
Dr. Ilona Linnoila who joined DCPC.
Dr. Michael Birrer who joined DCPC.
Dr. Powel Brown who joined DCPC.
Dr. Frank Cuttitta who joined DCPC.
Dr. James Mulshine who joined DCPC.
Dr. Eva Szabo who joined DCPC.
Dr. Dennis Sanders who took a position at Boston University Hospital as a Staff physician.
Dr. Francine Foss who took a position at Boston University Hospital as a Senior Staff physician.

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 was 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 was 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 used 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 follow-up of study of 5.3 years, the event-free survival is projected at between 55 and 60 percent at four 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 treated 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 follow-up on study is 5-1/2 years. A total of 176 patients were 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. NCI-CCG 1911: 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).

This new ALL treatment protocol was initiated in January 1992. 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 new protocol, we are evaluating 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 is being carried out collaboratively with selected institutions of the Childrens Cancer Study Group (CCG).

4. Intrathecal Diaziquone (AZQ): AZQ is a lipid soluble aziridinyl benzoquinone designed for enhanced CNS penetration of the CNS to treat CNS neoplasms. We have evaluated the feasibility of intrathecal AZQ in a Phase I-II trial in patients with refractory meningeal malignancy. Two schedules of administration were originally 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 was 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.

5. 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 carried out 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) were evaluated. To date, 4 of 9 patients with CNS ALL treated on the twice weekly schedule have achieved complete responses. The remission duration ranges from two to five months. No significant toxicity has been observed. These results indicate that intrathecal 6-MP is safe and active against meningeal leukemia.

6. 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 is in progress.

7. 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 has been 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 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 been initiated.

8. Studies with Thiotepa: We carried out a multi-institutional Phase II study designed to assess the therapeutic efficacy of Thiotepa against brain tumors. Sixty patients have been entered (57 are evaluable for response). Three of 13 PNET tumors have had a partial response. Nine of the patients in the remaining tumor categories have had stable disease; no other responses have been noted.

9. 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 All-*trans* Retinoic Acid (all-*trans*-RA)

All-*trans*-RA is an agent which has demonstrated activity *in vitro* as a tumor differentiating agent. *In vivo* all-*trans*-RA has demonstrated significant activity in patients with acute promyelocytic leukemia. We recently initiated and subsequently completed a Phase I trial of all-*trans*-RA in pediatric patients with refractory malignancies. All-*trans*-RA was given orally on a q 12 hour schedule for 28 days. The starting dose was 45 mg/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$^2$. Pseudotumor cerebri was the dose limiting toxicity. Complete responses were observed in two patients with multiply relapsed acute

promyelocytic leukemia. Pharmacokinetic studies demonstrated that the plasma half-life of all-*trans*-RA was significantly shorter than that for 13-*cis*-RA. Furthermore, in the seven patients studied on day one and again on day 28 of therapy, a marked decrease in the plasma AUC of all-*trans*-RA was observed. These findings suggested that the metabolism of all-*trans*-RA differs from that of 13-*cis*-RA, as the 4-oxo-all-*trans*-RA metabolite was detected only in low concentrations, indicating that other metabolic pathways must be active. Further studies to evaluate this phenomenon were pursued in a primate model and demonstrated that all-*trans*-RA is metabolized by an inducible capacity-limited process, which accounts for decreasing plasma drug concentrations observed following chronic administration of all-*trans*-RA. These results have had profound implications on the planning of dose schedules for 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. A phase I pediatric trial is currently being performed to (1) determine the acute toxicity and MTD of amifostine (WR2721) in pediatric patients with refractory malignancies, (2) determine the maximum tolerated dose of melphalan when administered in conjunction with amifostine and to (3) study the pharmacokinetics of amifostine in pediatric patients.

Phase I Study of Topotecan

Topotecan, 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 multi-drug 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 has recently been completed. The dose-limiting toxicity was thrombocytopenia. A safe dose (5.5 mg/$m^2$) was identified for subsequent Phase II trials which will be carried out in the pediatric sarcomas, bone tumors and CNS malignancies. Interest in this agent as treatment for brain tumors is significant since our studies in primates have demonstrated

that parenteral Topotecan penetrates the blood/brain barrier to a significant degree (CSF/plasma ratio ≈ 30%).

Phase I trial of Pyrazoloacridine (PZA )

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. Extensive pre-clinical studies were performed in the primate which demonstrated that the proposed starting dose for PZA identified from studies in mice and dogs was significantly less than that which was found to be safe in the primate. These identified interspecies differences suggest the importance of preclinical evaluation of new agents in the primate model. Based on the results of our preclinical pharmacology studies a Phase I trial of PZA in children has been initiated. In the first part of this trial, the maximum tolerated dose of PZA given as a 1 hour infusion will be determined. Subsequently, the MTD of a 24 hour infusion will determined.

Phase I Trial of Intravenous 6-Thioguanine

6-thioguanine (6-TG) has been used for the treatment of cancer for more than 30 years, but has undergone only limited testing in pediatric cancer patients. 6-TG requires fewer metabolic activating steps to produce cytotoxicity than does the more widely used thiopurine, 6-MP. *In vitro* cytotoxicity studies with human lymphoblastic leukemia cells and cell lines were performed to compare the concentrations and duration of exposure of 6-MP and 6-TG required for cytotoxicity. Cytotoxicity was assessed in three human leukemia cell lines. These studies indicated that 6-TG has a distinct cytocidal advantage over 6-MP against lymphoblastic leukemia cells. With 6-TG, cytotoxicity occurred with 10-fold lower concentrations and was achieved with a considerably shorter duration of exposure. Review of published studies indicated that 6-TG has not previously been administered on a schedule which achieves and maintains the desired cytocidal plasma concentrations. Therefore, a pediatric phase I trial is being performed to determine the rate of continuous IV infusion of 6-TG required to achieve steady state plasma drug concentrations of approximately 1 $\mu$M (the maximum cytotoxic concentration). Following determination of the required dose rate, the maximum tolerated duration of exposure will be determined by increasing the duration of infusion in cohorts of patients by increments of 12 hours, starting with a 24 hour infusion.

Phase I Study 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) were studied following i.v. bolus and continuous i.v. infusion in rhesus monkeys. These studies defined significant differences in the pharmacokinetics of CPE-C in the primate compared to rodents and dogs. These interspecies differences in the disposition of CPE-C are important and information from these studies has been incorporated into the selection of the starting dose for this agent. Based on these Pediatric Branch findings an adult phase I study which incorporates a pharmacologically directed dose escalation strategy has been initiated. Preliminary pharmacokinetic results from this adult phase I study are similar to those predicted from the preclinical pharmacokinetic studies in our nonhuman primate model. A pharmacologically guided pediatric phase I trial recently has been initiated.

Phase I Study of Taxotere in Pediatric Patients with Advanced Neoplastic Disease

Taxotere, a semisynthetic analogue of taxol, is an antimitotic agent which induces microtubule polymerization resulting in nonfunctional microtubules. The antitumor activity of taxol, the naturally occurring analogue of taxotere, has become evident in phase I and II human trials. In preclinical studies, taxotere has demonstrated a broad spectrum of antitumor activity against both murine leukemias and solid tumor xenografts. These studies have demonstrated that taxotere has equivalent or superior *in vivo* activity versus taxol in some murine tumor models. Furthermore, the supply of taxotere is significantly more stable and predictable than that of the parent compound, taxol. We have developed a Pediatric Phase I Study of Taxotere. The purpose of this study is to determine the maximum tolerated dose and the dose-limiting toxicity of this agent (which has a novel mechanism of action) in pediatric patients with malignancies refractory to standard therapy. Taxotere will be administered to patients every 3 weeks as a 1 hour intravenous infusion using a starting dose equivalent to 80% of the adult MTD.

Phase I Study of EPOCH/R-Verapamil

Preliminary evidence suggests that drug resistance of pediatric sarcomas may be mediated through the MDR gene product p170 glycoprotein. R-Verapamil has been demonstrated to reverse MDR *in vitro*. In collaboration with the Medicine Branch we have initiated a trial in pediatric

sarcoma patients with recurrent disease to determine the MTD of R-Verapamil given with EPOCH (etoposide, prednisone, vincristine, cyclophosphamide and adriamycin). EPOCH is given without the reversing agent for the first two cycles. If there is no evidence of response the subsequent cycles of EPOCH are administered with R-Verapamil. This will allow us to determine the dose of R-Verapamil to use in future studies and to assess the response rate to EPOCH + R-Verapamil in patients resistant to EPOCH alone.

10. Late Effects. We have instituted a new protocol to study late effects of patients treated on all previous lymphoma protocols. Particular attention will be paid to growth and development, reproductive function and long term cardiac affects. In addition, a survey will be conducted to determine whether inherited genetic abnormalities (e.g., heritable p53 abnormalities) can be found in patients, and if so, in their families.

11. Protocol for HIV-associated lymphomas. A new protocol has been designed for the treatment of patients with lymphomas occurring on the background of an inherited or acquired immunodeficiency syndrome. The major emphasis will be on patients with HIV infection. The protocol, which will include G-CSF administration, is designed to ensure delivery of maximum dose intensity of the drugs predicted to be most effective -- namely methotrexate and cyclophosphamide.

12. Protocol 89-C-41. This protocol is designed for the treatment of small noncleaved cell lymphomas and large cell lymphomas and consists of low risk and high risk arms. There is a randomization to receive or not receive GM-CSF. Thirty patients have been entered to date. Two patients are presently on therapy and not yet evaluable, and a third who died within a week from RSV pneumonia, present prior to protocol entry, is not evaluable. Of the remaining 27 patients (five low risk and 22 high risk), all but one (stage III) achieved a complete response. Six of these patients had stage IV (marrow and/or CNS disease). One patient (stage IV) has relapsed so far at four months from completion of therapy. Nineteen patients have been followed for one year or more and remain free of disease. Their risk of recurrence at this time is very small. At present, there is no difference in the toxicity between GM-CSF treated or control patients.

13. 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.

14. 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 short duration 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. Accrual is nearing completion.

The pilot protocol for the treatment of high-risk sarcomas, 86-C-169, continues to monitor patients. There have been 80 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, 35 patients have been enrolled in this study.

15. 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. Thirty-five patients have been entered on this study. Preliminary data shows no significant increase in toxicity for patients receiving the ICRF-187.

16. 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 $\mu$M Ara-C induces growth arrest, 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.

17. 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 $\mu$g/ml and 200 $\mu$g/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.

18. 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.

19. 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 carboplatin or cyclophosphamide in order to prevent rapid, progressive disease, and receive additional IFN-g. Once sufficient TIL are grown, patients are treated with IFN-g followed by TIL and IL-2 administered in the intensive care unit. Five patients have been entered on this study. One of two patients completing therapy has responded.

20. 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, 23 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 has determined the regimens active in PNET without malignant glioma.

21. To determine the role of new b-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. An in-depth analysis of the data accumulated in this study is in progress, with emphasis on comparing NCI and Immunocompromised Host Society evaluation criteria, as well as a cost analysis of the two antibiotic regimens.

The recent development of potent, highly bioavailable oral antibiotics which provide a broad antibacterial spectrum has allowed us to initiate a study exploring the use of such oral agents as empiric therapy for neutropenic patients who become febrile. We have begun a double-blind, placebo-controlled, randomized comparison of oral ciprofloxacin plus amoxicillin/clavulanate with intravenous ceftazidime, our current standard therapy. The study population is limited to febrile neutropenic cancer patients who are clinically stable on presentation and are anticipated to have less than 10 days of neutropenia from the time of their initial fever. The two regimens will be evaluated with regard to their ability to provide safe, effective initial antibiotic therapy for febrile neutropenic patients, and specifically to determine whether the numbers of modifications of each primary antibiotic regimen will vary, reflecting any differences in the antimicrobial coverage provided. If the oral regimen proves to be comparable to standard intravenous therapy, there is the potential for neutropenic patients to be treated as outpatients during all or part of their febrile episode, with a large projected savings in

hospitalization costs and an improvement in quality of life for the patients. This study is anticipated to accrue over 200 patients in a 2 to 3 year span, in order to produce statistically evaluable data.

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.

- These experimental antifungal studies completed in our laboratory in rabbit models of acute, subacute, and chronic disseminated candidiasis provided the scientific rationale for design of an ongoing multicenter clinical trial to test the concept of early empirical antifungal therapy with fluconazole and for the first phase I-II trial of a systemic antifungal triazole (fluconazole) in children.

- We recently completed the first phase I-II pharmacokinetic study of fluconazole in children with cancer. This study found that fluconazole was safe and well-tolerated but had a shorter mean plasma half-life than that of adults, thus warranting the underscoring the need for separate clinical trials of fluconazole in children with cancer.

- In order to be able to monitor these patients receiving fluconazole for the emergence of resistance, we completed a multi-center trial for the standardization of methods for *in vitro* susceptibility of fluconazole.

- Applying these same concepts in high-risk adults with prolonged granulocytopenia, a phase I-II trial and a randomized, double blind placebo-controlled clinical trial of saperconazole (a broad spectrum antifungal triazole with activity against candidiasis and aspergillosis) is being initiated.

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 nearly 300 children, enrolling the majority into clinical trials.

23. 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, 32 patients have been enrolled.

24. We initiated a Phase I/II protocol to evaluate the effect of subcutaneously administered granulocyte colony-stimulating factor (G-CSF) in pediatric patients who developed an absolute neutrophil count below 0.8 x $10^9$/L while receiving AZT despite dosage reductions to 120 mg/$m^2$ every six hours. With doses of G-CSF ranging from 1 to 20 mcg/kg/d, 17 of 19 patients were able to tolerate 120 - 180 mg/$m^2$ of AZT every six hours. We conclude that G-CSF therapy enables patients who have had AZT related neutropenia to receive therapeutic doses of AZT. Since this initial evaluation an additional 13 patients have been treated with G-CSF according to this protocol. We are also studying the effect of human erythropoietin on overcoming AZT-induced anemia. To date 14 patients have been treated and we continue to assess the role and value of this treatment modality.

25. 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, 114 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/$m^2$/day. 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. Median CD4 cell count increased from 218/$mm^3$ at baseline to 327/$mm^3$ at 24 weeks (P=-0.001). Patients with baseline CD4 cell counts greater than 100/$mm^3$ 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. Pancreatitis

occurred in eight patients. Optic atrophy has been observed in four patients. Two patients stopped drug altogether and the atrophy has remained stable and has not affected their sight. One patient continued drug and another stopped drug and recently re-started. Both remain stable and their sight remains unaffected. All patients are monitored frequently for this.

26. "A randomized study to evaluate the safety and efficacy of AZT versus ddC/AZT in children with symptomatic HIV infection" had been initiated. This study will be performed in close collaboration with Dr. Jose Santos, Hospital Infantil de Mexico, Mexico City. We plan to enroll most patients in Mexico City. The protocol compares AZT 180 $mg/m^2$ q6 hrs po (Arm A) with AZT 180 $mg/m^2$ q 6 hrs po x 21 days, followed by ddC 0.03 mg/kg q 6 hrs po x 1 week (Arm B). A third arm combines AZT 120 $mg/m^2$ q 6 hrs po with ddC 0.01 mg/kg q 6 hrs po daily (Arm C). We plan to enroll approximately 50 patients into each arm.

27. 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 66 children have been enrolled at doses ranging from 60 to 180 $mg/m^2$ every 6 hours of AZT, and 60 to 180 $mg/m^2$ 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). This combination has been well-tolerated over the full range of doses, without evidence of new short or long term toxicities or enhancement of known toxicities, with over 20 children having been on study for over one year. 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 at all dose levels at both the 12 and 24 week major evaluation points. These data suggest that this combination is active *in vivo*.

28. All cases of *Mycobacterium avium-intracellulare* infection in our HIV patient population were reviewed and clinical and laboratory characteristics of infected children were determined. Nineteen 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 mean CD4% 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.

29. We completed a Phase I-II dose escalation trial of oral clarithromycin for pediatric patients with disseminated *Mycobacterium avium* complex infection. This study was being conducted in collaboration with the Children's Hospital of Los

Angeles. A total of 25 patients were enrolled at doses of 7.5, 15, and 30 mg/kg/day of clarithromycin. A decrease in low frequency hearing in a single patient is the only significant possible toxicity observed to date. Preliminary analysis indicates that clarithromycin suspension does not appear to affect AZT or ddI pharmacokinetics in children.

Short term improvements in energy levels, appetite and decreased fever were observed in most patients, however recurrence of symptoms has occurred after several weeks in most patients at all dose levels. Significant decreases in mycobacteremia were observed only at the highest dose level. Tolerance and toxicities of this agent, as well as the durability of clinical response, remain to be determined. The effective management of HIV-infected children with *Mycobacterium avium* infection will require multi-drug regiments, earlier treatment or prophylaxis, and prevention through better immune preservation. Analysis of quantitative mycobacterial cultures, clarithromycin susceptibility patterns and correlation with symptoms is currently underway.

30. 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 $\mu$g/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.

31. In April, 1992 we initiated a Phase I/II investigation of 2'-deoxy-3'-thiacytidine (3TC), a new nucleoside analog antiretroviral agent. This agent is most similar to ddC but exhibits less cytotoxicity *in vitro* than either ddC or AZT while retaining good antiviral activity. The study is designed as a two-armed trial of 3TC in previously untreated, relatively well HIV-infected children (Group A) and in more severely ill children who have experienced either disease progression or unacceptable toxicity on other therapeutic regimens (Group B). This dose-escalating study will investigate safety, pharmacokinetic profile, optimal dosing and preliminary efficacy of the drug in pediatric patients. To date similar adult trials have shown an excellent safety profile but have not yet identified either the maximal tolerated dose or the most efficacious dose. At present we have enrolled 4 children in the lowest dose level, 2 in each group and all have tolerated drug well; none have reached the 12 week evaluation point as yet.

32. We have demonstrated a relation between levels of CSF Quinolinic acid (an excitatory neurotoxin) and neuropsychological function: CSF Quinolinic Acid may function as a marker for HIV associated encephalopathy. In addition, we observed decrease in

CSF Quinolinic Acid with a concurrent increase in general cognitive function after anti-retroviral treatment: thus CSF Quinolinic Acid may function as a measure of therapeutic response.

33. We have demonstrated the specific vulnerability of expressive language to the effects of HIV infection in children. A significant discrepancy between expressive and receptive language was found both in encephalopathic and non-encephalopathic children. The magnitude of this discrepancy was related to the degree of CT-brain scan abnormality in the encephalopathic children. In addition, poorer immune status was associated with more impaired language functioning.

34. We have demonstrated that the level of psychological functioning in symptomatic HIV infected children prior to anti-retroviral treatment is related to the integrity of the CNS as visualized by CT-brain scans. CT brain-scans showed a lack of lateralized abnormalities, which seems to support the hypothesis, based on an analysis of intellectual profiles, that HIV encephalopathy in children has a global, stage like character, with stepwise decline in function.

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 is the topic of a prospective investigation in the new NCI 1911 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 which amplify the hypervariable region of Ig heavy chain also is 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.

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 suggests 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 which amplify the hypervariable region of Ig heavy chain rearrangements also is being evaluated.

3. 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 systematically 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 >10$\mu$M. 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 $CPDG_2$ 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. 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.

4. Pediatric Branch investigators 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. 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.

5. 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 Topotecan, a topoisomerase I inhibitor, 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, all trans-retinoic acid, piroxantrone, an anthracycline derivative synthesized to have decreased cardiac toxicity, taxol and taxotere 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.

6. We are attempting to define mechanisms of pathogenesis in the small noncleaved cell lymphomas, and in particular to determine the functional consequences of the myc-IG translocations and the presence of EBV sequences. We have demonstrated that Ig enhancers are able to increase transcription from all myc promoters (P1, P2, and P3). However, the ability of different tumor cell lines to support enhancer function varies markedly. Interesting, in those with good enhancer function, myc transcription in the absence of an enhancer is very low. The reverse is true in lines with little ability to support enhancer function. These experiments strongly suggest that while myc deregulation is partly dependent upon the juxtaposition of enhancer sequences, at least one additional factor plays an important role.

7. Following our observation that EBNA-1 can transactivate c-myc via immunoglobulin enhancers, and therefore can cooperate with the 8;14 translocation in the pathogenesis of the small noncleaved cell lymphomas, we have demonstrated that antisense molecules (provided in a stably transfected vector), under the control of an inducible vector will markedly inhibit proliferation and rapidly cause death of an EBV positive cell line, strongly suggesting that EBNA-1 is essential for viability of the tumor cells. No effect was seen in control cells transfected with a sense plasmid. When grown as a tumor in nude mice, complete regression can be accomplished by antisense induction. These observations confirm the importance of EBNA-1 to tumorigenesis in EBV containing cells, and also indicate that EBNA-1 is a potential target for therapy.

8. We have demonstrated that in a small fraction of EBV positive Burkitt's lymphomas, some cells permit the initiation of the lytic cycle. This is almost certainly the explanation for the high EBV antibody titers in patients with EBV positive tumors, and for the association of antibody titer with prognosis.

9. We have looked for scl (tal-1) deletions and translocations in T cell leukemias in patients from India, and found that the frequency is somewhat lower than that reported in the USA.

10. We have initiated a study of bcl-2 rearrangements in follicular and diffuse lymphomas from Pakistan (where follicular lymphomas are uncommon.) Preliminary results suggest that the rarity of follicular lymphomas is not a consequence of rapid transformation, but due to the paucity of tumors bearing 14;18 translocations.

11. We have demonstrated that p53 mutations occur seldom, if at all in primary anaplastic large cell lymphomas, but are present in relapse tumors, suggesting that p53 mutation in this tumor is associated with progression.

12. We have demonstrated that in some heterozygous p53 mutations the notion that the mutant protein sequesters the normal protein and prevents it from exerting its suppressor effect on cell growth (i.e., the mutation is dominant negative) does not hold. This strongly supports the idea some p53 mutations are "gain-of-function" mutations. In heritable mutations, our data suggests that the normal protein is able to sequester the mutant protein and prevent its accumulation.

13. We have initiated studies of the association of EBV with Hodgkin's disease in different world regions. Preliminary data indicate a very high (close to 100%) association rate in children in Pakistan, but a very low rate in adults in India.

14. 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.

15. 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 two 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) and neuropeptides (somatostatin, neuropeptide Y, CRF). 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.

16. The central nervous system pharmacology of antiretroviral agents is being studied. We have 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.

17. 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.

18. Since retinoic acid (RA) controls cell proliferation, induces differentiation and inhibits tumorigenicity, we have used it as a model to study genes important in regulating these processes in neuroblastoma tumors. 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.

19. Continued studies on retinoic acid treated NB cells has identified an increase in $TGFb_1$ coinciding with decreased levels

of NMYC. These studies indicate TGFb$_1$ may mediate growth inhibiting effects of retinoic acid. Continued studies on genes controlling differentiation of NB indicate involvement of trk genes.

20. 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.

21. 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 $G_2$.

22. 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.

23. 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. We have defined a model system to study retinoic acid induced regulation of IGF II gene transcription.

24. Developed a normal human fetal olfactory neuroblast cell line that can be induced to differentiate *in vitro*. Cell line will be utilized to study effects of oncogenes and growth factors on tumorigenicity. Model will also be used to study mechanisms of HIV neuropathy *in vitro* as well as regulation of HIV expression in neural cells.

25. 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.

26. 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. We have demonstrated the IGF-II induced motility of RMS is mediated through the type II IGF receptor, while the IGF-II induced mitogenic response is induced through the type I IGF receptor.

27. 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.

28. 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.

29. We have begun to characterize the frequency and diversity of p53 mutations in RMS cell lines and tumors. To date, 3/5 cell lines have been shown to have homozygous mutations that lead to significant alterations of protein structure. In addition, 2/6 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.

We have established subclones of a human RMS cell line with high metastatic potential and are currently characterizing the molecular alterations that lead to this phenotype.

30. We have successfully reconstituted the superoxide production capacity of lymphocytes from patients with chronic granulomatous disease by stable transfection of a shuttle vector which expresses the wild type cDNA. We have done this in two forms of CGD, both autosomal recessive, p47-phox and p67-phox deficient.

31. With the development of this new system for whole cell reconstitution, we have constructed a series of mutants to study the structural and functional characteristics of the gene products. Initially, we have deleted the SH3 domains of the p67 and recently, the p47. We have also constructed a series of mutants based on theoretical sites of phosphorylation. Lastly, we have substituted the SH3 domains from homologous regions derived from src-like sequences.

32. We have cloned, sequenced and analyzed the 2.5 kb upstream region of the p47-phox gene, a myeloid specific gene integral to the NADPH-oxidase system. We have identified several sequences that are homologous to a loose consensus sequence for interferon-gamma responsiveness.

33. We have begun to map the cis-elements of the p47-phox 5' upstream region by constructing a series of deletion mutants in reporter gene constructs. Initial studies suggest several regions are necessary for transcription in myeloid cell lines.

34. We characterized the molecular defect of several new patients with p47-phox autosomal recessive CGD and described new mutations.

35. The lab has been investigating the immunosuppressive effects of recombinant gp41 and gp120 proteins of HIV on several functions of peripheral polymorphonuclear cells, specifically, superoxide generation, chemotaxis, and bactericidal activity.

36. We have finished characterizing the cDNA of a late interferon-gamma responsive element. We have found that this cDNA is expressed hours after stimulation with interferon-gamma and is expressed in cells that contribute to an immunologic response, B and T lymphocytes, myeloid cells, mast cells, megakaryocytes and vascular endothelial cells. We have recently isolated the genomic clones and plan to map the structure of the gene including the start of transcription and intron/exon borders.

37. We have coordinated a study of the bactericidal and fungicidal *in vitro* activity of polymorphonuclear cells isolated from patients receiving intravenous M-CSF. To complement this, we have looked at the regulation of several myeloid specific genes which are integral to microbicidal activity.

38. We have observed that ascorbic acid is significantly taken up by monocyte cell lines, specifically in response to stimulation with cytokines. We have begun a long-term cloning project to identify and characterize the transporter for ascorbic acid by oocyte injection and the subsequent screening of a constructed cDNA library.

39. Using a monocyte cell line, we have studied the effect of numerous cytokines on intracellular triphosphorylation of AZT.

40. Because increased frequency of bacterial infections was noted in patients treated with the antineoplastic agent suramin, we studied the effects of suramin on neutrophil function *in vitro*. Pretreatment with suramin impaired the phagocytic and bactericidal activity of normal neutrophils against *Staphylococcus aureus* but not the oxidative burst and the phagocytosis of *Candida albicans*.

41. We demonstrated that cilofungin, the model compound of the class 1,3-b-glucan synthetase 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.

42. We also 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.

43. We found 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.

44. 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.

45. 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.

46. Pursuant to these immunohistochemical findings, we developed a new animal model of esophageal and gastrointestinal candidiasis and found that methylprednisolone and non-absorbable antibacterial antibiotics, alone and in combination, increase the intensity and duration of mucosal colonization by *Candida albicans*. Studies further revealed that when cytotoxic chemotherapy due to cytosine arabinoside was administered to such colonized rabbits that invasion of the gastrointestinal tract led to dissemination of Candida to multiple tissues. These *in vivo* systems of esophageal and gastrointestinal candidiasis, which pertain to problems of patients with HIV-infection, as well as granulocytopenia, are currently being studied for investigation

of new antifungal strategies, recombinant cytokine therapy, and diagnostic methods.

47. *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 (DTI), we developed models of disseminated and gastrointestinal infection in persistently granulocytopenic rabbits. Antigenemia cross-reactive with cryptococcal polysaccharide (described in cases of DTI) were reproduced. We further demonstrated the immunohistological origin of cryptococcal antigenemia in disseminated *Trichosporon* infection as arising from cell wall and matrix of *Trichosporon beigelii*. DTI developed in rabbits with *T. beigelii* gastrointestinal colonization following cytotoxic chemotherapy.

48. We further demonstrated in this model 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 *Trichosporon beigelii* infection. Antigenemia declined during the course of antifungal therapy. These studies have afforded new understanding of this and other emerging resistant fungal pathogens.

49. In order to further understand the pathogenesis and molecular epidemiology of *Trichosporon* infection, we further identified and characterized the biochemical and physiological factors that regulate germination, an important virulence factor of *T. beigelii*. We 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. This amplified fragment is now being employed in new strategies for PCR-based diagnostic methods.

50. In view of the complexity of nomenclature of new and emerging invasive fungal infections in immunocompromised patients, the Infectious Diseases Section contributed to an internationally convened consensus panel for the development of uniform terminology in describing disease processes due to these organisms.

51. 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. We further 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.

52. We developed, characterized, and implemented a rapid enzymatic assay for the detection of d-arabinitol in high risk cancer patients for the early diagnosis and therapeutic monitoring of invasive candidiasis in a multi-center clinical study coordinated by the Pediatric Branch. The data indicate that this enzymatic system may permit the rapid daily monitoring of high risk patients of an entire oncology unit. Correlative studies in our rabbit models of disseminated candidiasis revealed a threshold effect in infected tissues, thus further elucidating the pathophysiological basis of expression of d-arabinitol in serum. These *in vivo* and clinical studies provide a foundation for a new approach to diagnosis and therapeutic monitoring of this disseminated candidiasis in cancer patients.

53. We have identified several oligonucleotide sequences, including those from C14-demethylase, aspartyl proteinase, and ribosomal RNA, of *Candida* and other species of pathogenic fungi for the development of polymerase chain reaction methods suitable for detection of small quantities of these organisms or their nucleic acids in blood, bronchoalveolar lavage specimens, and other normally sterile body fluids.

54. 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.

55. Little is known about the daily dosage, total dose, duration, and dose intensity of amphotericin B, the mainstay of systemic antifungal therapy. We therefore completed a series of *in vivo* studies to investigate the pharmacodynamic properties both amphotericin B and amphotericin B lipid complex (ABLC) in our rabbit model of chronic disseminated (hepatosplenic) candidiasis. These findings indicate that among the three parameters, duration, daily dosage, and total dose, extended duration and high daily dosage, rather than total dose, are the important determinants of antifungal response by amphotericin B and ABLC.

56. We further developed the concept of "reticuloendothelial loading" of ABLC in our rabbit model of chronic disseminated candidiasis, demonstrating (1) that high doses of ABLC (5 mg/kg/d) achieved high concentrations in the reticuloendothelial tissues (liver and spleen), (2) that these concentrations persisted long after discontinuation of ABLC, (3) and that

lesions in liver, spleen, and other tissues continued to resolve after discontinuation of ABLC.

57. These pharmacodynamic studies of ABLC in chronic disseminated (hepatosplenic) candidiasis have led to a nationwide protocol of ABLC, wherein a child with hepatosplenic candidiasis may be referred from anywhere in the United States to receive either 3 or 6 weeks of ABLC, thus potentially reducing the length of treatment of this infection from a approximately 6 months to more than 1 year of conventional therapy to 1 to 2 months.

58. We recently reviewed all cases of hepatic abscesses at the NCI and reported that bacterial and fungal hepatic abscesses tend to be distinguishable on the basis of epidemiologic and radiologic features but that biopsy of suspected fungal lesions is clearly warranted in order to be establish a definitive diagnosis of candidiasis and to exclude other processes, particularly the primary neoplastic disease.

59. In order to better understand the management of catheter-associated fungemia, we studied 155 consecutive episodes of this infection at the NCI. The data indicated that virtually all cases of catheter-associated fungemia in cancer patients were clinically significant, require prompt therapy with amphotericin B, and are optimally managed by removal of the vascular catheter.

60. We recently described the association of pulmonary cryptococcosis simulating metastatic soft tissue sarcoma in children being monitored by CT scans for potential neoplastic relapse. Excisional biopsy of new nodules is warranted to exclude pulmonary cryptococcosis and other opportunistic mycoses causing nodular asymptomatic lung lesions.

61. 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.

62. 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.

63. 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) ABLC. The positive outcome of this patient, who received the first compassionate release of this compound in the United States, led to a nationwide program for compassionate release of this agent in selected mycoses.

64. A subsequent study in our rabbit model of invasive pulmonary aspergillosis investigated a cholesterol sulfate based liposomal formulation of amphotericin B that is under consideration for clinical trials against invasive aspergillosis in the United States. This study clearly demonstrated a highly significant optimal safety and efficacy dosing regimen at 5 mg/kg/d, which may facilitate determination of the appropriate dose in these future trials.

65. We found that the *Aspergillus* metabolite, d-mannitol, as measured by mass spectroscopy-gas-liquid chromatography (MS-GLC) and gas-liquid chromatography-flame ionization detector (GLC-FID) was present in serum and bronchoalveolar lavage specimens obtained from persistently granulocytopenic rabbits with primary pulmonary aspergillosis.

66. We found that empirical low dose amphotericin B was not always 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.

67. 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.

68. Invasive fungal infections and in particular invasive aspergillosis have emerged as serious infections in HIV-infected patients. PMNs from HIV-infected children with low CD4 count (<25% of normal median) were shown to have significantly impaired fungicidal capacity against *Aspergillus fumigatus* hyphae. This defect was acquired by normal PMNs after *in vitro* incubation with sera from HIV-infected patients as compared to sera from healthy donors. *In vitro* incubation of defective PMNs with G-CSF improved the fungicidal impairment. These findings may explain the increased susceptibility of these patients to invasive aspergillosis, and suggest a potential therapeutic role of G-CSF and potentially other cytokines.

69. In studies of human monocyte-derived macrophages from children with HIV infection we found that these cells do not ingest or inhibit the germination of *Aspergillus* conidia as well as normal controls. This finding suggests that HIV-infected patients are at increased risk to develop invasive aspergillosis as compared to control healthy population.

70. To evaluate the potential therapeutic role of interferon-g (IFN-g) in prevention of disseminated candidiasis, we studied the *in vivo*_and_*ex vivo* effects of recombinant rabbit IFN-g in normal and methylprednisolone-treated rabbits with disseminated infection due to *Candida albicans*. IFN-g enhanced $O_2$- production in normal and corticosteroid-treated rabbits *ex vivo* and augmented *in vivo* clearance of *C. albicans* from reticuloendothelial tissues in normal rabbits, but not in corticosteroid-treated rabbits.

71. The effects 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* were studied and compared with those 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. These findings suggest a potential beneficial role of the two cytokines in host defenses against *Candida*.

72. The effects of G-CSF and IFN-g on the hyphal killing capacity of normal PMNs against nonopsonized hyphae of *C. albicans* were studied and compared with those against non-albicans *Candida* species (*C. tropicalis* and *C. parapsilosis*). G-CSF and IFN-g enhanced the killing of *C. albicans* and *C. parapsilosis* whereas the killing of *C. tropicalis* was marginally affected. This study illustrated the differences in modulatory effects of cytokines that exist among different species of *Candida*.

73. 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. The implications of this finding are important for the prevention and treatment of invasive aspergillosis in immunocompromised cancer patients. In other experiments, we found that both hydrocortisone and dexamethasone inhibit the antihyphal capacity of normal PMNs but pretreatment of the PMNs with G-CSF or IFN-g appear to prevent this steroid-induced defect. The combination of the two cytokines together showed greater effect than each of them separately.

74. Because transfusions of large numbers of elutriated monocytes may be beneficial for neutropenic patients with invasive aspergillosis, we studied the *in vitro* effects of GM-CSF and IFN-g on the fungicidal activity of human elutriated monocytes against *Aspergillus fumigatus* hyphae. The two cytokines augmented the fungicidal activity of those cells and the combination of them in low concentrations was more effective than each of them in the same concentrations. Similar experiments with dexamethasone-treated monocytes showed that GM-CSF and IFN-g eliminated the dexamethasone-induced suppression

of the fungicidal activity. The role of M-CSF in the fungicidal activity of human elutriated monocytes as well as monocyte-derived macrophages against *Aspergillus* is being studied currently.

75. Bacterial infections are 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.

76. We assessed T helper cell 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. 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.

77. 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 by the CD8+ T cells in the supernatant without being the virus itself.

78. 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 younger than 13 months of age but not in neonates. These findings suggest a unique maturational process of the T helper cell function and antigen-presenting function possibly influenced by the presence of various maternal cytokines in the neonates.

79. In collaboration with other investigators we evaluated the presence of antibodies against putative epitopes of the V3 loop of the HIV envelope protein in sera of HIV-infected children as well as the capacity of these sera to neutralize the MN strain of HIV-1. Most children had antibodies against various epitopes of the V3 loop especially the principal neutralizing domain. However, the existence of those antibodies did not correlate with the clinical status of the patients. Higher neutralizing capacity, however, correlated with better clinical status of the

patients and predicted a period with lower number of serious disease-related events.

80. 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 IC50 in post-therapy isolates remains to be determined. Small decreases in sensitivity to ddI in HIV from patients being treated with this agent, however, were observed. Selective PCR was used to demonstrate the presence of a known mutation related to AZT resistance in 5 to 7 patients who received long-term AZT/ddC therapy, and a known mutation related to ddI resistance in 5 of 7 patients who received long-term ddI therapy. We have begun to use the selective PCR technique to evaluate the occurrence of resistance mutations in HIV from patients receiving simultaneous combination therapy with AZT and ddI.

81. We have attempted to assess the efficacy of antiretroviral therapy by following the changes of viral load in blood as determined by quantitative viral culture using 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. Currently, we are planning to employ quantitative polymerase chain reaction (PCR) technology to determine the level of plasma viremia in these patients.

82. Using PCR technology, we have also quantitatively evaluated the amount of HIV viral DNA in tissues obtained from autopsy of 14 patients with AIDS or ARC. Among 13 organs tested, lymph nodes contained the highest numbers of HIV-1 DNA (copies/mg total DNA) followed by spleen, colon, skin, lung, etc. We then examined the level of HIV-1 RNA and DNA in PBMC's and biopsied lymphoid tissues by PCR in combination with reverse transcription (RNA PCR). We found that the numbers of HIV RNA were substantially greater than those of HIV DNA in lymphoid tissues. These data suggested that lymphoid tissues represent one of the main sites for active infection as well as replication of HIV-1 in patients with AIDS or ARC.

In addition, we have developed a quantitative HIV-1 RNA assay for small (4 ml) whole blood samples using a direct competitive PCR strategy. This detects both virion RNA and cellular mRNA. The utility of this for monitoring viral burden will be compared to p24 antigen detection (standard and with immune-complex disruption), age-adjusted CD4 counts and the PCR plasma viremia assay.

83. 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.

84. 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.

85. 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.

86. We produced the first comprehensive, fine-scale map of regulatory sequences present in the HIV LTR. This map identified several previously unknown LTR regulatory sequences active in unstimulated and stimulated lymphocytic cells. The identification of these additional regulatory sites should provide useful insights into basic HIV biology as well as suggest additional targets for possible future therapies.

87. We showed that some of the well-characterized HIV LTR regulatory sequences like NF-kB and TATA behave differently in unstimulated, stimulated, and particularly in tat-expressing lymphocytic cells. For example, we showed that the two NF-kB sites are not equivalent in that the 3' site appears to be functionally much more important than the 5' site in the presence of tat.

88. We made a contribution to understanding the function of tat by showing that the sequences close to the transcription start site are required for maximal expression in the presence of tat, while sequences 5' to the NF-kB sites provide no contribution to wild type LTR activity in the presence of tat, providing support for the "flip-back" model for tat-mediated transactivation.

89. We identified additional sequences at the extreme 5' end of the HIV LTR that are required for maximal expression and transcription complex formation in HeLa cells.

90. We collaborated in studies that identified sequences in the R region of the HIV LTR between the TATA element and the transcription start site, 5' to the AAUAAA, that are required for wild type levels of HIV RNA polyadenylation. This result helps to explain why HIV produces a full-length RNA instead of the very short transcript that would be produced if the polyadenylation signal in the 5' LTR were active.

91. HIV makes use of the transcriptional apparatus of its host cell. Since the regulation of gene expression changes dramatically during development and differentiation, largely as the result of changes in the regulation of transcription, we performed experiments designed to examine the effects of differentiation-dependent changes in the regulation of cellular gene expression on HIV gene expression.

Through these experiments we hoped to understand some of the unique features of HIV infection in developing infants and children. We used our comprehensive collection of HIV LTR linker-scanning mutants in a human embryonal carcinoma (EC) cell model to look for LTR regulatory elements that function differently during the course of differentiation. We found that the HIV LTR contains several previously uncharacterized regulatory sequences active in the differentiated EC cells, but not in lymphocytic cells. Some of the regulatory sequences active in the differentiated EC cells behave differently depending on the cell's state of differentiation. These differentiation-dependent regulatory sequences include some previously well-characterized sequences like NF-kB, as well as several previously unknown sequences. The existence of regulatory sequences active in differentiated cells, but not in lymphocytic cells suggests that the ability of the virus to replicate in cells other than lymphocytes and monocytes may be important for virus survival and transmission. Such findings also suggest that LTR sequences, as well as the better characterized envelope sequences, may contribute to HIV tropism. Finally, the existence of differentiation-responsive HIV LTR regulatory elements may help to explain some of the distinctive features of pediatric AIDS.

## RADIATION ONCOLOGY BRANCH

### *Description*

The Radiation Oncology Branch (ROB) is dedicated to the multidisciplinary care of the cancer patient, allied to an active clinical and laboratory research effort which, we believe, is unique in the field. Out treatment effort has centered around the development of novel means of optimizing the use of radiation therapy and related disciplines, in the primary treatment of

certain malignancies. We have also participated and fully supported the integrated use of radiation therapy with the therapies of the other branches and institutes of the National Institutes of Health (NIH) in the management of the patient with cancer. State of the art radiation therapy using photon and electron beam treatments, allied with intercavitary and interstitial therapies continue to be employed. In addition, the clinical evaluation of promising radiation sensitizers has continued; novel therapies evaluating the role of dose rate effects in radiation therapy has been initiated; and the use of photodynamic therapy and radio-immunotherapy (entirely novel cancer therapies) continues to be explored. The three stated goals of the branch continue to be:

1) Performance of clinical trials of a combined modality nature, in collaboration with the other branches of the cancer institute, and with other institutes of the NIH.

2) Development of a strong laboratory program, emphasizing basic scientific projects with potential applications in the clinical arena.

3) Training residents and fellows in the practice of Radiation Oncology in an ACGME approved program to Board Certification level.

*Accomplishments and New Initiatives: Clinical*

We believe the following report will show that the ROB continues to meet its stated goals. Despite major changes in clinical personnel we continue to provide care of the highest quality, and pursue our research goals with diligence. Exciting new initiatives pave the way for future studies in the Branch.

Radiation sensitizers continue at the heart of our research efforts. The improvement of the local control of primary solid malignancies is one of the central aims of all radiation oncology efforts. We have continued to accrue patients on protocols involving patients with sarcomas and other unfavorable neoplasms. The apparent lack of benefit seen in our prior study of Glioblastoma Multiforme patients, although disappointing, provided the impetus to further select and refine this approach in other tumors. Tumor biopsies of selected brain tumor patients revealed very poor sensitizer uptake. This may well explain the poor results seen in this setting. A pilot study of patients with head and neck squamous cell malignancies showed good sensitizer uptake and exceptionally good clinical responses suggesting that these agents require further study.
We have recently opened a protocol evaluating the role of the radiation sensitizer iododeoxyuridine (IdUrd) with low dose rate brachytherapy in the management of Cervix Cancer. The easy accessibility of these tumors allow us to biopsy at selected intervals to assess the incorporation of the sensitizer in the tumor. Furthermore we plan to compare low and high dose rate

radiation in these patients, a study which will provide unique and important information in the field.

Photodynamic therapy (PDT) studies continued to progress. Unique work in the use of PDT in the management of intraperitoneal and intrapleural malignancies was undertaken, and Phase I studies defining the technique in these complex surface cancers was completed. In collaboration with Dr. Harvey Pass, of the Cardiothoracic Section of the Surgery Branch, a study in patients with Mesothelioma is nearing completion. Further details are found in the surgery branch report. Dr. William Sindelar of the Surgery Branch collaborated in a similar study involving various intra-abdominal malignancies, and this study is now providing the basis of Phase II studies in Intraperitoneal Sarcomatosis and Ovarian Cancer. Laboratory evaluation of laparoscopy guided light delivery are proceeding in the animal model, and plans are underway to bring this to the clinic to allow the delivery of repeated light doses in this setting. A Phase I trial in Superficial Bladder Cancers is likewise nearing completion. Further refinement of the dosimetry of PDT with on line light diode calibration, and computerized light delivery systems are being developed to allow the treatment to be more easily administered.

Low dose rate radiation has been piloted in the treatment of Small Cell Lung Cancer. The rationale for such an approach derives directly from the laboratory observations of Dr. Mitchell and others, showing a lack of the ability of Small Cell Cancers *in vitro* to repair low dose rate radiation. When this is contrasted with the ability of normal tissue counterparts to efficiently repair such damage, it becomes theoretically possibly to improve the therapeutic ratio in treated patients, and limit the expected toxicity of radiation in this lethal disease.

Continued progress in the development of effective therapy incorporating the use ofradio-immunotherapy has been made. Collaborative studies with the Metabolism Branch have been accorded the Cancer Institute's highest priority. The Inorganic and Radioimmune Chemistry Section has developed the chemistry necessary for linkage of the high energy beta-particle emitter Yttrium-90 to the monoclonal antibody anti-Tac. Clinical grade doses of the ligand-linked anti-Tac prepared by this Section were employed by Dr. Thomas Waldmann, Chief, Metabolism Branch, NCI, in a Phase I clinical trial of $^{90}$Y-radioimmunotherapy of Adult T-cell Leukemia which until now has been universally fatal. Eight of 15 patients survive today; all but four patients who were failing aggressive chemotherapy and one patient treated compassionately within a day of expiration, had positive responses to the therapy. A Phase 11 trial was recently initiated. Most recently, doses of chelator-linked, genetically engineered and humanized anti-Tac conjugates were prepared by this Section for a new Phase I clinical trial. This trial

will make use of Granulocyte Colony Stimulating Factor (GCSF) to reduce the toxicity induced by the therapy. GCSF has proven effective in our laboratory in reducing the toxicity of the 90Y-anti-Tac employed to prolong graft survival in primate allograft transplantation.

An important bladder conserving protocol involving the use of combined modality therapy in invasive bladder cancer has been approved and will soon begin to enroll patients.

Important follow up information, including toxicity evaluation and cosmesis, continues to be gained from the randomized study of breast conserving therapy versus mastectomy in early breast cancer. This study is now closed to further accrual.

The Clinical Physics has made good progress on the Macintosh based Treatment Planning System. The system is in the process of being tested and phased-in clinically. A collaboration has been established with DCRT to explore a novel idea: linking our Mac II TPS and an Intel massive parallel processor at DCRT. The objective would be a massive shortening of processing time in "true 3-D" three dimensional treatment planning.

In addition to these studies, we continue to provide important collaboration with the Medicine, Surgery, and Pediatric Branches of the Cancer Institute, in a wide variety of malignancies.

*Accomplishments and New Initiatives: Laboratory*

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 source 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. We have established a CRADA with American Cyanamid to evaluate the biological potential (using *in vitro/in vivo* model systems) of aqueous chemiluminescent systems in Photodynamic Therapy (PDT) (light/photoactivatable compound-mediated cell killing). Preliminary *in vitro* results are encouraging with more details to be worked out regarding maximal

concentrations of both the chemiluminescent reagents and light sensitizer to achieve maximal cell kill.

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 in white 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. *In vitro* photodynamic treatment of normal and human papilloma virus-transfected keratinocytes with photofrin II and red light revealed that papilloma infected cells are responsive to PDT and therefore PDT may represent a potential treatment option.

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 during 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. More detailed experiments are underway involving multiple treatments and the use of a variety of sensitizers.

Studies in the Radiobiology Section has 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. Recent studies have shown that taxol-mediated cytotoxicity can be markedly reduced if tumor cells are depleted of GSH using buthionine sulfoximine. These results are exciting in that they may be instructive in elucidating the mechanism(s) to taxol-mediated cytotoxicity. Other studies relating to taxol include the finding that taxol pretreatment of human breast cancer cells (MCF7) followed by adriamycin significantly reduces adriamycin cytotoxicity. The reason for this is still unclear, but the results of the study may have implications for current clinical trials exploring this combination. We have demonstrated that taxol treatment of some, not all, human tumor cell lines results in a significant G2/M block in the cell cycle with subsequent radiosensitization. We have observed an unique taxol cytotoxicity dose response relationship in a variety of cell lines which shows that maximal

taxol cytotoxicity is achieved between 10-250 nM; higher concentrations result in actually increased levels of survival. Radiosensitization seems to parallel this dose response. We are currently pursuing these interesting findings; however, if such *in vitro* results mirror the *in vivo* situation dose intensification which is currently under investigation for a number of chemotherapy drugs would not seem advisable for taxol. These preclinical studies will provide the framework for a new clinical trial in ROB combining taxol and radiation with the aim of tumor radiosensitization.

The use of halogenated pyrimidines as radiation sensitizers remains a focus for both laboratory and clinical research. *In vitro* studies have shown that tumor cell radiosensitization increases as the percentage of IdUrd incorporation increases. The laboratory has developed appropriate techniques to support clinical studies, namely to determine the percent of IdUrd replacement in tumor cell DNA from biopsy material taken from patients on IdUrd infusion. These studies are important to possibly link tumor radiosensitization and response to the percentage of incorporation of IdUrd into tumor DNA. Our findings to date reveal that IdUrd is poorly taken up by glioblastoma tumors (0-4%). This finding correlates with the failure to improve survival of patients with glioblastoma treated with IdUrd and high dose radiation therapy. In contrast, in a pilot study in head/neck carcinoma we have found excellent local control with IdUrd/radiation with IdUrd replacements ranging from 5-15%. The laboratory will continue to support clinical evaluation of IdUrd/radiation. This will be of particular importance for a new clinical protocol to evaluate IdUrd and low dose rate brachytherapy for cervical carcinoma. Not only will IdUrd replacements be conducted, but also an estimate of the potential doubling time of tumor ($T_{pot}$) will be possible at the beginning, during, and at the end of radiation treatment. This will provide new biological information for this tumor type and provide unique radiobiological information regarding the effect of low dose rate radiation and tumor proliferation.

We have made considerable progress toward transfecting cells with inducible genes both in the sense and anti-sense direction for enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (GPX). Positive GPX transfected cell lines have been isolated and upon induction we have demonstrated that GPX activity can be enhanced by 30 fold. These cells once induced demonstrate a 2 fold resistance to hydrogen peroxide treatment; however, are not resistant to adriamycin or x-rays. Work continues to isolate cell lines with enhanced superoxide dismutase and catalase activity. 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.

We have spent considerable effort toward the characterization of a class ofstable free radical agents known as nitroxides. Nitroxides such as tempol protect cells against superoxide, hydrogen peroxide, and ionizing radiation. The findings of laboratory establishes nitroxides as a completely new class of non-thiol radioprotectors. Tempol has been shown to protect mice against whole body radiation and protect when topically applied against acute radiation-induced alopecia. Preliminary studies reveal that tempol also protects against fractionated radiation-induced alopecia. Preclinical studies continue with the prospect of bringing tempol to clinical trial for possible protection against alopecia for patients receiving whole brain irradiation. Interestingly we have shown that tempol does not provide radioprotection to tumor in a rodent tumor model suggesting that tempol radioprotection may be selective to normal tissues. The reason for this may reside in the ability of tumor to more rapidly reduce tempol to the hydroxylamine, a species that does not protect against radiation cell killing. Further, we have shown that tempol affords significant protection to mitomycin C mediated cytotoxicity. Preliminary results suggest that tempol protects against mitomycin induced skin damage *in vivo* thereby establishing such agents a possible candidates for clinical application in the setting of mitomycin drug extravasation. Further chemical characterization of the superoxide dismutase mimic activity of nitroxides revealed that nitroxides are first oxidized to an oxoammonium cation intermediate before a two electron reduction to the hydroxylamine. This important finding will be used to rationally screen a series of nitroxides for maximal protective capacity based on their oxidation potential and charge. Tempol has been shown to protect cells against mutation induction mediated by superoxide, hydrogen peroxide, and radiation. 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 paramagnetic resonance (EPR).

Our laboratory is currently involved in the development of both a pulse wave (PW) EPR spectrometer and PW-EPR imaging device. Because the electron decay times are in the order of microseconds as compared to the vastly longer nuclear relaxation times, the use of nanosecond PW-EPR with extremely rapid signal averaging and processing offers the possibility of surmounting the previous limitations imposed by limited signal. The project is multi-disciplinaryrequiring state of the art electronics, computation, gradient design, etc. The project is designed to create a prototypical PW-EPR instrument for *in vitro* biologic studies and to establish the foundational framework to demonstrate the application of PW-EPR to *in vivo* imaging. Hopefully it will ultimately yield insight into cellular events

that occur at the microsecond time scales and should because of the inherent physics of rapid free induction decay of excited electrons yield practically real time imaging with voxel resolution not attainable by conventional nuclear magnetic resonance based magnetic resonance imaging. It is anticipated that the prototype will be functional in late 1992.
The Inorganic and Radioimmune Chemistry Section has explored the efficacy of the alpha-particle emitter $^{212}$Bismuth in treatment of mice infected with the Rauscher leukemia virus. A single dose of 150 mCi was effective in inducing complete remission of disease, yet no histological evidence for toxicity was seen upon examination of major tissues. This is first successful treatment of a systemic cancer by antibody therapy. Currently, we are in the process of scaling-up the doses of $^{212}$Bi-labeled antibodies for use in clinical trials. We have also examined chelators for using the long-lived $^{212}$Lead ($^{212}$Pb) parent of $^{212}$Bi. It was found that the DOTA ligand complexes lead efficiently and is stable *in vivo* when linked to monoclonal antibody. When the radionuclide $^{203}$Pb was linked to monoclonal antibody B72.3 and injected into mice bearing LS-174T tumors, clear and distinct tumor images were obtained by scintigraphic methods. Current investigations center on employing $^{212}$Pb-DOTA antibody conjugates for radioimmunotherapy of cancer.

Surgery Branch

Laboratory and clinical efforts of the Surgery Branch are concentrating on the development of new 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 genes coding for neomycin phosphotransferase, tumor necrosis factor (TNF) and interferon-gamma. The molecular and functional aspects of these TIL have been characterized in detail.

2. TIL have been isolated from patients with melanoma that exhibit unique MHC restricted lytic specificity for the tumor from which they were derived and not for other normal tissues or other allogeneic tumors. These TIL traffick to tumor deposits following intravenous injection.

3. TIL transduced with genes for chimeric T-cell receptors (TcR) composed of the constant region of the TcR and the variable region of a monoclonal antibody recognize new targets in a non-MHC restricted fashion.

4. Improved techniques for detecting TNF secretion by transduced TIL have been developed using soluble TNF receptors.

5. A new method has been described for distinguishing between the MAGE 1, 2 and 3 genes that code for melanoma antigens.

6. Studies have demonstrated that shared melanoma specific antigens recognized by human T lymphocytes are commonly expressed in the melanoma patient population. Recognition of shared melanoma antigens was demonstrated in lysis assays and by secretion of the cytokines TNF-alpha, IFN-gamma, and GM-CSF by TIL. Transfection of the HLA-A2.1 gene into non-HLA-A2 melanomas permitted recognition by HLA-A2 restricted melanoma TIL, showing that some melanoma antigens can be shared among HLA disparate individuals. In addition, HLA-A2 restricted melanoma TIL recognized select other neural crest derived tumors, demonstrating that these tumor specific antigens are not limited to the melanoma histology.

7. Studies have shown that CD4+ TIL derived from some melanomas are capable of specific recognition of autologous cultured tumor cells via cytokine secretion. An EBV-transformed B cell line has been identified which can process and present melanoma antigens to CD4+ T cells. Studies are in progress to directly identify the tumor derived peptide recognized in one CD4+ TIL system using immunoaffinity chromatography to isolate MHC II-peptide complexes.

8. Tumor specific recognition by TIL derived from 4 of 10 colon carcinomas, 2 of 12 lymphomas, and 3 of 11 breast carcinomas has been documented. Recognition was manifested by secretion of TNF-alpha, TNF-gamma, and/or GM-CSF from TIL populations cocultured with tumor. Some TIL cultures were almost exclusively CD4+. Studies are now in progress in melanoma patients to correlate TIL cytokine secretion with clinical responses to TIL therapy.

9. We have molecularly engineered murine tumor cells to secrete various cytokines. TNF and IL-2 cDNAs have been introduced into both weakly- and non-immunogenic tumors and the host cellular immune response to these cytokine-secreting tumors has been characterized. Interferon-gamma gene-modification corrects an antigen presentation defect in a non-immunogenic sarcoma and elicits therapeutically effective CD8+ TIL reactive against the wild-type tumor, which had not previously produced effective TIL.

10. We have demonstrated a potent role for IL-6 in the treatment of established solid tumors in mice that have the capacity to elicit both CD4+ and CD8+ T cell responses. IL-7 has been shown to mediate the generation and expansion of murine antitumor CTL. Adoptively transferred, IL-7 activated CTL have potent antitumor effects in vivo.

11. We have demonstrated that epidermal Langerhans cells and splenicdendritic cells (DC) process and present tumor-associated antigens toprimed CD4+ T cells in mice. Tumor-pulsed DC are extremely potent stimulators of helper T cell proliferation in vitro.

12. Demonstrated that the injection of interleukin-6 (IL-6) in a collagen matrix depot with tumor cells augments the therapeutic efficacy of TIL generated from that tumor in a murine model.

13. Preliminary characterization and purification of a novel heparin-binding chemotactic factor for murine TIL.

14. Developed a method, based on flow cytometry to measure calcium flux, to detect individual T cell reactivity to tumor antigens. The method also detects conjugate formation between the T cell and the stimulator cell and allows selection of the reactive population.

15. In studies performed to localize the tumor suppressor genes associated with initiation and progression of familial as well as sporadic renal cell carcinoma, 365 patients either affected or at risk for von Hippel-Lindau (VHL) disease have undergone a comprehensive screening evaluation. The VHL disease gene is linked to two new polymorphic DNA markers, D3S18 and 1963. In situ hybridization, genetic and physical mapping localized D3S18 and the VHL disease gene to the 3p25.5 locus. Analysis of variations in phenotypic suggests the presence of more than one mutant allele at the VHL disease gene locus.

16. In studies performed to identify the familial kidney cancer disease gene associated with VHL disease, molecular probes isolated from a chromosome 3-specific cosmid library have been used for cosmid walking as well as other genetic and physical mapping studies. A candidate gene, which codes for a previously identified member of a family of genes involved in signal transduction has been identified. Full length cDNA cloning and germ line mutational analysis are currently underway.

17. A large, multi-generational kindred with a potentially new form of inherited renal cell carcinoma has been identified. DNA sequence deletion and linkage analysis suggests that the molecular events associated with initiation of this form of renal cell carcinoma is different from that encountered in either sporadic renal cell carcinoma or the familial form of kidney cancer associated with VHL disease.

18. Over 40 human tumor cell lines have been derived from tumors removed from patients undergoing surgery in the Surgery Branch, NCI. By RFLP analysis loss of heterozygosity was determined 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 on chromosome 18.

19. We have established an in vivo metastatic model utilizing intra-prostatic injection of three different human prostatic carcinoma cell lines. A human prostate carcinoma cell line has been transfected with a pCMV-neo vector containing the nm23 gene construct for use in in vivo and in vitro studies evaluating metastasis and hormonal regulation of growth.

20. Suramin was found to inhibit bone resorption induced by parathyroid hormone, epidermal growth factor, tumor necrosis factor, and by a tumor-produced factor, PTH related-protein.

21. Demonstrated that TNF is partially responsible for the toxicity and anti-tumor efficacy of IL-2 biologic therapy in mice and for the toxicity of inhaled high-dose oxygen therapy in mice.

22. Antibody to interferon-gamma can protect mice against the lethal effects of endotoxin (LPS).

23. Interferon-gamma is partially responsible for the toxicity and anti-tumor efficacy of TNF in mice.

24. D-factor pretreatment protects mice against the lethality of endotoxin.

25. Demonstrated increased PDT efficacy with the use of monoclonal antibody conjugated to photosensitizer.

26. Demonstrated downregulation of TNF toxicity with a potassium channel activator, in vitro.

27. Demonstrated that TNF and IL-2 induce TNF gene expression and TNF production but do not act synergistically in growth inhibition of human breast cancer cells.

28. Demonstrated that IL-2 and IL-6 act additively to inhibit growthand modulate estrogen and progesterone receptor content of breast cancer cells in vitro.

29. Demonstrated that TNF enhances secretion of transforming growth factor beta in a time and dose-dependent manner by human breast cancer cells.

30. Demonstrated that monoclonal cell cultures could be established in vitro from solid breast cancers, and characterized these cell lines.

<u>Significant clinical accomplishments of the Surgery Branch include the following:</u>

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 seven patients have been treated without toxicity.

2. Clinical trials using autologous tumor cells transduced with the genes by TNF or IL-2 to immunize cancer patients have begun. Five patients have been treated thus far.

3. 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 phosphotransferase, 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.

4. 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 of patients will undergo a 50% or greater regression of malignancy.

5. 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 trend 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.

6. Pilot trials utilizing TIL plus IL-2 in over 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.

7. 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.

8.Phase I clinical trials evaluating the therapeutic efficacy of M-CSF have been initiated.

9. A randomized, prospective clinical trial has been initiated comparing the efficacy of low-dose bolus IL-2 to high-dose bolus IL-2 in patients with metastatic renal cell carcinoma. 84

10. Demonstrated that single-dose cyclophosphamide augments the localization of human TIL into tumor deposits in patients with metastatic melanoma and that such localization is correlated to clinical responses to TIL therapy.

11. Demonstrated that local radiation in patients with high-grade extremity sarcoma controls local recurrence, but does not impact on overall survival (when adjuvant systemic chemotherapy is also given).

12. Conducted a prospective quality of life evaluation showing that theadministration of adjuvant radiotherapy to patients with high-grade extremity sarcomas causes measurable, consistent decreases in their quality of life, but these are not of significant magnitude to alter the day-to-day functioning of these patients.

13. Conducted prospective randomized clinical trial evaluating efficacy of intensive preoperative chemotherapy for treatment of stage II breast cancer.

14. Characterized hepatic abscesses in cancer patients according to nature, distribution, and relationship to underlying malignancy.

15. Demonstrated that perioperative blood transfusion does not increase the risk of future failure following primary treatment for stage I, II breast cancer.

16. A prospective clinical trial to ascertain whether or not we could perform presymptomatic detection of VHL disease by DNA polymorphism analysis has recently been completed. There was convergence of results of risk calculations and clinical assessment in 41 of 42 informative patients. This work represents the first report of use of presymptomatic DNA analysis to predict which at risk patients carry the disease gene for VHL.

17. Clinical trials performed in collaboration with investigators in the Clinical Pharmacology Branch, NCI evaluated the effect of suramin in 38 patients with advanced, hormone refractory prostate carcinoma. 6/17 patients underwent a >50% decrease in their evaluable soft tissue disease and 71% of those with bone pain had marked symptomatic improvement. Survival in these patients strongly correlated with decline in pre-treatment PSA.

18. In order to evaluate the effect of new forms of therapy for patients with recurrent superficial bladder cancer, two Phase I trials have been performed. In collaboration with the Radiation Oncology Branch, NCI, 12 patients with recurrent superficial bladder have been treated with intravesical photodynamic therapy. In collaboration with Dr. Ira Pastan in the Laboratory of Molecular Biology, seven patients with superficial bladder cancer have been treated with TP40, a recently developed TGF-alpha-pseudomonas exotoxin fusion protein.

19. Established the maximum tolerated dose of intrapleural photodynamic therapy in humans.

20. Completed Phase I trial of intra-abdominal photodynamic therapy for patients with disseminated intraperitoneal malignancies.

21. Developed and described a method to easily and quickly dilate ureteral strictures in patients undergoing placement of indwelling ureteral stents for ureteral obstruction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07202-9 BDMS

PERIOD COVERED
October 1, 1991 through September 30, 1992

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 |

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION
Biostatistics and Data Management Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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.)*

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 as well as a unified system for tracking basic information on all patients registered on COP treatment protocols.

1. 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) Analyzed data from the randomized trial of GM-CSF in pediatric patients with cancer.
(7) Analyzed data on the relationship between GST , progesterone receptors, and estrogen receptor status in patients with breast cancer, and their effect on prognosis.
(8) Analyzed data from patients on a Phase II trial of suramin for treatment of prostate cancer.
(9) Performed analyses of data from a randomized trial comparing IL-2 alone to IL-2 + LAK for treatment of advanced malignancy.
(10) Analyzed data from the randomized early stage breast cancer trial, focussing on the effect of c-erb2 expression on prognosis.
(11) Performed analyses of data from the Phase II trial of 6MP in children with ALL or AML.
(12) Performed analysis of data from a Phase I trial of 5-FU, leucovorin, and alpha-interferon for treatment of gastrointestinal carcinomas.
(13) Presented a series of three seminars on statistical methodology in clinical trials to the Pediatric Branch.
(14) Provided advice regarding analysis and presentation of data from a trial of CPC chemotherapy +/- WR 2721 for treatment of ovarian cancer.

(15) Performed analysis of data on the effect of MDR expression on time on study in patients with breast cancer.
(16) Analyzed data from a study of the effect of several factors on survival in patients with LN3 type mycosis fungoides.
(17) Performed analysis of ICE chemotherapy for treatment of lymphoma.
(18) Performed detailed analyses of the quality of life in patients with high-grade soft tissue sarcomas.
(19) Analyzed data on patients with low-grade soft tissue sarcomas treated over many years at NCI.
(20) Performed analyses of data from the randomized trial evaluating somatostatin analog and its ability to control drainage following pancreatic tumor surgery.
(21) Prepared statistical considerations for randomized trial treating fever and neutropenia.
(22) Analyzed data on the effect of PTH and suramin and osteoblast counts in experiments with mouse calvaria.
(23) Performed analyses of data on T helper cell function in HIV(+) patients.
(24) Analyzed data from the locally advanced breast cancer protocol.
(25) Performed analyses of data on HLA antigens in melanoma patients treated with IL-2.
(26) Examined the relationship between EBV serology and lymphoma occurrence in patients receiving AZT for HIV.
(27) Performed analyses of data on DHE photosensitizer in patients with disseminated intra-abdominal cancer.
(28) Analyzed the effects of P53 mutations on survival in patients with NSCLC.
(29) Provided advice and consultations regarding design and conduct of quality of life studies in patients with cancer or with AIDS.
(30) Performed analyses of data on infections in patients treated with IL-2.
(31) Prepared statistical considerations for a Phase II study for treatment of mesothelioma.
(32) Prepared statistical considerations for a randomized phase II study evaluating two agents for treatment of metastatic androgen-resistant prostate cancer.
(33) Performed analyses of toxicities noted in patients on a clinical trial for treatment of pediatric ALL.
(34) Analyzed data for a study to examine the effect of perioperative transfusions on survival and disease free survival among patients with early stage breast cancer.
(35) Performed analyses of survival and disease free survival from a study of patients with male breast cancer, and compared the data to that obtained from a cohort of matched patients with the same disease selected from the SEER data registry.
(36) Performed analyses of data from a study comparing use of colloid vs crystalloid for patients receiving IL-2 therapy.
(37) Analyzed data on the relationship between p24, HIV virions, and other factors in patients with AIDS.
(38) Performed analysis of data on the attained suramin concentrations in patients treated on the Phase II study of this agent.
(39) Analyzed data concerning serial measurements of tumor size in rats with varying drug doses.
(40) Performed analysis of vital signs in rats treated with an IL-1 receptor antagonist after cecal ligation and puncture.
(41) Analyzed data from a study of parental coping with children who are HIV positive.
(42) Performed analyses of data from a study of surfactant associated protein in NSCLC as a discriminant between biological subsets.
(43) Performed analysis of data from a study of infections in patients with mycosis fungoides treated on two protocols.

(44) Provided advice to a physician regarding analysis of data on hemodynamic changes in patients treated with IL-2.
(45) Analyzed data from a study of the relationship between suramin dose and a form of mild neurotoxicity.
(46) Performed updated analyses of response, disease-free survival, and overall survival in a protocol using FLAC chemotherapy + GM-CSF to treat breast cancer.
(47) Provided consultation regarding the derivation of estimates of half maximal growth inhibition from a curve fitting procedure and suggested a modified method to obtain estimates and standard errors directly.
(48) Performed analyses of data on the association of clinical outcome in HIV(+) patients with relative concentrations and antibody titers of several epitopes on the HIV envelope.
(49) Analyzed data on the occurrence of lymphoma in patients receiving ddI for HIV infection, and on associations with IL-6 levels.
(50) Derived estimates of yearly hazards of the development of second tumors and tested potential risk factors for a study of long-term survival of small cell lung cancer patients.
(51) Provided advice on the statistical design of a randomized trial of anti-diarrheal agents for patients treated with IL-2.
(52) Performed analyses of data on the randomized multi-modality therapy trial for limited non small cell lung carcinoma.
(53) Analyzed data from a Phase II trial for treatment of mycosis fungoides using alpha-interferon plus deoxycoformycin.
(54) Performed analyses of data on dose intensity in two consecutive breast cancer protocols.
(55) Conducted sensitivity analysis of data concerning the association of clinical improvement and HIV neutralizing antibody titer.
(56) Performed survival analyses using calcitonin assay data from small cell lung cancer patients.
(57) Provided sample size information for a protocol to treat small cell lung cancer patients with combination chemotherapy.
(58) Prepared statistical considerations for a protocol on development of second primary tumors in patients with non small cell lung cancer.
(59) Provided advice regarding design of a phase I trial using IuDR in cervical cancer.
(60) Performed analyses of data on a study of abnormalities in bone marrow and their relationship to patient characteristics and outcome in patients with mycosis fungoides.
(61) Performed analyses of data regarding characteristics of AIDS patients with MAI infections vs patients who were symptomatic but did not have MAI.
(62) Analyzed p24 and CD4 data in relation to development of AZT sensitivity and clinical outcome.
(63) Performed analyses of data from a study of dose intensity in patients with small non-cleaved lymphoma.
(64) Analyzed data for the ability of three calcitonin assays to discriminate between normal non-smokers, normal smokers, and small cell lung cancer patients.
(65) Provided advice regarding parametric models and curve-fitting procedures for use in a new assay of neutrophil activity.
(66) Conducted an analysis of the relationship between CD4 cell count and development of non-Hodgkins lymphoma in HIV patients on AZT and ddI protocols.
(67) Provided advice to investigators on the localization of insulinomas by portal venous sampling of insulin levels.
(68) Analyzed data on the independent and combined effects of corticosteroids and cytokines on fungal killing by human neutrophils.
(69) Tested several models in a curve-fitting study of the killing efficiency of human neutrophils at different effector-target ratios.
(70) Performed analyses of the effect on DPD levels in patients treated with interferon or GM-CSF.
(71) Provided statistical considerations for a study of photodynamic therapy.

(72) Performed analysis of data on a Phase III trial of 5-FU vs leucovorin in patients with colorectal cancer.
(73) Analyzed data from a large trial of patients with non small cell lung cancer.
(74) Performed analyses of CD4 changes since baseline comparing sequential vs alternating AZT.
(75) Determined sample size requirements for a study comparing growth rates between drug and control among children with HIV disease.
(76) Provided advice regarding power and sample sizes for a potential randomized Phase II trial of taxol and p-glycoprotein blocker for breast cancer.
(77) Discussed response rates in trials treating pediatric brain tumors with thiotepa or cyclophosphamide and participated in the meeting which closed the trials.
(78) Provided advice to a researcher regarding statistical considerations for a questionnaire to be administered to patients of pediatric patients regarding their child's development while on therapy.
(79) Performed analysis of ELISA data from colon cancer cell lines.
(80) Analyzed data on the association of platinum-DNA adduct formation and tumor response.
(81) Provided advice on the statistical considerations for a protocol to treat lymphoma in patients with AIDS.
(82) Developed statistical considerations for a Phase I/II trial of taxol in relapsed breast cancer and lymphoma.
(83) Prepared statistical considerations for a Phase II trial to evaluate whether ddI or Rhogam could lead to improvement in thrombocytopenia in patients with HIV who developed this condition whether on ddI or on other agents.
(84) Developed statistical considerations for a proposed protocol of photodynamic therapy in patients with advanced ovarian cancer.
(85) Analyzed data on the effects on cell survival of several nitroxide-based radioprotectants.
(86) Performed analyses on the relationship between CD4 cell counts and the incidence of tumors and opportunistic infections in AIDS patients on AZT or ddI protocols.
(87) Provided advice regarding design of a study for the treatment of recurrent breast cancer with tamoxifen and 4-HPR.
(88) Performed analyses of data for a study of hyponatremia in patients with SCLC.
(89) Performed analyses of data from three trials of pediatric sarcomas.
(90) Provided advice on the development of a questionnaire to elicit information about how women on the randomized early breast cancer trial are feeling several years after treatment.
(91) Analyzed data on the age of onset of sporadic renal cell carcinoma and an inherited form of von Hippel-Lindau disease.
(92) Provided advice on the analysis of survival data with crossing hazards from an experiment using ectopic ACTH.
(93) Performed analyses on the in vitro effects on neutrophil superoxide production of interferon and Medrol administered to rabbits.
(94) Analyzed data for a Phase I PALA/5-FU trial.
(95) Performed analyses of data from a Phase II trial of 5-FU + leucovorin + alpha-interferon.
(96) Prepared statistical considerations for a randomized equivalence trial of two suramin treatments.
(97) Analyzed data on the effects of G-CSF and interferon on the in vitro killing of Aspergillus hyphae by normal human neutrophils.
(98) Performed analyses of Rb type and clinical correlations in patients with lung cancer.

B. Data Management Activities

The Section has continued the development and maintenance of several systems which facilitate the monitoring of protocols:

(1) Designed and currently completing the development of a microcomputer Clinical Data Registry (CDR) system that will replace the mainframe system. The system is designed to run on a 486 machine using Paradox as the underlying database. The database structure consists of three tables, patient information, on study and follow up. Data from DB2 databases on the mainframe was converted to Paradox format.

(2) Edit programs were developed for the microcomputer CDR system and run against the existing database. Currently researching and correcting data.

(3) Users and system documentation is currently being prepared for the microcomputer CDR system.

(4) Continued enhancements and modifications to the Protocol Database Management System (PDMS) in 4th Dimension on the MacIntosh for the Medicine Branch. Maintained and debugged various reports and utilities including downloading and uploading from and to mainframe files.

(5) 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.

(6) Continued enhancements and modifications to the Metabolism Branch Data Management System as requested by branch staff.

(7) Continued enhancements and modifications to the Pathology Tracking System and Navy Patient Listing System as requested by branch staff.

(8) 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) 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.

(5) Created, modified and updated mainframe and microcomputer databases for branches of the COP.

(6) Maintained various computer packages and hardware used by the Section.

(7) 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.

(8) 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.

(9) 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.

(10) 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.

(11) 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.

(12) 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.

(13) Provided registration support for all Navy Medical Oncology Branch patients, including setting up all support materials and registering all eligible patients.

(14) Created a system to provide registration support for all Radiation Oncology Branch patients, including setting up support materials and registering all eligible patients.

(15) Began providing registration support for all Metabolism Branch patients, including setting up all support materials and registering all eligible patients.

(16) Researched approach to provide registration support for all Pediatric Oncology Branch patients.

(17) Developed and implemented system for the secure storage of clinical data collected in the branches by clinical data management project staff. Also storing documentation for data management system.

(18) 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.

(19) Served as the coordinating center for two multi-center ovarian cancer protocols, including data collection and maintenance of protocol databases.

(20) Provided extensive data collection, data processing and programming support to the COP Clinical Resource Allocation Program for the Administrative Office.

2. Projects Outside Cop

1. Performed analyses of data to determine factors influencing development of second tumors in patients treated in Brazil over a thirty year period.

2. Served as statistical and data management advisor to a senior scientist in the National Center for Nursing Research.

3. Analyzed data on variations over time of a new aminopeptidase in rat brain for an investigator in Bilbao, Spain.

4. Served as a referee for a manuscript submitted for possible consideration for publication in Biometrics.

5. Performed analyses of data on dyspnea in three groups of patients for a researcher in the NIH Clinical Center Nursing Department.

6. Provided advice to two investigators from DCPC regarding methods for testing for synergy between two drugs in experiments with cell lines.

7. Advised a scientist in LTCB, DCE regarding the sample size needed for a study of HIV prevalence in Nigeria.

8. Served as a member of a committee evaluating proposals for computer support for an investigator in DCRT.

9. Provided advice to a senior physician in NIDDK regarding how to monitor a multi-center clinical trial of two doses of methotrexate vs placebo for treatment of primary biliary cirrhosis.

10. Determined needed sample size for a study of breathing treatment vs muscle strengtheners to improve walking speed for a researcher working with the NIH Clinical Center Critical Care Medicine Department.

11. Performed analysis of data on bone marrow transplantation from Hadassah Hospital in Jerusalem, at the request of a visiting scientist to NHLBI.

12. Analyzed data for a scientist from the Experimental Immunology Branch of DCBDC regarding changes in the function of T helper cell subsets during the progression of HIV infection.

13. Provided advice to an investigator from Harvard regarding design of a Phase II study with 6 disease groups and limited drug availability.

14. Delivered a presentation to senior NIAMS scientists and chairmen of university departments of dermatology regarding statistical issues in the design of trials for treatment of skin disease.

15. Performed review of an article submitted for consideration for publication in Blood.

16. Prepared statistical considerations for a randomized trial to treat pudendal neuralgia being proposed by a senior investigator of Dermatology Branch, DCBDC.

17. Analyzed data on the changes over time in the levels os several intracellular enzymes in rat adrenal glands for an investigator in Bilbao, Spain.

18. Analyzed data for an investigator in NIDR regarding long-term sequelae of pneumococcal meningitis in patients from a hospital in Greece.

19. Reviewed an article submitted for consideration to Archives of Internal Medicine.

20. Advised an investigator from Experimental Immunology Branch of DCBCD regarding the analysis of data on the perinatal transmission of HIV.

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) Development of data management systems which may serve multiple purposes.
(2) Development of models for testing in-vitro synergy of chemotherapeutic agents.
(3) 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 06523-08 CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 |
| | N.M. Benchekroun | Visiting Fellow | 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

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|---|---|---|
| 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 mechanisms 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-demthylated 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 VP-16 metabolite, dihydroxy VP-16, formed from O-demethylation of VP-16, chelates Fe3+ ions and in the presence of H2O2 or reduced glutathione forms hydroxyl radicals which induce topo-II-independent DNA cleavage. In addition, Cu2+ is also an excellent catalyst for inducing DNA cleavage in an oxygen radical-dependent pathway. We have shown that O-demethylated compounds bind to topo-II and that the binding is similar to that of VP-16 and induce DNA cleavage.

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 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. Further, 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. In order to further define the mechanisms of VP-16 resistance, we used multi-drug resistance 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 antibody to topoisomerase II, we have found that topoisomerase II level was 2-3-fold lower in the drug-resistant cell line compare to WT cells. Moreover, we found that VP-16 induced significant amounts of DNA double strand breaks (measured by alkaline unwinding assay) in the parental cell line compare to the resistance 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 resistance to the drug-dependent DNA breakage thatn the intact cells (2.5 uM vs 175 uM VP-16 for 50% damage in WT and 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.

Recent studies have shown that there are no significant differences in the activities of detoxification enzymes, e.g., superoxide dismutase, catalase, glutathione perozidase and glutathione transferase between the drug-sensitive and -resistant cells, indicating that VP-16 resistance and decreased DNA damage was not related to VP-16 detoxification in these cells. VP-16 activating enzyme, myeloperozidase activity was however, was 2-3 -fold higher in VP-16 sensitive HL60 cells and the same difference was observed in nuclei of these cells, indicating VP-16 activation by myeloperozidase in sensitive cells may be important in VP-16 action and possibly in DNA damage.

Work is also in progress to identify other factors that may be involved in VP-16 resistance in HL60 cells. We have initiated studies to define the roles of topo II phosphorylation in VP-16 resistance and DNA damage.

We have recently initiated studies to define the molecular mechanisms of drug resistance in vivo. We have found that drug-sensitive MCF-7 cells when inplanted in nude mice were resistant to VP-16.

We have recently found that the combination of VP-16 and IL-1 was more cytotoxic to human A375 melanoma cells than either drug alone. These synergistic interactions appear to result from upregulation of IL-1 receptors by VP-16, and subsequent increase in IL-1 binding, and internalization IL-1. Further studies have indicated that the synergistic interaction between VP-16 and IL-1 is not at the topo II level because IL-1 failed to increase VP-16-dependent DNA damage. Futhermore, IL-1 had no effects on VP-16-induced DNA repair in A375 cells, indicating that the synergistic interaction between IL-1 and VP-16 is due to increase in drug-dependent DNA damage.

**Publications:**

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06524-02 CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 | Branch Chief | 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 Branch

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF 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 06525-02 CP

PERIOD COVERED

October 1, 1991 to September 31, 1992

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 | Senior Staff Fellow | CPB, 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 STAFF 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 kisases. 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 nouse-human xenograft model, we observed a significant reduction in tumor mass following subcutaneous drug administration. Tumors studied were prostatic carcimona (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.

**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 bromo-deoxyuridine 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.

5. Tumor cell sensitivity appears to be related to src and/or family kinase activity. Relatively non-responsive 3T3 cells can be made sensitive to the cytotoxic effext of the ansamycins by infecting them with v-src expressing retrovirus. Already sensitive cells can be made at least one log more sensitive by this process.

6. While cytotoxicity can be achieved with as little as a 30 minute exposure to drug, no immediate effect on src knase activity or protein level is observed. An alteration in the association of src with several substrate signal transduction proteins can be seen however, suggesting that the ansamycins may interfere with SH2 and/or SH3 interactions, a novel method of interference in tyrosine kinase activity.

7. A panel of glioblastoma cell lines have also proven to be sensitive to the cytotoxic effects of the ansamycins at doses of drug similar to those used with the meduloblastomas and neuroepitheliomas.

**Publications:**

Whitesell L, Shifrin SD, Schwab G, and Neckers LM. Benzoquinonoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res 1992;52:1721-1728.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06526-04 CP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 | Senior Staff Fellow | CPB, COP, DCT, NCI |
| | E. Kyle | Microbiologist | CPB, COP, DCT, NCI |
| | B. Fahmy | Guest Researcher | CPB, COP, DCT, NCI |
| | C. Chavany | Visiting Fellow | CPB, COP, DCT, NCI |
| | R. Bergan | Medical Staff Fellow | 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 STAFF 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 modifies 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 comfirmed that N-myc inhibition leads to reduction in growth secondary to alteration in differentiative status of neuroectoderm-derived cell lines. We have demonstrated that interruption of TGF and autocrine loops is cystostatic for epithelial and mesenchemal cells.

(3) We have demonstrated that continuous subcutaneous perfusion of an oligo can significantly affest 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 hav demonstrated the feasability of continous perfusion intrathecal model to study the clinical efficacy of antisense in a more relevant pre-clinical in vivo model system.

**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 vivo 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; (3) and 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.

7. The bcr/abl, src, PDGF receptor and EGF receptor tyrosine kinases can be inhibited in vitro by oligonucleotides in a sequence specific, non-antisense, aptomeric manner with ki's of 1 uM or less.

8. We have determined that unmodified oligonucleotide is stable in CSF for up to 24 hours and that intrathecally administered phosphodiester oligonucleotide (bolus or continuous infusion) has a clearance rate (t1/2) of 10-20 minutes. By continuous infusion of 20 mM oligonucleotide one can maintain a steady-mate level of 20 uM oligonucleotide in the CSF.

9. Oligonucleotide can be imaged in the CSF of the intact animal with MRI by covalently attaching a gadollinium chelate to the oligonucleotide.

10. Mycoplasma in mycoplasma-infected cells, or free in culture, avidly take up oligonucleotide, process the material and reincorporate it into the RNA of the mycoplasma, thereby severely limiting antisense effectiveness in mycoplasma-infected cells or organisms.

**Publications:**

Rosolen A, Whitesell L, Ikegaki N, Kennett RH, Neckers LM. Antisense inhibition of single copy N-myc expression results in decreased cell growth without reduction of c-myc protein in a neuroepithelioma cell line. Cancer Res. 1990;50:6316-6322.

Rosolen A, Whitesell L, Neckers LM. Antisense oligodeoxynucleotide inhibition of N-myc expression in a neuroectodermal cell line. Advances in Neuroblastoma Research. 1991;3:29-36.

Schwab G, Siegall CB, Aarden LA, Neckers LM and Nordan RP. Characterization of an interleukin 6-mediated autocrine growth loop in the human multiple myeloma cell line, U266. Blood 77:587-593, 1991.

Whitesell L, Rosolen A, and Neckers LM. Episome generated N-myc antisense restricts the differentiation potential of neuroectodermal cell lines. Mol. Cell Biol. 1991;11:1360-1371.

Whitesell L, Rosolen A, Neckers LM. N-myc expression is required for neuroectodermal transdifferentiation in vitro. Advances in Neuroblastoma Research. 1991;3:45-54.

Neckers LM, Whitesell L, Rosolen A. Antisense inhibition of gene expression: a tool for studying the role of N-myc in the growth and differentiation of neuroectoderm-derived cells. In: Gene regulation by antisense nucleic acids (The Raven Press series on molecular and cellular biology, R. Erickson and J. Izant, eds.) 1992;1:295-32.

Neckers LM, Whitesell L, Rosolen A, and Geselowitz D. Antisense inhibition of gene expression. In: CRC Critical Reviews in Oncogenesis. 1992;3:175-231.

Stein CA, Neckers, LM, Pal R, Nair BC, Mumbauer S, Hoke G Phosphorothioate oligodeoxycytidine inhibits binding of HIV-1 gp120 to CD4. J. Acquir Immune Defic Syndr. 1991;4:686-693.

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)

Whitesell L, Rosolen A. and Neckers LM. In vivo modulation of NMYC expression by continuous perfusion with an antisense oligonucleotides. Antisense Res Dev. 1991;1:343-350.

Geselowitz DA, and Neckers LM. Analysis of oligonucleotide binding, internalization and intracellular trafficking utilizing a novel radiolabled crosslinker. Antinsense Res Dev. 1992;2:17-25.

Geselowitz DA, Olsen LD, and Neckers LM. Incorporation of radiophoshorus from labeled oligodeoxynucleotides into RNA of mycoplasma in cell cultures. Antisense Res Dev 1992;2:41-49.

Neckers LM. Cellular internalization of oligonucleotides. Antisense Res Applications, S.T. Crooke and B. Leblue, eds., CRC Press, Boca Raton, FL, in press, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06527-02 CP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncologic Aspects of Tyrosine Kinsases and Epithelial Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Oliver Sartor | Senior Investigator | CPB, COP, DCT, NCI |
| Others: | C. McLellan | Chemist | CPB, COP, DCT, NCI |
| | S. Kim | Stay-in-School | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
LCDO, NIDR, NIH (Dr. Keith Robbins)

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Molecular Oncology Group/ Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, MD 20892

| TOTAL STAFF 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.)*


The focus of the laboratory is two fold: To study tyrosine kinases and pharmacologic agents which may potentially alter their activity and to study, in human prostate cancer patients, pharmacologic regimens that can potentially lead to improved therapeutic outcome.

1) We have identified suramin as an agent which alters tyrosine phosphorylation and other growth-associated events in a wide variety of epithelial carcinoma cells. In addition, we have identified human B cell tumor cell lines as being particularly susceptible to suramin-induced growth inhibition.

2) We have identified protamine sulfate and pentosan, in addition to suramin, as being potent inducers of tyrosine phosphorylation in an early B lymphocyte cell line.

3) We have identified an interaction between transforming src-family kinases and a tyrosine phosphorylated protein.

4) We have identified a novel transforming mutation for lck.

5) We have identified aminoglutethimide as a particularly active agent in suramin-pretreated prostate cancer patients.

**Objectives:**

To determine the mechanisms whereby pharmacologic agents can modify cancer cell growth and to exploit these mechanisms in a manner that leads to improved therapeutic efficacy.

**Methods Employed:**

We have employed a variety of methodologies including transfection, PCR, and immunoblotting as well as studying prostate cancer patients in the clinic.

**Major Findings:**

1. We have identified novel transforming mutations of the lck proto-oncogene.

2. We have described the tyrosine-phosphorylation modulating properties of suramin and other polysulfated compounds.

3. We have identified novel interactions between transforming versions of src-family kinases and tyrosine-phosphorylated proteins.

4. We have identified aminoglutethimide as an agent with unexpected activity in hormonally un-responsive suramin-pretreated patients with metastatic prostate cancer.

**Publications (Abstracts excluded):**

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, and Allegra CJ. A pilot Study of Interferon alpha-2a in combination with 5-Fluorouracil plus High-Dose Leucovorin in Metastatic Gastrointestinal Carcinoma. J. Clin. Oncology. 1991;9:1811-1820.

Sartor O, Book Review for: Molecular Genetics in Cancer Diagnosis, edited by J. Cossman. J. Natl. Cancer Institute. 1992;83:877.

Sartor O, Moriuci R, Sameshima J, Severino M, Gutkind JS, and Robbins KR. Diverse Biologic Properties Imparted by the c-fgr Proto-oncogene. J. Biol. Chem. 1992;267:3460-3465.

Cardinali M, Sartor O, and Robbins KR. Suramin, an Experimental Chemotherapeutic Drug, Activates the Receptor for Epidermal Growth Factor and Promotes Growth of Certain Malignant Cells. J. Clinical Investigation. 1992;89: 1242-1247.

Sartor O, McLellan CA, Myers CE, and Borner MM. Suramin Rapidly Alters Tyrosine Phosphorylation in Prostate Cancer Cell Lines. Submitted, 1992.

Sartor O, McLellan CA, and Chiueh T. Comparison of src-family CDNAs Reveals Mechanisms Underlying Focus Formation in Transfected Fibroblasts. Submitted, 1992.

Schlaifer D, Cooper MR, Attal M, Sartor O, Trepel JB, Laurent G, and Myers CE. Myeloperoxidase: An Enzyme Involved in Vincristine Resistance in Human Myeloblastic Leukemia. Submitted, 1992.

Myers CE, Cooper MR, Ranson M, Sartor O, Sausville E. Antitumor Activity of Polyanions. In Holland and Frei, Cancer Medicine (In Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06532-01 CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Analysis of autocrine growth of follicular lymphoma cells in vitro

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: | M. Blagosklonnyi | Visiting Fellow | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

None.

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Tumor Cell Biology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF 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.)*

Follicular lymphoma is an indolent disease which eventually results in a rapidly proliferating lymphoma. Growth factors which regulate the growth of follicular lymphoma cells are not well described. We have undertaken to identify the autocrine growth factors, if any, which are responsible for follicular lymphoma growth in vitro. We have made the initial observation that follicular lymphoma cells will condition medium, which can then be used to support their growth when the cells are plated at low density. In this way we have been able to demonstrate that autocrine factors are produces by these cells. Size fractionation of serum-free conditioned medium reveals that a greater than 10 kd fraction is responsible for the growth promoting activity of unftactionated conditioned medium. Reverse transcription/PCR analysis of follicular lymphoma cell mRNA reveals that these cells produce several interleukins, including IL1β, IL2, GM-CSF and IL-5. IL1β and IL2, when added to cells at low density, potentiate their growth to the same degree as conditioned medium. When antibodies to IL1β and IL2 are added to conditioned medium, its ability to stimulate cell growth at low cell density is markedly inhibited. Thus, follicular lymphoma cells appear to possess autocrine growth loops for IL1β and IL2.

**Objectives:**

The objective of this study is to uncover the autocrine growth loops characteristic of follicular lymphoma cells growing in vitro. Once these are known, attempts will be made to inhibit them with antisense technology. If this proves possible in vitro, in vivo studies will be undertaken.

**Methods Employed:**

Standard molecular and cell biological techniques are used in these studies including RNA isolation, reverse transcription and PCR analytic methods (for other methods used, see project Z01 CM 06526-03 CP).

**Major Findings:**

1. Follicular lymphoma cells are able to condition serum-free medium to support their growth when cells are split to low density (such that, in the absence of conditioned medium, there is a lag phase of several days during which growth is minimal).

2. Size fractionation of conditioned medium reveals that the growth promoting fraction is greater than 10 kd.

3. Reverse transcription/PCR analysis demonstrates that follicular lymphoma cells make detectable amounts of IL1ß, IL2 and several other interleukins.

4. Addition of IL1ß and IL2 to cells plated at low density mimics the effect of conditioned medium addition.

5. Antibodies to IL1ß and IL2 added to conditioned medium can abrogate the growth promoting ability of conditioned medium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06533-01 CP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modulation of myeloid tumor cell growth in vitro by estrogen & progesterone analogs

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: | M. Blagosklonnyi | Visiting Fellow | CPB, 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 STAFF 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.)*

Myliod leukemias have proven to be particularly unresponsive to glucocorticoid therapy, a regimen shown to be quite useful in the treatment of T and B cell leukemias. We have investigated the in vitro sensitivity of several myeliod tumor cell lines, including HL60 and U937, to various sex steriods, including estradiol, diethylstilbestrol (DES), progesterone and testosterone. All cell lines tested proved to be killed by amounts of estradiol, DES and progesterone ranging from 10-100uM. DES proved the most effective. The method of cell death appears to involve cytoplasmic swelling and extrusion of nuclei, accompanied by DNA degradation. Leukemias sensitive to hydrocortisone and dexamethasone, such as T and B cell leukemias, were also quite sensitive to DES, estradiol and progesterone. All cell lines tested were found to posses receptors for estradiol (which are shared by DES), but no receptors for progesterone could be detected. in vivo studies of drug effectiveness against myeliod tumor xenograph growth in athymic nice are underway.

**Objectives:**

The objective of this study is to identify a steroid or steroids which are cytotoxic for myeloid leukemias in vitro and in vivo. Since these tumors are not sensitive to glucocorticoids either in vitro or in vivo, their sensitivity to certain estrogen and progesterone analogs could be a clinically relevant finding.

**Methods Employed:**

Standard cell and molecular biological techniques are used in these studies (see project Z01 CM 06525-01 CP for a description).

**Major Findings:**

1. Myeloid leukemia cell lines are sensitive to clinically achievable levels of estradiol, DES and progesterone.

2. Cell death occurs within 24-36 hours and is preceded by cytoplasmic swelling, nuclear extrusion and DNA degradation.

3. The cells studied posses receptors for estradiol but not for progesterone. Thus, progesterone may be functioning through a non-steriod receptor driven mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06534-01 CP

PERIOD COVERED: October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Molecular Basis of Tumor Radioresistance and Therapeutic Implications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | D. Samid | Senior Investigator | CPB, COP, DCT, NCI |
| Others: | C.E. Myers | Branch Chief | CPB, COP, DCT, NCI |
| | S. Shack | Special Volunteer | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*: Biochemistry Department, AFRRI (A. Miller, E. Clark)

LAB/BRANCH: Clinical Pharmacology Branch

SECTION: Differentiation Control Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD

| TOTAL STAFF 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.)*

Understanding the molecular basis of intrinsic radioresistance is fundamental to the design of effective radiotherapy protocols. The goal of this project is to investigate the contribution of oncogenes to tumor radioresistance in pursuit of identifying sensitive target(s) for pharmacological intervention. The major observations are:

1. Oncogenes and Radioresistance. Altered expression of the *ras* gene family, resulting from either missense mutations or transcriptional activation, has been implicated in intrinsic resistance to ionizing radiation. Using *ras*-transformed mouse and human tumor cell lines we demonstrated: (a) dosage-dependent correlation between the amounts of ras-encoded protein, $p21^{ras}$, and radioresistance; (b) the effect on radiation response is independent of neoplastic transformation by *ras*; and, *(c)* localization of $p21^{ras}$ to the inner side of the plasma membrane is critical for maintenance of the radioresistant phenotype.

2. $p21^{ras}$ As a Therapeutic Target. Cellular p21 is subject to a series of posttranslational modifications, of which isoprenylation is obligatory for its membrane localization and biological activity. Biosynthesis of the required isoprenoids is part of the mevalonate pathway of cholesterol biosynthesis; the early and rate-limiting step is conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) into mevalonate, catalyzed by HMG-CoA reductase. Our studies indicate that inhibitors of HMG-CoA reductase, including lovastatin and limonene, prevent $p21^{ras}$ attachment to the cell membrane with subsequent reduction in tumor radioresistance.

Lovastatin and limonene are prototype inhibitors of HMG-CoA reductase suitable for clinical use. Such drugs are of particular interest since they might suppress tumor growth *in vivo* and increase the efficacy of radiotherapy.

**Objectives:**

The goal of this project is to investigate the role of the ras oncogenes in tumor radioresistance in pursuit of identifying sensitive target(s) for pharmacological intervention. Specifically we aim to:

1. Explore the association of $p21^{ras}$ production and membrane localization with resistance of human malignancies to killing by ionizing radiation.

2. Determine whether agents that affect $p21^{ras}$ biosynthesis/post-translational processing might affect radiation responses.

2. Identify modulators of radioresponses suitable for clinical use.

**Methods Employed:**

Analysis of radiation responses of cultured tumor cells (e.g., survival curves); tumor biology, including growth in semi-solid agar and matrigel. Molecular analyses of DNA, RNA and proteins based on nucleic-acid probe technology and immunochemistry. Recombinant DNA and gene transfer techniques; animal models will be used in future studies in in vivo radiation responses.

**Major Findings:**

Using as a model mouse and human tumor cell lines transfected with genes of the *ras* family (Ha-*ras* , Ki-*ras* ) modified by eithe a point mutation, amplification, or transcriptional activation, we demonstrated:

1. Dosage-dependent correlation between the amounts of *ras* -encoded protein, $p21^{ras}$, and radioresistance.

2. The effect on radiation response is independent of neoplastic transformation by *ras* .

3. Localization of $p21^{ras}$ to the inner side of the plasma membrane is critical for maintenance of the radioresistance phenotype.

4. Inhibitors of HMG-CoA reductase, such as lovastatin and limonene, markedly enhance tumor radiosensitivity; the effect could be attributed to prevention of $p21^{ras}$ isoprenylation and its attachment to the cell membrane.

**Significance:**

Lovastatin and limonene are prototype inhibitors of HMG-CoA reductase suitable for clinical use. Such drugs are of particular interest since they might suppress tumor growth in vivo and increase the efficacy of radiotherapy.

**Publications:**

Rimoldi D, Miller AC, Freeman SE, and Samid D. DNA Damage in Cultured Human Skin Fibroblasts Exposed to Excimer Laser Radiation. J. Invest. Dermatol. 1991;96:898-902.

Rimoldi D, Flessate D, and Samid D. Modulation of Gene Expression by Subablative Doses of Excimer Laser Radiation. Radiation Res. In press, 1992.

Samid D, Miller AC, Rimoldi D, and Clark E. Increased Radiation Resistance in Transformed and Nontransformed Cells with Elevated ras Protooncogene Expression. Radiation Res. 1991;126:244-250.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06535-01 CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Phenylacetates in Differentiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | D. Samid | Senior Investigator | CPB, COP, DCT, NCI |
| Others: | C.E. Myers | Branch Chief | CPB, COP, DCT, NCI |
| | P. Prasanna | Special Volunteer | CPB, COP, DCT, NCI |
| | V. Cioce | Special Volunteer | CPB, COP, DCT, NCI |
| | S. Shack | special Volunteer | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)* Surgical Neurology Branch, NINDS (Z. Ram, E.H. Oldfield); Lab. of Chemical Biology, NIDDK (G. Rodgers, E. Fibach), Pediatric Department, John Hopkins (S. Brusilow, G. Dover)

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Differentiation Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD

| TOTAL STAFF 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.)*

Sodium Phenylacetate (NaPA) was found to induce tumor cell differentiation *in vitro* at concentrations that have been achieved in humans with no significant adverse effects. The goal of this project is to explore the mechanisms of action and the efficacy of phenylacetate, its pro-drug phenylbutyrate, and analogs in treatment of malignant and non-malignant disorders of cell differentiation. The major observations are:

1. Differentiation Models. NaPA induced granulocyte differentiation in promyelocytic leukemia HL60 cells following rapid decline of *myc* oncogene expression; erythroid maturation in K562 leukemia; and, adipocyte conversion in immortalized mesenchymal C3H 10T1/2 cultures. NaPA was neither cytotoxic nor carcinogenic.
2. Molecular Marker of Cell Response. In K562 cells, differentiation was associated with increased production of the fetal form of hemoglobin (HbF). With HbF as a marker, we were able to demonstrate activity in **humans.** Patients with urea cycle disorders treated with the pro-drug, phenylbutyrate, were examined for red cells containing HbF (F cells); the mean % F cells was found to be significantly higher than that of normal subjects, indicating the validity of our experimental models.
3. Activity in Solid Tumors. Impressive activities were observed in cell lines established from hormone-refractory prostate carcinoma, glioblastoma, lung adenocarcinoma, and melanoma NaPA caused selective cytostasis and phenotypic reversion, as evident by altered gene expression, and loss of invasiveness and tumorigenicity. A pilot study involving systemic NaPA treatment of rats with intracerebral gliomas showed tumor suppression *in vivo.*

Our data suggest that phenylacetates, used alone or in combination with other drugs, may offer safe and effective new approach to treatment of some hematopoietic and solid neoplasms, and of severe hemoglobinopathies. Clinical protocol "Phase 1 Trial of Intravenous Phenylacetate in Adults with Cancer" has been IRB approved (#92-C-140A) and is IND pending.

**Objectives:**

The goal of this project is to explore the mechanisms of action and the efficacy of phenylacetate, its pro-drug phenylbutyrate, and analogs in treatment of malignant and non-malignant disorders of cell differentiation. Specifically we aim to:

1. Examine phenylacetate activity in solid tumors (with a prime focus on prostate carcinoma , giomas and melanoma) in both the preclinical and clinical setting.

2. Explore further the activity range of phenylacetate.

3. Identify the mechanisms of drug action and tumor resistance.

4. Develop active analogues.

5. Design effective combination treatment protocols.

**Methods Employed:**

Cell, culture, analysis of tumor biology, including growth in semi-solid agar, invasion thorough reconstituted basement membranes, proteolytic activity. Molecular analyses of DNA, RNA and proteings based on nucleic-acid probe technology and immunochemistry. Recombinant DNA and gene-transfer techniques. In vivo studies using rats and mice tumor models.

**Major Findings:**

Sodium phenylacetate (NaPA) was found to induce tumor cell differentiation in vitro at concentration that have been achieved in humans with no significant adverse effects. Preliminary studies indicate activity in vivo in experimental brain cancer. Specifically:

1. Treatment of promyelocytic leukemia HL-60 cells wtih NaPA 3-6 mM resulted in rapid decline of *myc* oncogene expression followed by growth arrest and granulocyte differentiation. NaPA also induced highly efficient adipocyte conversion in immortalized mesenchymal C3H 10T1/2 cultures; yet, unlike the differentiating chemotherapeutic drug 5-aza-2'-deoxycytidine, phenylacetate did not cause neoplastic transformation in susceptible cells.

2. NaPA induce erythroid differentiation in leukemic K562 cells, with increased gamma globin expression and accumulation of the fetal form of hemoglobin (HbF). Using HbF as a molecular marker, we were able to demonstrate phenylacetate activity in humans: Patients with urea cycle disorders treated with the pro-drug, phenylbutyrate, were examined for red cells containing HbF (F cells). The mean % F cells in these patients was found to be significantly higher than that of normal subjects, indicating the validity of our experimental models.

3. Impressive antitumor activities were observed in cell lines established from solid tumors including hormone-refractory prostate adenocarcinoma, glioblastoma, lung adenocarcinoma, and melanoma. NaPA caused selective cytostasis and induced cell maturation, as indicated by alterations in gene expression and cell phenotype (e.g., loss of invasiveness and tumorigenicity in recipient animals). Results of a pilot study involving systemic treatment of rats with intracerebral gliomas show that NaPA can suppress tumor growth in vivo.

**Significance:**

Our data suggest that NaPA might offer a safe and effective new approach to treatment of some hematopoietic and solid neoplasms, as well as of severe hemoglobinopathies. Phase I clinical trials with phenylacetate/ phenylbutyrate in treatment of advanced prostatic cancer and gliomas are IND pending.

**Publications:**

Samid D, Yeh T, and Shack S. Interferon in Combination with anti-neoplastic Phenyl Derivatives - Potentiation of Interferon A Activity In Vitro. Brit. J. Hematol. 1991;79:81-83.

Samid D, Shack S, and Sherman LT. Phenylacetate - A Novel Tumor Inducer of Tumor Cell Differentiation. Cancer Res. 1992;52:1988-1992.

Samid D, Yeh A, and Prasanna P. Induction of Erythroid Differentiation and Fetal Hemoglobin Production in Human Leukemic Cells Treated with Phenylacetate. Blood. In press, 1992.

Dover GJ, Brusilow S, and Samid D. Fetal Hemoglobin and Sodium 4-Phenylbutyrate. New England J Med. In press, 1992.

NCI Clinical Protocol No. 92-C-140A. Phase I Trial of Intravenous Phenylacetate in Adults with Cancer.

Patent Application. Compositions and Methods for Therapy and Prevention of Cancer and Severe Anemias. Application No. 07/779,744 filed in the US Patent and Trademark Office on October 1991 by USUHS (D. Samid previous employer).

Research Grants. Studies funded by a grant to D. Samid from Elan Pharmaceutical Corporation (G174ED), administered through the Henry Jackson Foundation. Total support for 1991-1994 $579,692. A CRADA between the NCI and Elan Corporation is pending review.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06719-04 CP

PERIOD COVERED: October 1, 1991 to September 30, 1992

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 |
| | F. Pirnia | Microbiologist | CPB, COP, DCT, NCI |
| | W.T. Niklinski | Biotechnology Fellow | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*: Molecular Pathophysiology Branch, NIDDK, NIH (A. Spiegel, A. Shenker) Laboratory of Chemoprevention, NCI, NIH (S.-J. Kim, M.B. Sporn); Dept. of Med. Urology, Stanford Univ. Med. Ctr. (D. Peehl); Dept. of Med. Neurosciences, Walter Reed Army Inst. of Research (M. Koenig); Digestive Disease 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 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.)*

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, (2) cytotoxicity through activation of $P_2$-purinergic receptors, and (3) the anticancer activity of the HMG-CoA reductase inhibitor lovastatin. We have found that elevation of intracellular cAMP is highly growth-inhibitory to all prostate carcinoma cell lines tested. To examine the mechanism of cAMP action in prostate carcinoma cells we tested the effect of the cAMP analog dibutyryl cAMP on the regulation of the potent negative growth factor TGF-ß. DbcAMP selectively induced the secretion of TGF-ß2 and not TGF-ß1 by prostate carcinoma cells. This TGF-ß2 was shown to be bioactive using the CCL-64 mink lung cell assay. Northern analysis showed that dbcAMP induced an increase in the five characteristic TGF-ß2 transcripts. Thus dbcAMP induces the expression of bioactive TGF-ß2 by prostate carcinoma cells, suggesting a new approach to the treatment of prostate cancer and a new molecular mechanism of cAMP action. $P_2$-purinergic receptor studies demonstrated that androgen-independent prostate carcinoma cells express $P_2$-purinergic receptors that are coupled to phospholipase C activation, acute $Ca^{2+}$ mobilization, prolonged cytoplasmic and nuclear $Ca^{2+}$ oscillations, growth arrest, and an increased rate of cell death. In contrast, androgen-sensitive cells have surface $P_2$ receptors that are uncoupled from phospholipase C, $Ca^{2+}$ mobilization and growth arrest. In collaboration with the Molecular Pathophysiology Branch we found that the androgen-sensitive cells lack expression of G protein alpha subunits that have been shown to couple to phospholipase C. These data strongly implicate phospholipase C activation and prolonged $Ca^{2+}$ mobilization in the growth-inhibitory efect of $P_2$ agonists, and provide a molecular mechanism for the uncoupling of the $P_2$ receptor in androgen-sensitive cells. Studies with the HMG-CoA reductase inhibitor lovastatin showed that lovastatin is cytotoxic to human prostate carcinoma cells. We found that lovastatin treatment results in marked deregulation of TGF-ß2 mRNA synthesis and in deregulation of protein expression of the tumor suppressor gene product pRB.

**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 three aspects of growth regulation in human prostate carcinoma cells: (1) effects of cAMP on growth and differentiation, (2) cytotoxicity through activation of $P_2$-purinergic receptors, and (3) the anticancer activity of the HMG-CoA reductase inhibitor lovastatin.

**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 $Ca^{2+}$ oscillations.

2. Protein expression studies are performed using immunocytochemistry and Western blot analysis. Morphologic studies are performed using light microscopy and transmission electron microscopy. Gene expression studies are performed using Northern blots, transient transfections and CAT assays.

**Major Findings:**

1. $P_2$-purinergic receptor agonists induce phospholipase C activation, acute $Ca^{2+}$ mobilization, prolonged $Ca^{2+}$ oscillations in both nuclear and cytoplasmic free $Ca^{2+}$, growth arrest, and an increase in cell death in androgen-independent prostate carcinoma cells.

2. 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. In these cells there is a defect in the expression of the

alpha subunits of the heterotrimeric G proteins that couple hormone receptors to phospholipase C. 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, and provide a molecular mechanism for the uncoupling of the $P_2$ receptor in hormone-sensitive cells.

3. Single-cell signal transduction studies showed that prostate carcinoma cells express two forms of $P_2$-purinergic receptors, $P_{2y}$ and $P_{2u}$, and that only the $P_{2y}$ receptor is coupled to growth arrest. These data will greatly facilitate the identification of a $P_2$ agonist with anticancer activity.

4. The HMG-CoA reductase inhibitor lovastatin is cytotoxic to human prostate carcinoma cells. Lovastatin treatment results in marked deregulation of TGF-ß2 mRNA synthesis and deregulation in protein expression of the tumor suppressor gene product pRB.

**Publications:**

Fang WG., Pirnia F, Bang YJ., Myers CE, and Trepel JB. $P_2$-purinergic receptor agonists inhibit the growth of androgen-independent prostate carcinoma cells. J. Clin. Invest. 1992:89:191-196.

Bergsagel PL, Victor-Kobrin C, Timblin CR, Trepel J, and Kuehl WM. A murine cDNA encodes a pan-epithelial glycoprotein that is also expressed on plasma cells. J. Immunol. 1992;148:590-596.

Myers C, Cooper M, Stein C, LaRocca R, Walther Mc, Weiss G, Choyke P, Dawson N, Steinberg S, Uhrich M, Cassidy J, Kohler D, Trepel J, and Linehan W. Suramin: A novel growth factor antagonist with activity in hormone-refractory metastatic prostate cancer. J. Clin. Oncol. 1992;10:881-889.

Bang YJ, Kim SJ, Danielpour D, O'Reilly MA, Kim KY, Myers CE, and Trepel JB. Cyclic AMP induces transforming growth factor ß2 gene expression and growth arrest in the human androgen-independent prostate carcinoma cell line PC-3. Proc. Natl. Acad. Sci. USA. 1992;89:3556-3560.

**Patent Applications:**

Myers CE, Kang W K, Whitesell LJ, Neckers LM, and Trepel JB. Anticancer activity of lovastatin and related compounds.

Whitesell L, Neckers L, Trepel J, and Myers C. Tumoricidal activity of benzoquinoid ansamycins against prostate cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06721-04 CP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 |
| Others: | F. Pirnia | Microbiologist | CPB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
Medicine Branch, NCI, NIH (K. Cowan, K. Dixon, T. Fojo, C. Herzog, S. Bates, P. Elwood, K.-N. Chung); NCI-Navy Medical Oncology Branch (C. Allegra, P. Johnson, J. Germ); Georgetown Univ. Med. Ctr. (R. Glazer, S. Ahmad).

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
Cell Signalling and Oncogenesis Group/Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF 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 strdies. 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:**

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.

Publications:

Dixon KH, Trepel JB, Eng SC, and Cowan KH. Folate transport and the modulation of antifolate sensitivity in a methotrexate-resistant human breast cancer cell line. Cancer Commun. 1991;3:357-365.

Yee LK, Allegra CJ, Trepel JB, and Grem JL. Metabolism and RNA incorporation of cyclopentenyl cytosine in human colorectal cancer cells. Biochem Pharmacol. 1992;43:1587-1599.

Herzog CE, Trepel JB, Mickley LA, Bates SE, and Fojo AT. Multi-method analysis of mdr-1/P-glycoprotein in human colon cancer cell lines. J Natl Cancer Inst. 1992;84:711-716.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06722-04 CP

PERIOD COVERED: October 1, 1991 to September 30, 1992

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

Characterization of IL6-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 |
| | M. Loeloff | Biologist | CPB, COP, DCT, NCI |
| | R. Brown | Guest Worker | CPB, COP, DCT, NCI |

COOPERATING UNITS (if any)

Lab. of Genetics, NCI, NIH (B. Mock); Lab. of Cellular and Developmental Biology, NIDDK, NIH (A. Greenberg); Department of Medicine, Univ. of Chicago (O. Colamonici)

LAB/BRANCH: Clinical Pharmacology Branch

SECTION: Tumor Cell Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF 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.)


A primary goal of this laboratory is to advance our understanding of cytokine-mediated tumor cell proliferation, with the ultimate goal of identifying targets for therapeutic intervention. For our studies we have focused on IL-6-dependent myeloma growth, a well characterized model of cytokine-dependent malignancy. We are performing studies aimed at (1) the characterization of the IL-6 receptor complex, (2) elucidating the mechanisms by which IL-6 regulates the action of growth-related genes, and (3) analyzing the mechanism by which tumor cells progress to a growth factor-independent phenotype.

We have continued the structural characterization the IL-6 receptor on various cell types. Affinity crosslinking studies in our laboratory indicate that (1) a 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. Studies using monoclonal antibodies developed in this laboratory also reveal that polymorphic forms of the IL-6 receptor complex exist on different cell types and may thus allow myeloma cells to be distinguished from normal cells.

We are characterizing IL-6 regulated genes that participate in the control of IL-6-dependent cell proliferation. The expression of the immediate-early response gene *jun*-B is known to be upregulated in IL-6-dependent myeloma cells. Using antisense oligonucleotides to *jun*-B we have found that *jun*-B is essential for proliferation and survival in these cells. We have initiated studies to identify and characterize the IL-6 responsive elements in the *jun*-B promotor. Initial results indicate these elements reside in a region 540 base pairs upstream of the transcriptional start site.

Our studies reveal that the progression to IL-6-independence is often mediated by a nonautocrine mechanism. Restoration of IL-6-dependence by the introduction of normal (wild type) DNA via cell fusion suggests that a negatively acting, growth regulatory gene is lost during the transition to IL-6-independence. In order to identify the chromosome on which this gene resides, we are screening a large panel of IL-6-dependent and IL-6-independent hybrid lines, derived by fusing IL-6-independent rat myeloma cells with normal mouse B lymphocytes. Identification of the chromosome will contribute to the molecular cloning of the gene responsible for transition to autonomous growth.

**Objectives:**

A primary goal of this laboratory is to advance our understanding IL-6-mediated myeloma growth with the ultimate goal of identifying areas that may provide targets for therapeutic intervention. Early studies by the senior 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 IL6-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 IL6-dependence representing a key step in the progression to a fully malignant tumor. We are performing studies aimed at (1) the characterization of the IL-6 receptor complex, (2) elucidating the mechanisms by which IL-6 regulates the action of growth-related genes, and (3) analyzing the mechanism by which tumor cells progress to a growth factor-independent phenotype.

**Methods Employed:**

To determine if IL-6-independent tumor cells have become autonomous by an autocrine or non-autocrine mechanism, supernatants were tested for IL-6 using a sensitive bioassay and cells were 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 and *jun*-B. *jun*-B promotor studies are carried performed using transient luciferase expression plasmids and include the use of deletions and site specific mutations, and DNase hypersensitivity analysis. Genetic composition of hybrid cell lines is performed by Southern blotting and polymerase chain reaction analyses.

**Major Findings:**

1. Non-autocrine, autonomous rat and murine myelomas can be restored to IL-6-dependence by the introduction of normal (wild type) DNA via cell fusion. The ability of normal DNA to restore IL-6-dependence suggests that the progression to a growth factor-independent phenotype may involve the functional loss of a negatively acting, growth regulatory gene. In order to identify this gene, we have created a large panel of IL-6-dependent and IL-6-independent hybrid lines derived by fusing IL-6-independent rat myeloma cells with normal mouse B lymphocytes. We are using this panel of cell hybrids to identify the chromosome on which this gene resides and we have thus far excluded twelve chromosomes as candidates. Knowledge of the chromosome location will contribute to the molecular cloning the gene responsible for transition to autonomous growth.

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. Furthermore, crosslinking studies using monoclonal antibodies developed in our laboratory indicate that polymorphic forms of gp80 and of the IL-6 receptor complex exist on different cell types and may allow myeloma cells to be distinguished from other IL-6 receptor bearing cell types.

3. *jun*-B is an immediate early response gene known to be upregulated by IL-6. Using antisense oligonucleotides to *jun*-B, we have found that *jun*-B is essential for the proliferation and survival of IL-6-dependent murine myeloma cells. We have initiated studies of the I,mL-6 responsive elements in the *jun*-B promotor. Initial results indicate these elements reside in a region 540 base pairs upstream of the transcriptional start site. Identification of the response elements may lead to the identification of transacting factors that contribute to *jun*-B regulation and increase our understanding of the regulatory pathways that control IL-6-dependent cell proliferation.

4. In continued collaboration with members of the Laboratory of Cellular and Developmental Biology, we have found that IL-6 is involved in the regulation of lipoprotein lipase activity in adipocytes and thus may play a role in cancer cachexia.

**Publications:**

Rieckmann P, D'Alessandro F, Nordan RP, Faucci AS and Kehrl JH. IL-6 and tumor necrosis factor-alpha. Autocrine and paracrine cytokines involved in B cell function, J. Immunol. 1991;146:3462-3468.

Schwab G, Siegall CB, Aarden LA, Neckers LM and Nordan RP. Characterization of an interleukin-6-mediated autocrine growth loop in the human multiple myeloma cell line, U266, Blood 1991;77:587-593.

Colamonici O, Pfeffer L, D'Alessandro F, Platanias L, Gregory S, Rosolen A, Nordan R, Cruciani R, and Diaz M. Multichain structure of the IFN-[illegible] receptor on hematopoietic cells, J. Immunol. 1992;148:2126-2132.

Greenberg A, Nordan R, McIntosh J, Calvo J, Scow R, and Jablons J. Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin 6 in cancer cachexia. Cancer Res. 1992;52:1-4.

Rosolen A, Colamonici OR, Pfeffer LM, Whitesell L, Nordan R, and Neckers LM. Detection of functional interferon alpha receptors in human neuroendocrine tumor cell lines using a new monoclonal antibody, Eur. Cytokine Netw. 1992;3:81-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06730-04

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 | Branch Chief | 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 Pharmaoclogy Branch

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD

| TOTAL STAFF 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 phosphorthioate obligodeoxynucleotides 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 06119-23 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

P.I. Jacqueline Whang-Peng, Head, Cytogenetic Oncology Section, MB, COP, DCT, NCI

Other: Turid Knutsen, Medical Technologist, MB, COP, DCT, NCI
Wei-Peng Zhao, General Fellow, MB, COP, DCT, NCI
Khalid Emara, Guest Researcher, MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
NCI/Navy Medical Oncology Branch, NCI; Surgery Branch, NCI; Pediatric Branch, NCI; Laboratory of Tumor Virus Biology, NCI; Environmental Epidemiology Branch, NCI

LAB/BRANCH
Medicine Branch

SECTION
Cytogenetic Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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 congential diseases, with special emphasis on AIDS patients who develop leukemia or lymphoma. Specific disease studies include lymphoma, renal cell carcinoma, small cell tumors of childhood, lung cancer, and leukemia (e.g., ALL, preleukemia, secondary leukemia), etc.

2. Cytogenetic studies of renal cell carcinoma. Preliminary analysis of 37 cases (11 direct and 26 cell lines) revealed a high incidence of chromosome 3 and 7 abnormalities; correlation of clinical and cytogenetic findings are being completed, based on the division of patients into four categories: (1) deletions of no. 3, (2) gain of no. 7, (3) abnormalities of 3 and 7, (4) no abnormalities of 3 or 7.

3. Cytogenetic studies in non-Hodgkin's lymphoma (NHL). Lymph node studies have been performed on 323 NHL NIH patients in the last ten years; 49/323 had serial lymph nodes, plus analysis of other tissues such as bone marrow, blood, and pleural fluids. Two reports are in progress, one on the total 323-patient population and the other on the serial cases; the chromosomal abnormalities are being correlated with histologic type, disease stage, and survival. Preliminary analysis in the serial studies revealed: 23/49 with t(14;18), 3 with t(8;14), and 20 with other clonal chromosomal abnormalities.

4. Localization of genes in normal chromosomes, using in situ hybridization ($^{3}$H-labeled and biotinylated probes).

5. Localization of chromosome abnormalities in resting cells, using the chromosome painting technique (FISH), in tumors with few or no dividing cells.

6. Use of FISH in interface cells to measure the distance between chromosomes 9 and 22 in normal individuals and CML patients to evaluate the relationship between these two chromosomes and formation of the 9;22 translocation.

7. Study of DNA topoiosmerase-mediated genome instability. Together with Dr. Leroy Liu of Johns Hopkins and Dr. Julang Huang of the Institute of Molecular Biology (Taiwan), we are studying the potential role of DNA topoisomerases in mediating illegitimate recombination in mammalian cells using the human c-ABL protooncogene as a model system.

8. Studies of genetic models of tumorigenesis of the uterine cervix. The development of cervical carcinoma progresses through several distinct stages from hyperproliferation of the epithelium to dysplasia, carcinoma in situ (CIN), and finally, invasive and metastatic carcinoma. Based on the current concept of the multi-step multi-factorial process of carcinogenesis, genetic mutation of specific oncogenes and tumor suppressor genes is probably associated with each step of tumorigenesis. We therefore propose to perform several studies to identify and isolate individual gene(s) involved in the various steps of tumorigenesis of the uterine cervix.

9. Cytogenetic studied in mesothelioma. Fresh specimens and cell lines are being obtained from Dr. Harvey Pass of the Surgery Branch, NCI. He has approximately 30 cell lines and can provide us with 1-2 fresh samples per week. Analysis of the first three specimens (pleural effusions) revealed cytogenetic abnormalities in all three cases, with chromosome numbers in the diploid range, and numerical (3/3) and structural (2/3) abnormalities; two cases had structural abnormalities involving chromosomes I and 6, and two cases had +22.

Projects Completed:

I. Chromosome studies in HTLV-I,II and HIV-I,2 cell lines infected in vivo and in vitro: 6 HTLV-I lines, 1 variant HTLV-I line, 2 HTLV-II lines, 5 T cell lines infected with HIV-I, 2 and cell lines infected with HTLV-II (lines infected in vitro were studied prior to and post-infection). All lines had multiple chromosomal abnormalities; of note were breakpoints at 6q11-13 (3/6 HTLV-I lines) (in 6/II HTLV-I patients studied previously, 6q deletions were associated with aggressive disease, short survival, and hypercalcemia), involvement of 2lp11 and +20 in both HTLV-II lines, and abnormalities of 17 (most frequent), 3, and 21 in the HIV-I infected lines (17q is the site of genes coding for NGL, CD7, HTLV-I, NGFR, and MIC6).

2. Two collaborative projects on the p53 recessive oncogene in leukemia with Dr. Carolyn Felix (formerly PB, NCI); we performed the cytogenetic studies. In the study of ALL patients, despite negative cytogenetic findings (no 17p abnormalities) and low level of mutation, the data supported the role of both hereditary and acquired p53 mutations in the pathogenesis and/or progression of some cases. In the second study, there was found to be an absence of hereditary p53 mutations in ten familial leukemia pedigrees.

3. Gene localization of HGPXI and RHOH 12. HGPXI had previously been mapped to 3q11-13.1 and RHOH 12 to 3p21. Our in situ hybridization studies localized both genes to 3p21, confirming the molecular studies conducted by Dr. Jeffrey Moscow (MB, NCI), which showed that the two genes are within 800 bp of one another. (Manuscript in preparation.)

4. Cytogenetic studies of KB cell lines (Dr. Clement Knight). Correlation was found between numerical and structural abnormalities of chromosome 11 and folate receptor resistence in the wild type (KB R10) and the subclone (KB 6B). The KB 6B displayed decreased expression of the folate receptor and fewer copies of chromosome 11q, the site (11q22.1-q23.1) of the gene family for the folate receptor. (Manuscript in preparation.)

5. Cytogenetic studies of cell line HL60 (Dr. Daniel Schlaifer). Multiple identical chromosomal markers were observed in the myeloperoxidase positive (A+) and negative (A-) clones, but the critical difference was the presence of chromosomal double minutes in the A+ clone, which displays resistance to VCR. (Manuscript in preparation.)

6. Chromosome studies in seven established cervical carcinoma cell lines (HT3, MEI80, MS751, C33A, SiHa, C4 I, C4 II) showed some clonal chromosome abnormalities. Chromosomes 2, 3, 11, and 14 were most frequently involved in these abnormalities. (Manuscript in preparation.)

PUBLICATIONS:

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Minna J, Maneckjee R, D'Amico D, Bader S, Bodner S, Broers J, Buchhagen D, Carbone D, Chiba I, Curiel D, Fedorko J, Geradts J, Jensen S, Knutsen T, Linnoila I, Mitsudomi T, Nau M, Oie H, Pass H, Russell E, Steinberg S, Takahashi T, Unger T, Viallet J, Whang-Peng J, Gazdar A. Mutations in dominant and recessive oncogenes, and the expression of opioid and nicotine receptors in the pathogenesis of lung cancer. In: Brugge J, Curran T, Harlow E, McCormick F, eds. Origins of Human Cancer: A Comprehensive Review. New York: Cold Spring Harbor Press, Cold Spring Harbor 1991; pp. 781-789.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06516 11 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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, M.D., Ph.D. | Chief, Breast Cancer Section | MB,COP,DCT,NCI |
| Others: | Charles Morrow | Biotechnician | MB,COP,DCT,NCI |
| | Jeffrey Moscow | Senior Staff Fellow | MB,COP,DCT,NCI |
| | Merrill Goldsmith | Microbiologist | MB,COP,DCT,NCI |
| | Kathy Dixon | Visiting Fellow | MB,COP,DCT,NCI |
| | Erasmus Schneider | Visiting Scientist | MB,COP,DCT,NCI |
| | Jean Gudas | Senior Staff Fellow | MB,COP,DCT,NCI |
| | Mary Jane Madden | Chemist | MB,COP,DCT,NCI |

COOPERATING UNITS *(if any)*
NIH Pathology, Surgery Branch, Radiation Oncology Branch, Pediatric Oncology Branch

LAB/BRANCH
Medicine Branch

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, Maryland

| TOTAL STAFF 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 cells as well as studies on the cell cycle regulation of human mammary epithelial cells. We have identified a number of changes associated with the development of multi-drug resistance including overexpression of the mdr1/P-glycoprotein drug efflux pump as well as changes in the number of drug metabolizing enzymes including glutathione transferase and the selenium dependent glutathione peroxidase gene. We have initiated studies on the role of these genes in development of resistance as well as on the regulation of expression of these genes in sensitive and drug resistant breast cancer cells. We have analyzed the transcription and posttranscriptions control mechanisms involved in the regulation of expression of the human mdr1 gene, the human π class glutathione transferase gene, and the human selenium dependent glutathione peroxidase one gene.

Other studies from our laboratory have identified other models of drug resistance. These include two models of non-P-glycoprotein associated with multidrug resistance. One of these is a mitoxantrone resistant breast cancer cell line with efflux in drug accumulation not associated with P-glycoprotein expression. The other is an etoposide resistant breast cancer cell line in which resistance is associated with non-P-glycoprotein associated decreases in drug uptake and alterations in toposomerase II activity. We also have studied a methotrexate resistant cell line that is defective in the reduced folate transport system. Additional studies in our laboratory have examined the cell cycle regulation of normal human mammary epithelial cells in comparison with estrogen receptor positive and estrogen receptor negative breast cancer cells.

Project Description

A. *mdr*1 Gene Regulation

We have cloned and sequenced the human mdr1/P-glycoprotein promotor and 4.7 kb of upstream sequences and studied the regulation of expression of this gene by fusing the promotor and flanking regions to the chloranphenoenichol transferase gene (CAT gene). These studies have demonstrated important sequences involved in both basal transcription and initiation of transcription of the human mdr1 gene. Current studies are addressing the nuclear protein factors that interact with these specific mdr1 DNA sequences.

B. Glutathione Transferases in Human Breast Cancer

Previous studies from our laboratory have shown that drug resistant breast cancer cells contain increased levels of π class glutathione transferase. Immunohistochemical studies have demonstrated that π class GST expression is inversely related to estrogen receptor and progesterone receptor expression in primary breast cancer cells. Furthermore, studies in node negative breast cancer (N=60) there was a correlation between increased GSTπ expression and poor prognosis. This association was not related to hormone receptor status, tumor size, patient age, or nuclear grade. Studies have also demonstrated that the regulation of GSTπ expression is predominantly posttranscriptional and, studies are underway to identify the genomic DNA sequences involved in the posttranscriptional control of the GSTπ expression.

C. Glutathione Peroxidase Expression of Breast Cancer

Multidrug resistance in human breast cancer cells is also associated with increased expression of glutathione peroxidase gene expression. Our laboratory has cloned glutathione peroxidase gene and sequence analysis revealed that 5' end of the glutathione peroxidase gene is only 2kb downstream of the human ras-related rho H12 oncogene. rhoH12 gene expression is related to cell growth and is differentialy expressed in some human tumors compared to adjacent normal tissues.

D. Non P-glycoprotein Mediated Resistance

We have isolated mitoxantrone resistant breast cancer cells that have decreased levels of drug accumulation and enhanced drug efflux in the absence of detectable P-glycoprotein/mdr1 gene expression. We have identified membrane proteins which are overexpressed in these cells suggesting the presence of a new drug efflux. We have also isolated a VP-16 resistant human breast cancer cell line that is cross-resistant to other topoisomerase II inhibitors. Resistance in this cell line is associated with a decrease in drug accumulation, in the absence of P-glycoprotein/mdr1 expression and altered topoisomerase II activity. The defect in topoisomerase II is being investigated.

E. Methotrexate Resistance

We have characterized a methotrexate resistant human breast cancer cell line that has a defect in the reduced folate carrier system. To examin the effect of increased folate receptor expression in this breast cancer cell line, we have in collaboration with Drs. Pat Elwood and Koong Nah Chung (Medicine Branch), transfected an expression vector for this gene into drug sensitive and resistant breast cells. Cells containing increased expression of the folate receptor display a marked increase in growth in low concentrations of folates including folic acid and reduced folates and enhanced sensitivity of folate receptor transfected cells to the lipophilic antifolate analog trimetrexate.

F. Cell Cycle Regulation in Human Mammary Epithelial Cells

We have established conditions using Lovostatin and/or growth factor deprivation to study the cell cycle regulation in normal human mammary epithelial cells grown in vitro, as well as for estrogen receptor positive and estrogen receptor negative breast cancer cells. We have found an inverse correlation between c-myb expression and in hormone receptor expression in human breast cancer cells. In order to study the function of myb expression in breast cancer cells, we have transfected expression vectors containing this gene into ER positive and ER negative breast cancer cells. We are also examining the cell cycle of P-53 expression in normal and malignant breast epithelial cells and as mentioned above we are exploring the changes in expression of the rho H12 oncogene protooncogene in cell cycle regulated normal and malignant breast epithelial cells.

Publications

Kantor, R.R.S., Giardina, S.L., Bartolazzi, A., Townsend, A.J., Myers, C.E., **Cowan, K.H.**, Longo D.L., and Natali, P.G. Monoclonal antibodies to glutathione S-transferase $\pi$ - immunohistochemical analysis of normal human tissues and cancers, Int. J. Cancer, 47(2):193-201, 1991.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06716 05 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
| PI: | Eddie Reed | Senior Investigator | MB, COP, DCT, NCI |
| Others: | Ricardo Parker | Biotechnology Fellow | MB, COP, DCT, NCI |
| | Meenakshi Dabholkar | Visiting Fellow | MB, COP, DCT, NCI |
| | Annette Bicher | Staff Fellow | MB, COP, DCT, NCI |
| | Terri Cornelison | Senior Staff Fellow | MB, COP, DCT, NCI |
| | Frieda Bostick-Bruton | Biologist | MB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
U.S.C. Cancer Center, Los Angeles, California
Laboratory of Molecular Pharmacology, DTP/DCT/NCI
Department of Molecular Epidemiology, Columbia University, New York

LAB/BRANCH
Medicine Branch

SECTION
Medical Ovarian Cancer Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.5 | 4.5 | 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.)*


**Overview:**
This unit conducts work on the clinical and molecular parameters of resistance to platinum compounds and other DNA damaging agents. Work is being performed using fresh human materials, and established cell lines of malignant and non-malignant origin.

**Project Description:**

**DNA Damage/Repair in Malignant and Non-Malignant Cell Lines:**

DNA damage and repair have been studied in three ways: studies of total genomic cellular repair of platinum-DNA adduct; studies of gene specific repair of platinum-DNA adduct; and studies of the repair of platinum-damaged plasmid DNA which has been transfected into target cells.

Our studies of total genomic repair in human ovarian cancer cells and in non-malignant human leukocyte cell lines, show that DNA repair appears to the primary determinant of cisplatin resistance in both settings. In collaboration with Vilhelm Bohr of LMP/DCT/NCI, we have shown that differences in gene specific repair are even more pronounced than differences in total genomic repair, when comparing sensitive and resistant human ovarian cancer cells. Studies of transfected platinum-damaged plasmid into human leukocyte cell lines, show that T cells appear to be an excellent target for the study of interindividual differences in in vivo DNA repair capability. In contrast, B cells and other non-T cells are not as useful.

Project Description (Cont.):

Studies of Human DNA Repair Genes in vitro and in vivo:

We have studied the ERCC family of human DNA repair genes to determine whether these genes may play a role in clinical resistance to DNA-damaging agents, and to learn more about the normal biology of human DNA repair processes. ERCC1 is a human excision nuclease, ERCC2 is a 3'-5' helicase, and ERCC3 is a 5'-3' helicase. The function(s) of ERCC6 have yet to be determined.

In tumor tissues taken from ovarian cancer patients treated with cisplatin or carboplatin based chemotherapy, the level of expression of ERCC1 was directly related to clinical resistance to platinum therapy, whereas ERCC2 expression was not. This finding is consistent with in vitro studies where transfection of ERCC1 into repair-deficient CHO cells confers resistance to cisplatin along with restored DNA repair capability, but this is not the case with ERCC2.

In non-malignant bone marrow samples taken from 52 patients with cancer, ERCC genes (ERCC1, ERCC2, and ERCC6) showed a bi-modal pattern of expression. That is, when ERCC1 expression was low, so was the expression levels of ERCC2 and ERCC6. This occurred in spite of the fact that ERCC6 is located on chromosome 19, whereas ERCC1 and ERCC2 are located on chromosome 10. This "coordinated" regulation of these three genes in human tissues has not been reported for human DNA repair genes before.

Studies of DNA Damage in Blood Cells from Patients:

In patient who receive cisplatin/carboplatin as their only therapy, the level of platinum-DNA adduct formation in leukocyte DNA is directly related to disease response. Previously, studies have shown this to be true using ELISA assays and AAS.

In a study recently completed, data generated in collaboration with Franco Muggia of USC show that the ELISA data of eight years ago fits very well with AAS data from current studies, in terms of the number of platinum lesions per unit DNA. That is, the precise amount of DNA damage that was associated with disease response as measured by ELISA, is totally consistent with current AAS data.

In another recently completed collaborative study, Federica Perera of Columbia University analyzed data from 36 patients with testicular cancer who received 3-5 drugs during the course of therapy. Such data included platinum-DNA adduct, SCE formation, and several other biomarkers. Although platinum-DNA adduct did not correlate with disease response, SCE (sister chromatid exchange) formation did correlate with disease response. SCE's are thought to be a reasonable representation of total DNA damage following carcinogenic exposures. These data suggest that total leukocyte-DNA damage correlates with disease response in patients receiving 3-5 agents, and supports the theory of the existence of a "parallel" between tumor tissue and hematopoietic tissues in the handling of DNA damaging agents.

Publications:

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Dabholkar M, Bostick-Bruton F, Weber C, Egwuagu C, Bohr V A, and Reed E. Expression of excision repair genes in non-malignant bone marrow tissues from cancer patients. Mutation Res, in press, 1992.

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Zhen W, Link Jr C J, O'Connor P M, Reed E, Parker R J, Howell S B, and Bohr V A. Increased gene-specific repair of cisplatin interstrand crosslinks in cisplatin resistant human ovarian cancer cells. Mol and Cell Biol, in press, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06718 04 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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, M.D. | Senior Investigator | MB, COP, DCT, NCI |
| Others: | Koong-Nah Chung, Ph.D. | Senior Staff | MB, COP, DCT, NCI |
| | Kirsten Price | Biologist | MB, COP, DCT, NCI |
| | Yutaka Saikawa, M.D. | Visiting Fellow | MB, COP, DCT, NCI |
| | Sue Roberts, Ph.D. | Senior Staff | MB, COP, DCT, NCI |
| | Sydelle Zinn | Medical Technician | MB, COP, DCT, NCI |
| | Tae-Hyun Paik, M.D. | Visiting Scientist | MB, COP, DCT, NCI |
| | Clement B. Knight, M.D. | Medical Staff Fellow | MB, COP, DCT, NCI |
| | William C. Owen, M.D. | Special Volunteer | MB, COP, DCT, NCI |
| | Rama Verma, Ph.D. | General Fellow | MB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Experimental Hematology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.8 | 4.3 | |

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.)*


Summary of Work:

Human folate binding proteins (FBP), or folate receptor (FR), bind and transport physiologic 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 Experimental Hematology Section is investigating the following:

1) Structure, Function, and Molecular Biology of Human FRs;
2) Role of the FRs in folate/drug transport and acquired resistance;
3) Expression of the FRs.

Major findings:

1. Structure, Function, and Molecular Biology of Human FBPs: **1A. Molecular Biology:** We have completed the characterization of the primary structures and genomic organization of a homologous placental FR cDNA (FR-P) and its respective gene. The longest GT11 cDNA (FR-P15) contains 125 bp 5'UTR, a 166 bp 3' UTR, and a 753 bp ORF. The 5' terminus of the FR-P cDNA is heterogeneous as determined by the sequence of 3 GTll and 11 PCR-cloned cDNAs, and the cDNA ($FR\text{-}P_R$) reported by Freisheim, et. al. The longest PCR-cloned cDNA (FR-PCR1) indicates that the 5' FR-P UTR is 151 bp. Cloning, PCR, and RNase protection assays demonstrate that FR-P cDNA represents the major transcript, and suggest that the divergent 5' $FR\text{-}P_R$ terminus may originate from gene polymorphisms.

Two of 5 human EMBL3 clones contain the full-length FR-P gene which is polymorphic relative to 5' BamH1 sites, spans 5148 bp, and contains 5 exons. Structural analysis of the FR-P gene boundaries predicts a transcript size of 1098 bp which correlates with the determined size (1100 bp) on Northern blots and cDNA sequences. The FR-P transcript is present in human placenta RNA but is not detectable in RNA from normal adult tissues by Northern analysis consistent with the hypothesis of differential fetal FR-P expression. Isolation of the FR-P gene will permit determination of the functional relevance of consensus promoter sequences juxtaposed to the FR-P gene transcription start site by standard techniques (e.g. reporter gene assays) and may further elucidate the transcriptional mechanisms resulting in tissue specific gene expression.

Using cDNA specific probes, we have determined the ultrastructure of the FR-KB (and FR-P) genes by Southern analysis and have isolated 2 non-overlapping hybridization positive genomic clones which are polymorphic relative to 5' restriction sites and contain the FR-KB gene. The KB cell gene spans 6156 bp, and is composed of 7 potential exons flanked by consensus splice sequences. The structure of the gene and cDNA is characterized by alternative splicing of exons I through IV accounting for the observed heterogeneity of FR-KB 5' UTRs and FR-KB10, a cDNA recently cloned in our lab. We are mapping the 5' boundary of these exons; determining the utilization of the putative alternatively spliced exons in normal human tissues; and studying the differences in translational efficiency of FR-KB transcripts. Preliminary data suggest that the transcriptional start site is heterogeneous and varies among normal human tissues. This heterogeneity may be involved in tissue differential expression, in translational efficiency, or in transcript stability. Transcriptional start sites utilized by normal human tissues are contained within exons I through IV. Numerous consensus regulatory elements are contained in the upstream DNA sequences which we are subcloning adjacent to reporter genes (CAT) to determine their functional relevance. Preliminary data indicate that sequence upstream to the major start site exhibits promoter activity.

Partial sequence analysis of 3 other EMBL3 genomic clones indicate that they contain DNA homologous to the FR cDNAs/genes and that 2 are probably unprocessed pseudogenes. These data indicate that the FR-P and FR-KB cDNAs/genes are homologous, have similar chromosomal organization, are conserved in evolution, and are transcriptionally active members of a gene family. Others have observed a unique, immunologically-related FBP in human saliva. Since comparison of the primary structure of this FBP to other FRs may provide insight into their structure and function, we have screened a human salivary gland with radiolabeled FR cDNAs under relaxed stringency. We have isolated > 100 hybridization positive clones and are selecting clones for further characterization by differential hybridization.

**1B. Cell Biology:** Human membrane-bound FRs may be anchored to the plasma membrane by either a transmembrane anchor or GPI tail. To study the membrane anchor, we have

transfected the FR-KB10 into mouse L(tk-) fibroblast cells which are unable to attach proteins via a GPI tail. Transfected cells express functional folate receptor on the plasma membrane as determined by surface ligand binding studies, ligand internalization, and immunohistochemical staining. Furthermore, the FR expressed in L(tk-) is resistant to release by PI-specific PLC. In contrast, >90% of the FR expressed by transfected MCF-7 cells and 50% of the KB cell FR is sensitive to PI-PLC release. These data confirm that functional FRs may be anchored by either mechanism and suggest that, depending on the cell type, a single cDNA can encode a functional protein anchored in the membrane by a transmembrane domain or a GPI tail.

We have identified residues which may be important in ligand binding by comparison of the primary amino acid sequences of FBPs and chicken riboflavin binding protein, and by computer analysis of the FR-KB sequence. Using PCR or transformer mutagenesis, we have subcloned site-directed mutant FR cDNAs into a mammalian expression vector to identify the ligand binding site, to study membrane anchoring, and to investigate the role of phosphorylation sites. Preliminary transfection experiments suggest that Trp residues are important in ligand binding. Experiments are ongoing to determine if altered binding results from changes in protein structure, changes in mRNA or protein stability, or whether the Trp residues constitute part of the binding site. We have mutated a conserved tyrosine residue and conserved clusters of charged residues to determine their role in FR function.

We have previously demonstrated in vitro phosphorylation of a serine residue in the KB cell FR protein mediated by PLC. Recent experiments demonstrated that the "KB cell" FR is phosphorylated in vivo by KB cells as well as transfected MCF-7 and CHO cells. Immunoprecipitated in vivo phosphorylated FR contains a phosphoserine. We will determine the specific serine residue which is phosphorylated and the relevance of phosphorylation on FR function by means of site directed mutagenesis and transfection studies.

2. **Role of the FBPs in Transport of Folates and in the development of Acquired Methotrexate Resistance**: We have characterized MTX resistant KB cells selected in folate replete and physiologic folate conditions. In a set of 9 clones selected in low folate conditions, defective transport was the most common phenotypic alterations (n=9), and was associated with increased DHFR expression in about 50% (n=4) of the mutants. The extent of reduction in transport Vmax was linearly related to their reduction in FR expression (R=0.9). These results indicate that the FR is important in MTX uptake in these cells, that modulation of FR expression is the most common mechanism whereby KB cells acquire MTX resistance, and suggest that reduction in FR levels in tissues expressing the FR may result in altered MTX cytotoxicity. We are cloning the 5' domains of KB cell gene from stable MTX-resistant mutants and will run nuclear run ons to determine the mechanism of decreased FR transcription in these cells.

Since selection may result in other alterations, we have transfected the FR cDNA into human MCF-7 and ZR-75 cells, and rodent CHO and L(tk-) cells and measured changes in MTX sensitivity. In all cell lines, transfection of the FR cDNA was associated with improved growth particularly in media containing reduced folates. Moreover, in all cell lines except the ZR-75 (in which the wild type will not grow in physiologic folate concentrations), expression of the FR is associated with increased methotrexate senaitivity by virtue of decreases in the IC50 and the IC99 supporting the relationship between the FR and MTX cytotoxicity.

In MTX resistant and folate deplete KB cells, we have identified a ~50 Kd, membrane-associated, non-glycosylated protein (pI=7.0) which crossreacts with a polyclonal anti-FR antisera and appears to copurify with the FR suggesting that MA50P may have an affinity for folic acid or for the FR. To study MA50P's relationship to the FR, and to MTX cytotoxicity and folate homeostasis, we have isolated 7 homologous clones which contain two distinct cDNA inserts (2100 and 2700 bp) from an expression library by immunoscreening. We

have sequenced a 2111 bp clone (CY.3) which contains a 363 bp 5' UTR; a 461 bp 3' UTR; and a 1287 bp ORF. The ORF encodes a 429 residue protein (MW=49,687) containing 7 consensus phosphorylation sites, 2 N-linked glycosylation sites, assumes an alpha secondary structure, and encodes a protein which crossreacts with the polyclonal anti-FR antisera.

3. **Expression of the FBPs:** In studies of in vitro transcription and translation of FR-KB32 and FR-KB10, we have demonstrated that in vitro transcription of each cDNA is similar. However, translation efficiency ($S^{35}$methionine incorporation in protein / ug cRNA added) of the cRNAs are 10-15 fold different. Identical results have been observed in vitro following transient transfection of each cDNA construct into CHO cells. To determine the nature of these differences, we are studying the transcriptional efficiency of each construct in detail and have designed deletion constructs of each KB cell cDNA for in vitro expression analysis in stable transfectants to study transcript expression and stability, translation efficiency, and FR stability.

Although FRs (FBPs) have been identified in numerous mammalian by binding assays, immunologic techniques, or northern analysis, these studies are unable to discriminate between the 2 members of the FR gene family and are difficult to compare or quantitate FR expression due to methodological differences or endogenous folate levels. In order to determine the relative expression of specific cDNA transcripts in human tissues, to determine the transcriptional start site(s) of each cDNA, and to determine differences in expression of the FR cDNAs during fetal development, we have employed cDNA-specific nucleotide probes or oligonucleotides in PCR and RNase protection assays. Preliminary results of these studies indicate that expression of either FR gene transcript is not restricted to fetal tissues, that transcript expression of each gene varies between tissues (e.g. they are not constitutively expressed), that the FR genes appear to be regulated non-coordinately, that the KB cell transcript is more commonly expressed than the placental FR gene, and that the KB cell gene is expressed in virtually all proliferating tissues.

Using the FR genes, we have begun studies of the transcriptional regulatory elements of each gene. We have identified the promoter of the major transcriptional start site of the KB cell gene in CAT assays. This will permit dissection of the transcriptional regulation of the KB cell FR gene.

Publications

None.

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# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06727 11 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

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COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 3.0 | 1.5 |

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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 have investigated the mechanisms of oncogenes regulation in human malignancies. A large body of evidence has implicated the c-myc oncogene in normal cell growth and differentiation as well as in the development of a wide range of human cancers. This project is designed to study both the mechanisms of transcriptional regulation of the c-myc gene and to examine functional properties of the c-myc protein product. The four major projects are the following:

A) Transcriptional regulation of the c-myc oncogene in normal and neoplastic cells.

B) Triplex-helix formation with the positive cis-acting DNA sequence to inhibit expression of the c-myc gene

C) Effect of differentiating agents on the function of transcription factors and their role in the regulation of the c-myc gene.

D) 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. 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 suggesting that the interaction among these four cis elements and their binding factors may be important in the control of c-myc expression. We found, however, that MIF-3 by itself is a strong negative regulator of the promoter activity of the c-myc gene and that MIF-3 is also frequently mutated in BL c-myc DNA. In addition, MIF-1 can reduce the suppression of MIF-3. Using methylation interference assay we have localized the contacts points to the six bp in the middle and to the 3'and 5' ends of the MIF-3 binding sequence, suggesting that MIF-3 protein requires the entire 34 bp for binding. We have employed expression library screening with the DNA recognition sequence to clone the gene which encodes the MIF-3 protein. Purification and cloning of the genes encoding these proteins will allow us to characterize protein binding region which interacts with the critical base pairs on the DNA molecules. In addition, we plan to determine whether the 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. DNA samples from colon, lung and follicular lymphoma are being examined by heteroduplex analysis for mutations in a 400 bp region of the intron I of the c-myc gene. Positive samples will then be sequenced to determine mutations in the four protein binding regions described above. These studies will explore 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) Triplex-helix formation with the positive cis-acting DNA sequence to inhibit expression of the c-myc gene.**

Work in several laboratories has indicated that triple helix formation can be used to target specific DNA sequences for transcriptional inactivation. It has been shown that a synthetic peptide can bind tightly to duplex DNA termed PuF within the human c-myc promoter and inhibit transcription of the c-myc gene. The PuF element has been shown to control transcription from the P1 promoter of the c-myc gene. Since in most normal and tumor cells the P2 promoter is predominant, our goal was to design triplex oligonucleotide to regulate the P2 promoter activity. Marcu has described DNA regulatory sequence in the murine c-myc gene (termed ME1) which positively regulate the P2 promoter activity. This sequence is conserved in human c-myc (18 out of 20 bp) consisting of a 20 bp stretch of purines which are required for triplex formation. In collaboration with Len Neckers (CPB, COP, NCI) we are examining the use of triplex formation to inhibit c-myc activity

through the ME1 site. Using mobility shift assay we have shown that the human ME1 recognition sequence can bind nuclear factor from HeLa cells. Parallel and anti-parallel oligonucleotides synthesized to the purine sense strand of the ME1 site will allow us to established the conditions for triplex formation in vitro and apply this information to triplex formation in vivo cell system.

**C) 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 and DMSO treatment, indicating that nuclear regulatory proteins that bind to the c-myc gene are affected by the agents used. Our goal was to determine, whether MIF-1 was post-transcriptionally modified following retinoic acid treatment or induced during the differentiation process. We have used cation-exchange and affinity chromatography to purified the MIF-1 from undifferentiated HL60 cells. Our results suggests that undifferentiated HL60 cells synthesize a protein(s) which has specificity for the MIF-1 sequence but is distinct from the MIF-1 protein purified from HeLa cells. Cross-linking analysis demonstrated that the undifferentiated HL60 cells contain two proteins p35 and p97 which interact with MIF-1 recognition sequence. Our data suggest that the 138kd MIF is induced during the differentiation of HL60 cells and it binds to the DNA recognitions sequence in presence of p97 and/or p35 proteins. The functional role of these distinct protein complexes which showed specificity for the same DNA binding sequence in the control of the differentiation and proliferation in HL60 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.

**D) Search for the function of the c-myc proteins.**

To understand how c-myc is involved in normal and aberrant cell proliferation the molecular function of the c-myc proteins needs to be elucidated. Our studies focus on c-myc proteins encoded by the open reading frame (ORF) of exonII/exonIII (p64,p67) as well as the c-myc protein encoded by the ORF of exon I (p58)

To elucidate the significance of the p58 c-myc protein. There are four potential open reading frames (ORF) in the c-myc gene, however, only two proteins (p64 and p67) have been well characterized in vivo . These proteins are translated from the predominant 2.2 kb mRNA species. An additional protein (p58) may also exist in vivo, but the corresponding mRNA has not been previously identified. We now have evidence for a novel mRNA species transcribed from the c-myc gene which may potentially translate the p58 protein. We have screen cDNA library and are analyzing the c-DNA phage clones.The full length clone will be used to determine whether it could be translated in vitro into appropriate size protein.The functional role this protein may play in cell growth and differentiation would be evaluated. This study may provide clues whether this p58 protein (which is disrupted or mutated in almost all cases of BL) also plays a role in development of the malignant phenotype.

The myc proteins p64,p67 have been implicated in cell proliferation, differentiation and neoplasia but their mechanism of function at the molecular level is unknown. A picture is emerging from work of several laboratories that the c-myc protein may be a transcriptional activator. The carboxyl-terminus of the Myc protein binds to related helix-loop-helix protein called Max (for myc associated protein "x") and this complex binds to a DNA sequence. The evolving knowledge of Max function, will lead to a better understanding of the c-myc protein. Our laboratory, however, is studing the role of the N-terminal portion of the protein which is required for transactivation and for transformation by the c-myc gene. To determine whether the c-myc protein can interact with other cellular proteins, we have fused the exon II ORF of the c-myc gene with gluthatione-S-transferase gene and the expressed fusion protein was than purified from bacterial proteins on gluthatione beads. Our results suggests that the myc fusion protein can interact with several proteins from HeLa and HL60 cells. Specifically, there are two abundant cellular proteins which bind to the c-myc gene. Deletional analysis of the c-myc gene exon II allowed us to map protein binding to region of the c-myc gene know to play a role in transactivation and cellular transformation. The identity of these cellular proteins are being investigated. In addition, frequent amino acid substitutions have been found within the N terminal region of the c-myc gene product in Burkitt's lymphoma tumor cell lines. Preliminary experiments showed that mutations found in c-myc protein from BL modulate binding to these cellular proteins. These studies may help to understand the molecular function of the c-myc product and may lead to development of trans-dominant mutants which may impede specific function of the c-myc gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06731 04M

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Expression, Regulation, and Reversal of Multidrug Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
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| PI: | Susan Bates | Senior Investigator | MB, COP, DCT, NCI |
| Others: | Bruce Dickstein | Biologist | MB, COP, DCT, NCI |
| | Stefania Scala | General Fellow | MB, COP, DCT, NCI |
| | Mary Spolyer | General Fellow | MB, COP, DCT, NCI |
| | Anika Lynch | SRTP | MB, COP, DCT, NCI |
| | Jong Sok Lee | Special Volunteer | MB, COP, DCT, NCI |
| | Yoon Koo Kang | General Fellow | MB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
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Dr. Yoon S. Cho-Chung, Laboratory of Tumor Immunology and Biology, DCBDC, NCI

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

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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.)*

The focus of the laboratory work this year has been on multidrug resistance, evaluating mechanisms of reversal, determining expression in human tumor samples, and performing functional studies in both in vitro model systems and in patient samples. The studies evaluating mechanisms of multidrug resistance reversal include principally two approaches. One, we have studied the effect of protein kinase C modulation on P-glycoprotein phosphorylation and function, demonstrating that inhibition of PKC results in dephosphorylation of Pgp and decreased transport of certain agents. Second, we have continued studies of the effect of 8-Cl-cAMP on multidrug resistant breast cancer cells, with the intent of discovering the mechanism underlying the decreased expression of mdr-1/Pgp after treatment. Concurrently, studies in growth factor biology in drug resistant cells have been initiated. Finally, a Phase I clinical study of PSC 833, a new P-glycoprotein antagonist, has received IRB approval and should enroll its first patient by September 1, 1992.

Studies of Expression, Regulation, and Reversal of Multidrug Resistance

1. Modulation of Pgp expression. Previous studies demonstrated that 8-Cl-cAMP was able to decrease Pgp by decreasing mdr-1 mRNA expression. This appeared to be linked weakly to a more differentiated phenotype in the multidrug resistant human breast cancer cell lines used as a model. In studies designed to unravel the mechanism underlying the 8-Cl-cAMP effect, we evaluated the effect of various activators and inhibitors of protein kinase A on mdr-1 expression. Pharmacologic modulation of PKA did not affect mdr-1 expression; studies of the effect of these agents on mdr-1 function are ongoing. In collaboration with Dr. Yoon Cho-Chung, studies are in place to evaluate the effect of transfection of antisense and sense constructs for the subunits to PKA to examine the effect of altering the levels of PKA in multidrug resistant cells.

2. Modulation of P-glycoprotein (PgP) function. Previous studies demonstrating that Pgp function could be impaired by treatment with sodium butyrate were extended to include the study of protein kinase C (PKC) modulation. The studies, performed in collaboration with Tito Fojo, have found that PKC inhibition by calphostin C, staurosporine, or prolonged treatment with phorbol ester results in decreased phosphorylation of Pgp. This decrease in Pgp phosphorylation is associated with impaired transport of vinblastine, actinomycin D, adriamycin, daunorubicin, rhodamine, azidopine, and cyclosporine. It is associated with increased transport of progesterone and verapamil. The increased Pgp transport of verapamil appears in preliminary studies to result in decreased Pgp antagonism when used to block rhodamine or vinblastine transport. The mechanism underlying the decreased transport appears to be loss of Pgp binding, as demonstrated by decreased photoaffinity labeling with azidopine after treatment of cells with calphostin C. Studies of PKC isoforms in multidrug resistant cells suggest that different isoforms, alpha and zeta, may phosphorylate P-glycoprotein. Finally, we have evaluated normal tissues to determine whether the detectable Pgp is capable of being modulated by alterations in PKC which are comparable to those observed in the model systems used in vitro. Studies of normal mouse liver and human T lymphocytes have demonstrated evidence of Pgp-mediated efflux. Human lymphocytes, like the multidrug resistant cells in vitro, demonstrate increased efflux after treatment with phorbol ester. Some samples demonstrate decreased transport after treatment with PKC inhibitors.

3. Expression of mdr-1 in human tumor samples. Using the quantitative PCR methodology which we previously developed in our laboratory, we have continued the determination of mdr-1 levels in human lymphoma samples. Levels from sixty patients have been examined. These have consistently shown low levels at the onset of chemotherapy. Serial samples from at least 12 patients are being studied, and precise quantitation is ongoing. Many of these demonstrate an increase in mdr-1 expression after treatment with a natural product chemotherapy regimen. When possible, these samples are being tested for alterations in Pgp function, as described in #4. Lymphoma cells from one patient who had progressive disease after treatment demonstrated efflux of rhodamine ex vivo suggesting that the low level of Pgp expression observed in the cells could result in decreased drug accumulation.

4. Use of FACS analysis to study Pgp function in cell lines from the NCI drug screen. We have collaborated with the Developmental Therapeutics Program to evaluate mechanisms of resistance present in the sixty cell lines of the NCI new drug screen. Studies of both mdr-1 expression and function have been performed in conjunction with the laboratory of Tito Fojo. Currently, my laboratory is engaged in evaluating Pgp function through the study of rhodamine transport by Pgp. Cells expressing no Pgp accumulate rhodamine, and retain it, despite placement in rhodamine-free media. Decreased accumulation occurs in some cells after treatment with phorbol esters, suggesting latent Pgp activity. Cell lines with higher levels of Pgp have both decreased accumulation and increased efflux of rhodamine. These results will be compared to cross-resistance data available from DTP. Using the characterized screen, evaluation of proposed Pgp antagonists will be possible.

5. Phase I clinical trial of PSC 833. Sandoz corporation has developed an analogue of cyclosporine which is putatively nontoxic; is not nephrotoxic and not immunosuppressive. PSC 833 in vitro is able to block Pgp function as well as cyclosporine. Multidrug resistant cells demonstrate return of drug accumulation to levels present in parental drug sensitive cells with simultaneous treatment with PSC 833 at concentrations which should be readily obtained in the serum of patients. The Phase I study will determine the pharmacokinetics and bioavailability of PSC 833. After an initial cycle in which both PSC 833 and vinblastine are given alone to allow independent pharmacokinetics, PSC 833 will be given in combination with a 5 day continuous infusion of vinblastine. Dose escalation of PSC 833 will be done in a standard Phase I fashion. It is expected that PSC 833 will decrease vinblastine clearance and increase the AUC (area under the curve) of vinblastine.

6. Growth factor biology in drug resistant cells. The onset of drug resistance in cancer cells is frequently accompanied by alterations in cell biology which may contribute to the observed drug resistance. Previous studies have examined breast cancer cell lines which demonstrate increased EGF receptor and decreased estrogen receptor. Efforts have been made in our laboratory to sensitize these cell lines to chemotherapy by adding agents which block the receptor. These studies continue, and have been extended to include characterization of other growth factor receptors, including the IGF-1 receptor and erb-B2 along with the proto-oncogenes c-fos, c-myc, and c-jun. The latter are known to be regulated by estrogen and growth factors in human breast cancer cells. How they are altered after the onset of drug resistance is unknown and is the subject of our current studies.

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3. Lai, G-M., Moscow, J.A., Alvarez, M.G., Fojo, A.T., and Bates, S.E. Contribution of glutathione and glutathione-dependent enzymes in the reversal of adriamycin resistance in colon carcinoma cell lines. Int.J.Cancer, 49:688-695, 1991.
4. Bates, S.E., Shieh, C.Y., Tsokas, M., Expression of mdr-1/Pgp in neuroblastoma. American Journal of Pathology, 139:305-315, 1991.
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6. Bates, S.E., Currier, S.J., Alvarez, M. and Fojo, A.T. Modulation of P-glycoprotein phosphorylation and drug transport by sodium butyrate. Biochemistry, in press, 1992.
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8. Bates, S.E. Clinical applications of serum tumor markers. Annals of Internal Medicine, 115:623-638, 1991.
9. Bates, S. and Herzog, C.E., Molecular diagnosis of multidrug resistance. In: Drug Resistance 3. Ozols, R.F. and Goldstein, L. (Eds) Kluwer Academic Publishers, Norwell, MA, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06732 04M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

| | | | |
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| Others: | Lyn A. Mickley | Biologist | MB, COP, DCT, NCI |
| | Manuel Alvarez | Guest Researcher | MB, COP, DCT, NCI |
| | Zhirong Zhan | General Fellow | MB, COP, DCT, NCI |
| | Oliver Rixe | Guest Researcher | MB, COP, DCT, NCI |
| | Hiroshi Takano | Guest Researcher | MB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
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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.)*

**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.**

I Multidrug Resistance

This field has been of interest to the principal investigator for nine years. Work in this field is continuing. The emphasis continues on understanding several basic aspects of this field with a final goal of applying the findings to clinical trials. Several projects have been ongoing in the laboratory.

1. Four years ago a project was begun whose goal was to determine the primary sequence of P-glycoprotein from a large number of cell lines. The original motivation was a desire to determine the frequency with which mutations would occur in the P-glycoprotein gene during the course of drug selection. We sought to determine this because we felt that if such mutations occurred with any significant frequency, the study of clinical samples should encompass methods for mutation analysis. To accomplish this, a full length cDNA encoding the P-glycoprotein gene was restricted and subcloned in smaller fragments, which were utilized in RNase protection analyses. To date, work has been completed on the primary sequence of over 100 P-glycoprotein from a wide variety of sources, including over 40 multidrug resistant sublines. The results unequivocally demonstrate the infrequent occurrence of acquired mutations during the selection of cells for multidrug resistance. In the drug resistant sublines studied, acquired mutations were found in only two, including the previously reported mutations in colchicine selected KB cells. In addition, two sites of genetic polymorphism were identified, with an otherwise high degree of conservation of primary sequence in P-glycoproteins from these sources. The acquired mutations and the sites of genetic polymorphism have allowed a detailed study of the process of drug selection and the evolution of genetic changes. These studies indicate that during the course of drug selection overexpression of one or both alleles can occur, with gene amplification following or coinciding with overexpression of an individual allele. Furthermore, during the course of selection, one clone usually becomes the predominant or exclusive member of a population as evidenced by identical patterns of overexpression and/or amplification in subclones, and the finding of acquired changes in all the RNA. Both of these observations are of potential clinical interest and the relevance of these observations in patient samples is under evaluation. We have begun to take advantage of the existence of genetic polymorphism and our ability to measure expression from individual alleles as a tool to understand the mechanisms responsible for drug resistance and the role of clonal selection during treatment. Preliminary observations indicate that in patient samples expression of one or both alleles can occur, suggesting different mechanisms of activation, the possibility of acquired mutations mediating multidrug resistance clinically, and the likelihood of clonal selection. As these studies continue, work is also underway attempting to understand the genetic changes responsible for the different patterns of gene activation.

2. The use of the RNase protection analysis as a method of screening for mutations, although not perfect, proved extremely valuable in our studies with P-glycoprotein, and we are now in the initial stages of a similar approach to screen for mutations in topoisomerase II. As subcloning progresses, work has already begun using adriamycin selected sublines, with one mutation already identified in a multidrug resistant subline. Both genetic polymorphism and acquired mutations will provide tools to examine expression of individual alleles, and will alow correlations to be made with our findings with P-glycoprotein. Similar studies are planned with the tubulin genes as we plan ahead to clinical studies with Taxol, especially in patients previously treated with vinca alkaloids. The possibility that acquired mutations in this gene may occur frequently or that certain mutations may predict for taxol sensitivity will be investigated.

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. In addition models of vincristine and of taxol resistance in both ovarian cancer and breast cancer are under

study.

A natural extension of this work has been a collaborative effort with Dr. Susan Bates and the staff of the Developmental Therapeutics Program, to utilize the NCI new drug screen panel as a tool for screening reversal agents and determining optimal combination of agents. As a first step, we measured P-glycoprotein expression in the sixty cell lines in the panel. To date these measurements have provided interesting correlations between drug resistance and P-glycoprotein expression and are guiding the establishment of a subpanel of cell lines that will be used for screening additional analyses and ongoing with the goal of identifying potential P-glycoprotein substrates - already a successful task - thus identifying agents with potential activity if given in conjunction with a P-glycoprotein antagonist as well as agents which might be useful as antagonists.

II Mechanisms of Cisplatin Resistance

This field has evolved into a major focus of the laboratory. This project began over five years ago with the initial selection of two cisplatin resistant cell lines and is currently taking advantage of a total of 15 cell lines isolated from ovarian, breast, colon and epidermoid parental lines, and including several drug sensitive revertants.

Several observations have been made to date, all of which are undergoing further studies. In collaboration with Drs. Eddie Reed and Ricardo Parker, we have demonstrated reduced cisplatin accumulation in all cell lines. In addition, a tight correlation has been demonstrated between constitutive metallothionein expression and cisplatin resistance. Finally, dramatic changes in cytoskeletal proteins and their organization have been demonstrated with a tight correlation observed between these changes and cisplatin resistance when parental, drug resistant, and drug sensitive revertants are examined.

Current efforts are directed at expanding and understanding these observations. The occurrence of these changes in independently selected cell lines suggests multiple mechanisms may be responsible for cisplatin resistance. The reduction in cisplatin accumulation is under further investigation, while the role of metallothionein is being tested by transfection in cell lines with and without reduced cisplatin accumulation. A role for the cytoskeleton as a target for cisplatin, as a mechanism of resistance or both is being actively pursued. In addition, in one cisplatin selected cell line cytogenetic studies have demonstrated an abnormally banding region consistent with gene amplification, and attempts are underway to isolate these sequences. The availability of excellent models, and the unequivocal findings to date promise that this field will become increasingly available to more in depth understanding of cisplatin resistance.

III Adriamycin Resistance Associated with Overexpression of a 95 Kilodalton Membrane Protein:

Attempts continue to further investigate this mechanism. Collaborative efforts are underway to isolate and sequence the protein. Expression has been found in clinical leukemia samples and lung cancer cell lines from previously treated patients, and also increased expression in an etoposide selected cell line.

PUBLICATIONS

1. Bates, S.E., Shieh, C.Y., Mickley, L.A., Dichek, H., Loriaux, D.L., and Fojo, A.T. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein) found in adrenocortical carcinoma. J Clin Endo and Metab 73: 18-29, 1991.
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3. Fojo, A.T.: Multidrug Resistance. In: Advances in Internal Medicine. Ed: Stollerman, G.H., Raven Press, pp. 195-218, 1991.
4. Lai, G.-M., Chen, Y.-N., Mickley, L.A., Fojo, A.T., and Bates, S.E.: P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma cell lines. Int J Cancer 49, 696-703, 1991.
5. Lai, G.-M., Moscow, J.A., Alvarez, M.G., Fojo, A.T., and Bates, S.E.: Contribution of glutathione and glutathione-dependent enzymes in the reversal of adriamycin resistance in colon carcinoma cell lines. Int J Cancer, 49, 688-695, 1991.
6. Bates, S.E., Currier, S.J., Alvarez, M., and Fojo, A.T.: Modulation of P-glycoprotein phosphorylation and drug transport by sodium butyrate. Biochemistry, in press, 1992.
7. Herzog, C.E., Trepel, J.B., Mickley, L.A., Bates, S.E., and Fojo, A.T.: Various methods of analysis of mdr-1/P-Glycoprotein in human colon cancer cell lines. JNCI, 84, 711-716, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06734 02 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
| P.I. | Maria Zajac-Kaye, Ph.D. | Senior Staff Fellow | MB, DCT, NCI |
| Others: | Noa Ben-Baruch, M.D. | Clinical Associate | MB, DCT, NCI |
| | Nazma Jahan | Senior Staff Fellow | MB, DCT, NCI |
| | Mellissa Blake | Biological Aid Tech | MB, DCT, NCI |
| | Ngockhanh Thi Nguyen | Stay-In-School | MB, DCT, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NIH, NCI Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 1.2 | 0.4 |

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.)*

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 cellular and as well as viral genes. Our data suggests that the raf kinase may activate cellular oncogenes which may then activate the HIV-LTR, the HTLV-LTR as well as the immediate-early (IE) promoter of the cytomegalovirus (CMV). The activation state of raf oncogene may not only play a role in cellular transformation but it maybe important determinant of HIV, HTLV and CMV latency .

Regulation of oncogenes expression and viruses by activated raf kinase.

The raf kinase is a necessary component of the mitogenic signal cascade pathway. Treatment of cells with agents such as phorbol esters or cytokines result in the activation of serine threonine kinase activity of raf. In cells which harbor latent HIV-provirus, these agents also activate the transcription factor NFkB. An increase of HIV-LTR transcription is observed, which leads to a switch to productive infection. In addition, the T lymphocytes may serve as a reservoir for human cytomegalovirus (CMV) during latency and it has been suggested that T cell activation plays a role in stimulating the CMV gene expression. These observation suggested that the activated raf kinase may play a role in activation of transcription from HIV-LTR as well as from the immediate early (IE) genes of CMV. Viral activation is in part controlled by host-cell encoded transcription factors which interact with DNA binding motifs in the promoter and enhancer region of the virus. Thus raf in its active form may activate cellular factors which are required for induction of viral gene expression. Using a transient cotransfection assay, we tested the ability of raf kinase to activate transcription from the HIV-LTR, HTLV-LTR as well as from the CMV IE promoter and enhancer. Our data demonstrates that raf kinase activates expression of all three viral promoters tested.

The viral LTRs and the CMV IE promoter enhancer region consist of NFkB binding site and several other consensus regulatory elements. To determine the mechanism by which raf kinase activates the viral promoter region, we first examined the DNA sequence of the CMV IE promoter-enhancer. The CMV IE promoter-enhancer region contains several repeats of NF-1 (NF-1 is a cellular factor which plays a role both in DNA replication and in RNA transcription) , NF-kB binding sequences ( NF-kB was first described as a protein which binds to the immunoglobulin k enhancer), the cyclic AMP (cAMP) responsive element termed CRE as well as the MDBP binding site ( MDBP is a methylation binding protein which have been implicated to be similar to myc intron factor; MIF-1).

To determine which of these enhancer elements mediates the raf kinase stimulation of promoter activity, oligonucleotides representing the NFkB, CRE and MDBP repeats of the CMV IE region were ligated to an IE core promoter in front of luciferase indicator gene. Two copies of the NFkB element enhanced expression from the IE promoter when cotransfected with the activated raf kinase expression vector, while no enhancement was observed with the CRE or MDBP elements. This result suggests that raf kinase activates IE CMV promoter activity through the NFkB binding site. The NF-kB 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-kB is bound to its inhibitor present in the cytoplasm. Phosphorylation of the inhibitor molecule dissociates the complex and NF-kB (which consists of two molecules; p50 and p65) relocates to the nucleus in its active form. Thus, the raf kinase may either phosphorylate the inhibitor molecule and activate the NF-kB factor and/or act directly on NFkB molecules. We are currently testing this hypothesis. The NFkB molecules have been recently cloned and found to be homologous to the rel family of oncogenes. An interesting possibility is that deregulated or mutated NFkB may turn into an oncogene. In addition, our finding of raf regulation of the NFkB interaction with its binding element will also have implication in the regulation of the HIV-1 which is also under strong influence of NF-kB 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06735 02 M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 O'Shaughnessy, M.D. | Senior Investigator | MB, COP, DCT, NCI |
| Others: | Kenneth H. Cowan, M.D., Ph.D. | Senior Investigator | MB, COP, DCT, NCI |
| | Andrea Denicoff, R.N. | Research Nurse | MB, COP, DCT, NCI |
| | Marianne Hillig, R.N. | Research Nurse | MB, COP, DCT, NCI |
| | Noa Ben-Baruch, M.D. | Visiting Associate | MB, COP, DCT, NCI |
| | Timothy Mulligan, M.D. | Staff Fellow | MB, COP, DCT, NCI |
| | Margo Aron | Psych Social Worker | CC, NIH |
| | Jorge Carrasquillo | Senior Investigator | DNM, NIH |

COOPERATING UNITS *(if any)*
Pediatric Oncology Branch; NIH Transfusion Medicine Department, Clinical Hematology Branch, NHLBI, 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 9.6 | 9.6 | 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.)*

Over the past year, the Medical Breast Cancer Section has significantly expanded its clinical trial treatment effort, increasing the number of clinical protocols and doubling patient accrual. The clinical focus of the Medical Breast Cancer Section since 1988 has been four-fold: the development of 1) dose-intensive therapies in combination with hematopoietic growth factors; 2) new cytotoxic agents; 3) targeted therapies against tumor-associated antigens; and 4) the study of drug resistance markers in breast cancer. The major questions we have been interested in studying are: 1) Do dose-intensive cytotoxic regimens improve patient outcome (response rates, disease-free survival [DFS], overall survival [OS])?; 2) Do hematopoietic growth factors sufficiently ameliorate myelosuppression to enable the delivery of more dose-intensive therapy?; 3) What is the anti-tumor activity of intensive doses of new cytotoxic agents in combination with hematopoietic growth factors?; 4) How can the antitumor activity of radioimmunotherapy be enhanced?; and 5) Does cytotoxic chemotherapy change the baseline expression of the drug resistance markers P-glycoprotein and glutathione transferase π (GSTπ) in primary breast cancer? The clinical trials addressing these questions are summarized below. Over the past year, we have completed our Phase II frontline dose intensity trial for metastatic breast cancer and three Phase I or II trials of new cytotoxic agents. We have initiated a second generation dose intensity trial with hematopoietic growth factors and have recently begun a Phase I study of taxol and the P-glycoprotein blocker, R-verapamil. In addition, a Phase I study of the monoclonal antibody CC-49 directed against the pancarcinoma antigen, TAG-72, radiolabeled with a novel Beta emitter Lutetim-177 is underway. Lastly, we have completed the review process and will soon begin a pilot study of the feasibility of obtaining bone marrow engraftment with marrow and peripheral blood stem cell autografts that have been transduced with the neomycin resistance marker gene. This trial will be the forerunner to an upcoming study of marrow reconstitution with mdr-1- transduced marrow and peripheral blood stem cells in patients with metastatic breast cancer.

Project Description

Additional Personnel Assigned on Project:

| | | |
|---|---|---|
| Stacey Berg | Senior Investigator | POB, COP, DCT, NCI |
| Frank Balis | Senior Investigator | POB, COP, DCT, NCI |
| Michele Fox | Senior Investigator | DTM, NIH |
| Monica Yu | Research Associate | DTM, NIH |
| Yoomie Chung | Research Nurse | COP, DCT, NCI |
| Mary McCabe | Clinical Trial Specialist | CTEP, DCT, NCI |
| Maria Merino | Pathologist | DCBDC, NCI |
| David Danforth | Senior Investigator | SB, COP, DCT, NCI |
| Lori Pierce | Senior Investigator | ROB, COP, DCT, NCI |
| Jeffrey Schlom | Chief | LTIB, DCBDC, NCI |
| Patricia Keegan | Senior Staff | CBER, FDA |

## I. Dose Intensity Treatment Trials in Breast Cancer

A. Phase II Study of FLAC/GM-CSF in Patients with Locally Advanced and Metastatic Breast Cancer

We have completed this study of dose-intensive FLAC chemotherapy (5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide) in combination with GM-CSF in patients with Stage III or IV breast cancer. The primary goals of this study were to determine the level of dose intensity achievable and the response rates and toxicity of this regimen. Twenty-six patients with Stage III and 55 patients with Stage IV disease have been entered on study. Toxicity has been primarily hematologic with 94% of patients developing Grade 4 neutropenia and 77% Grade 4 thrombocytopenia. For Stage III patients, the clinical response rate was 100% with 20% pathologic CR's determined by multiple tru-cut biopsies of the original primary site. The median DFS and OS have not been reached with a median follow-up time of 16.4 months. The overall response rate for Stage IV patients is 85% with 23% CR's. The average durations of these responses are 10.5 months for the CR's and 12.3 months for the PR's. The median time to progression for the Stage IV patients is 16 months and median survival is 24 months. The average delivered dose intensity on this study is cyclophosphamide 194mg/M$^2$/week, doxorubicin 14.5 mg/M$^2$/week and 5-fluorouracil 335 mg/M$^2$/week. 97% of the intended FLAC doses were therefore delivered for all patients and all cycles in combination with GM-CSF. No correlations between dose intensity and the likelihood of achieving a response or with DFS or OS have been found for the Stage III or IV patients in this study.

B. Phase I Trial of FLAC Plus GM-CSF Plus Dose Escalation of IL-3 in Metastatic Breast Cancer

We are conducting this Phase I study of dose-intensive FLAC chemotherapy in combination first with interleukin-3 (IL-3) alone and then with IL-3 plus GM-CSF in order to determine the maximal tolerated dose(s) of the hematopoietic growth factors. We are also studying the patterns of circulating peripheral blood progenitor cells (PBPC) in response to IL-3 with and without GM-CSF following FLAC chemotherapy. The Phase I study of FLAC plus IL-3 alone has been completed. No dose limiting toxicity from IL-3 occurred at the highest dose level studied (10μg/kg). Accrual to the Phase I study of sequential IL-3 and GM-CSF administration is continuing. No dose limiting toxicity even at the highest dose level of IL-3 (10μg/kg) has been encountered in this part of the study. To complete this trial, we will next conduct a Phase I study

of combined IL-3 and GM-CSF with both growth factors administered daily following FLAC chemotherapy. Analysis of neutrophil and platelet recoveries and of circulating levels of PBPC following FLAC and IL-3, with and without GM-CSF, are underway.

C. Pilot Study of High-Dose Ifosfamide, Carboplatin, Etoposide (ICE) Chemotherapy, with Autologous Bone Marrow Support for Treatment of Metastatic Breast Cancer Patients: Use of the Retroviral Markers to Study the Biology of Bone Marrow Reconstitution

Prior to undertaking an mdr1 gene transfer study, we will conduct a pilot study in collaboration with Dr. Nienhuis which will attempt to reconstitute breast cancer patients' bone marrows with marrow stem cells that have been transfected with the neomycin phosphotransferase (neo) gene. The primary objective of this study will be to determine the feasibility of obtaining bone marrow engraftment with neo-transfected human stem cells. Serial samples of peripheral blood and bone marrow will be obtained during and after engraftment to study the trafficking patterns of the neo-transduced stem cells. Harvested bone marrow CD34+ stem cells will be transduced with high titer, helper virus-free neo retrovirus in the presence of hematopoietic growth factors (IL-3 and IL-6) to promote stem cell cycling and successful gene transfer. Patients will be treated with high dose ICE chemotherapy and the neo-transduced stem cells will be reinfused. The trafficking patterns of the stem cells will be studied by PCR analysis of peripheral blood and engrafted marrow stem cells and progeny. This clinical trial has passed review and has been approved by NCI's IRB, NIH's Institutional Biosafety Committee, and NIH's Recombinant DNA Advisory Committee. It will begin in the next 1 - 2 months. Once a clinical grade mdr-1 retrovirus is available, the $neo^R$ gene will be replaced by the mdr-1 gene to study whether patients' marrow can be reconstituted with cells containing a functional drug resistance gene.

## II. New Cytotoxic Agents

A. Phase I study of Taxol and Doxorubicin with G-CSF in Previously Untreated Metastatic Breast Cancer

We have completed this Phase I study of 72-hour continuous infusion taxol and doxorubicin with G-CSF. The MTD of Taxol in combination with Doxorubicin 75mg/$m^2$ and G-CSF is 160mg/$m^2$. The DLT of Doxorubicin 75mg/$m^2$ and Taxol 180mg/$m^2$ and G-CSF was typhlitis in 3/3 patients. The MTD of Taxol in combination with Doxorubicin 60mg/$m^2$ and G-CSF is 180mg/$m^2$. The DLT of Doxorubicin 60mg/$m^2$ and Taxol 200mg/$m^2$ and G-CSF is grade 3 or 4 diarrhea and abdominal pain without CT evidence of typhlitis in 3/6 patients. Other significant toxicities include grade 4 neutropenia in all patients, grade 4 thrombocytopenia in 7% of patients and hospitalization for fever and neutropenia in 47% of cycles. The average cycle length with G-CSF was 23 days. No significant allergic, cardiac or neurologic toxicity was seen. The overall response rate (RR) on this Phase I study has been 57% with 9% CRs. Eleven patients are still being treated and are not finally evaluable for response. We are conducting pharmacokinetic studies of taxol and doxorubicin to determine whether significant alterations in drug clearance occur when given in combination.

B. Phase I Study of High-Dose Piroxantrone Alone and In Combination with G-CSF

Piroxantrone is a new anthrapyrazole, an anthracycline derivative, designed to decrease cardiac toxicity while maintaining significant antitumor activity. We have completed this Phase I study and have defined the MTD of Piroxantrone alone as 185mg/$m^2$ given as a one-hour infusion every 21

days. The dose limiting toxicity at this dose level was neutropenia. The MTD of piroxantrone in combination with G-CSF was 335mg/m2 with the dose limiting toxicity being thrombocytopenia at the 455mg/m2 dose level. Six patients who were previously treated with doxorubicin (325mg/$m^2$ minimum cumulative dose) developed Grade 4 congestive heart failure and two of these patients died. The total cumulative piroxantrone doses in these patients ranged from 855mg/$m^2$ to 1245mg/$m^2$. This cardiac toxicity developed precipitously with left ventricular ejection function falling from normal to significantly clinically impaired within one cycle of therapy. No significant cardiac toxicity was observed in patients previously untreated with doxorubicin even at the highest cumulative piroxantrone dose of 2220mg/$m^2$. Five out of 28 breast cancer patients had a response to therapy (3 MR, 2 PR); no responses were seen in the 10 patients with other malignancies (renal, colon, esophagus, pancreas cancers). The mean t 1/2 alpha was 3.4 mins, t 1/2 beta 115 mins and total body clearance 826ml/min/$m^2$. A Phase II study of piroxantrone and G-CSF in breast cancer patients previously untreated with doxorubicin is recommended as a follow-up to this study.

C. Phase II Study of Fazarabine in Patients with Metastatic Breast Cancer

We have completed a Phase II study of Fazarabine, a synthetic pyrimidine nucleoside containing the structural features of ara-C and 5-azacytidine. Twenty-eight patients were treated with a starting dose of 2mg/$m^2$/hr as a continuous 72-hour infusion every 21-28 days with subsequent dose escalation to achieve Grade 4 hematologic toxicity. The major DLT was neutropenia with no other significant toxicities. There were 3 PR's lasting 5-11 months, all in nodal or soft tissue sites. Fazarabine is a well-tolerated antimetabolite with a low degree of antitumor activity in metastatic breast cancer.

## III. Targeted Therapies for Breast Cancer

A. Upregulation of Tumor-Associated Antigens (TAA's) by Interferon-Alpha in Metastatic Breast Cancer

We are studying the ability of systemically-administered interferon-alpha (IFN) to upregulate the expression of TAA's including the pancarcinoma antigen TAG-72. Patients with breast cancer metastatic to the chest wall undergo a biopsy of their tumor on Day 1, receive Interferon-alpha, 5MU/$m^2$ SC, Days 1, 2, 3, and have a repeat biopsy of the skin disease on Day 4. Six patients have been treated to date; data is currently available for three patients. The preliminary results suggest that IFN had some effect in increasing TAG-72 expression in two patients' tumors that constitutively express the antigen but was ineffective in the one patient whose tumor did not have constitutive TAG-72 expression. Accrual to this study is continuing. IFN may be useful as an adjunct to radioimmunotherapy to help enhance uptake of the radioimmunoconjugates.

B. Phase I Study of Intravenously Administered 177-Lutetium ($^{177}$Lu) Murine CC49 Monoclonal Antibody (MAb) in Patients with Advanced Adenocarcinoma

We are conducting this Phase I study of $^{177}$Lu-CC49 with the goals of defining the MTD and DLT of the radiolabeled antibody and of studying its ability to image known metastases. CC49 is a murine MAb directed against the pancarcinoma antigen TAG-72 and $^{177}$Lu is a novel beta (and low

level gamma) emitter. Three patients have been treated to date at the lowest $^{177}Lu$ dose level of $10mCi/m^2$. No toxicity has been observed. Lung and bone metastases in the three breast cancer patients have been successfully imaged with the gamma camera. Accrual to this study is continuing.

## IV. Multidrug Resistance Studies in Breast Cancer

### A. P-glycoprotein (Pgp) and Glutathione Transferease (GSTπ) Expression in Primary Breast Cancer

We are studying expression of the clinical drug resistance markers Pgp and GSTπ as well as the proto-oncogene erbB-2 in Stage II and III primary breast cancers. Biopsies of patients' breast cancers are obtained prior to initiating neoadjuvant FLAC/GM-CSF chemotherapy and at the completion of five (Stage II) or 10 (Stage III) cycles of therapy, prior to surgical resection of residual disease. We are interested in studying the baseline expression of these proteins in primary breast cancer as well their expression after treatment with FLAC/GM-CSF. In addition, we will analyze the prognostic significance of these markers regarding degree of clinical and pathologic response to chemotherapy, disease-free and overall survival. Pre- and post-treatment biopsies have been obtained on 30 patients to date and the immunohistochemical analysis is underway.

### B. Phase I Study of Taxol plus R-verapamil and G-CSF in Patients with Metastatic Breast Cancer

This Phase I study will begin in August, 1992 with the goal of defining the MTD and DLT of combined Taxol, R-verapamil (a Pgp blocker) and G-CSF. Patients with metastatic breast cancer will be treated initially with taxol to stable best response. Patients with residual disease will then receive taxol plus R-verapamil to study both the toxicity of the regimen as well as the ability of R-verapamil to induce further tumor shrinkage. Biopsies of accessible disease will be obtained prior to beginning taxol and again prior to the addition of R-verapamil to study whether Pgp, GSTπ and other markers correlate with response to treatment.

Publications

Lichter AS; Lippman ME; Danforth DN Jr.; d'Angelo T; Steinberg S; deNoss E; MacDonald HD; Reichert CM; Swain SM; Cowan KH; Gerber LH; Bader JL; Findlay PA; Schain W; Gorrell CR; Straus K; Rosenberg SA; Glatstein E. Mastectomy versus Breast Conserving Therapy in the Treatment of Stage I and II Carcinoma of the Breast: A Randomized Trial at the National Cancer Institute. J. Clinical Oncol. 10:976-983, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06736 02M

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 |
| Others: | Elise Kohn | Senior Investigator | MB, COP, DCT, NCI |
| | Gisele Sarosy | Senior Investigator | MB, COP, DCT, NCI |
| | Charles Link | Senior Staff Fellow | MB, COP, DCT, NCI |
| | Debra Adamo | Research Nurse Spec. | MB, COP, DCT, NCI |
| | Patricia Davis | Research Nurse Spec. | MB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
Clinical Pharmacology Branch, COP/DCT/NCI
Laboratory of Pharmacology, FDA
Laboratory of Pathology, DCBD/NCI

LAB/BRANCH
Medicine Branch

SECTION
Medical Ovarian Cancer Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*


**Overview:**

**This unit focuses on the development of new treatment strategies for patients with advanced stage ovarian cancer. Studies are conducted with patients with newly diagnosed disease, as well as with recurrent disease. Generally, studies have focused on the development of "dose intensity" strategies and the use of colony stimulating factors. Studies have also been conducted using non-traditional agents with novel mechanisms of action, such as suramin and CAI (carboxy amido imidazole).**

**Project Description:**

**Studies in the Initial Therapy of Advanced Stage Disease:**

**We have completed a phase II platinum dose intensity study in advanced stage disease, as initial systemic therapy after surgery. Recent studies elsewhere suggest that dose intensity may be important in small volume disease, but there is some question is large volume disease. In our study, drug doses were; carboplatin 600 mg/m2, cisplatin 100 mg/m2, and cytoxan 250 mg/m2. Carboplatin and cytoxan were given on day 1, cisplatin was given on day 8, and the cycle was repeated every 28 days. The pathologic complete response proportion was 11 of 25 overall (44%), and was 7 of 18 (39%) in suboptimal patients. This compares with an overall PCR rate of 26%, taken from a collected series detailed in DeVita's textbook of oncology. Platinum dose intensity in our study was more than twice that used in standard types of regimens. Patient tolerance was good.**

**We have initiated a phase I study of taxol, cisplatin, and cytoxan in newly diagnosed patients. As of this writing, we have completed accrual to dose level two. Following this phase I, we will initiate a limited phase II study to assess potential response rate.**

Project Description (Cont.)

Studies with Taxol in Recurrent Disease:

Taxol is a diterpenoid product of the bark of the Western yew tree, Taxus brevifolia. This agent was demonstrated by others to have an approximate 30% objective response rate in human recurrent ovarian carcinoma. We have conducted studies to determine if taxol can be dose intensified with the use of granulocyte-colony stimulating factor (GCSF).

We first conducted a phase I study and showed that with GCSF support, a taxol dose of 250 mg/m2/21 days was well tolerated. Further, at a dose of 300 mg/m2/21 days, dose-limiting peripheral neuropathy developed. We then conducted a phase II study of taxol with GCSF support. At a taxol dose of 250 mg/m2, the objective response rate was 50% (22 of 44 evaluable patients). When compared with collective 30% response rate rate reported in the literature to date (33 of 110 evaluable patients), the difference is statistically significant, $p2 = 0.019$

These clinical studies have been supplemented with taxol pharmacokinetic laboratory studies. Clearance and renal excretion were determined to occur in our cohort to the extent that has been described previously. However, two novel findings were made that have not been previously reported. The percentage of drug bound to plasma proteins was determined to be 97%. Also, the peak drug level was found to correlate well with the occurrence of mucositis ($p2 = 0.002$), but did not correlate with the occurrence of neuropathy or with myelosuppression (note that patients received GCSF).

Evaluation of GCSF Usage:

Since GCSF has been used to dose intensify taxol therapy, we have evaluated the utility of "flexible" GCSF dosing as a tool to maintain taxol dose intensity. In concept, if "flexible" GCSF dosing is shown to work, the principle could be applied to a large number of myelosuppressive agents.

We have found that by using flexible GCSF dosing, taxol dose intensity could be maintained in the majority of our phase II cohort. Forty-two (42) percent of these patients would have otherwise been dose reduced with respect to taxol, with subsequent compromise of tumor response.

Studies with Traditional Types of Agents:

We have completed a phase II study of 5FU and leucovorin in advanced stage recurrent disease. Our results suggest that this combination offers meaningful disease management in up to 50% of patients who are clinically resistant to DNA damaging agents such as cisplatin. Although objective response occurs in ~ 10% of patients, disease stablilization for periods up to 2 years occurs in ~ 40% of patients.

We have completed a phase I study of tetraplatin. Multiple tumor types were entered onto study. Through dose level 9 (90 mg/m2/28 days), there were no objective responses to therapy, although several patients experienced stable disease for periods up to 6 months. Pharmacokinetic studies showed a biphasic pattern of drug clearance similar to cisplatin and carboplatin, and platinum-DNA adduct levels were not measurable at any dose level studied.

Studies with Non-Traditional Agents:

Several studies have investigated the role of anti-cancer agents which have novel mechanisms of action. Suramin, an anti-proliferative agent, was shown by this group to have modest activity in platinum-refractory recurrent ovarian cancer but was associated with unacceptable toxicity in this cohort. CAI, a signal transduction inhibitor, is currently in phase I trial and only a small number of patients have been treated to date.

Publications:

Reed E, Cooper M, LaRocca R, Bostick-Bruton F, and Myers C E. Suramin activity in advanced stage platinum-refractory ovarian cancer. Eur J Cancer, 28A(No.4/5);864-866, 1992.

Reed E, Jacob J, Ozols R F, Young R C, and Allegra C. 5-Fluorouracil and leucovorin in platinum-refractory advanced stage ovarian cancer. Gynecol Oncol, in press, 1992.

Bicher A, Sarosy G, Kohn E, Adamo D O, Davis P, Jacob J, Chabner B A, and Reed E. Age does not influence taxol dose intensity in recurrent ovarian cancer. Cancer (suppl), in press, 1992.

Cornelison T L, and Reed E. Dose intensity analysis of high dose carboplatin in refractory ovarian carcinoma relative to age. Cancer (suppl), in press, 1992.

Rothenberg M L, Ozols R F, Glatstein E J, Steinberg S M, Reed E, and Young R C. Dose-intensive induction therapy with cyclophosphamide, cisplatin, and abdominal radiation in advanced stage epithelial ovarian cancer. J Clin Oncol, 10:727-734, 1992.

Sarosy G, Kohn E, Stone D A, Rothenberg M, Jacob J, Adamo D O, Ognibene F P, Cunnion R E, and Reed E. Phase I study of taxol and G-CSF in patients with refractory ovarian cancer. J Clin Oncol, 10(No 7):1-6, 1992.

Christian M C, Spriggs D, Tutsch K D, O'Rourke T, VonHoff D D, Jacob J L, and Reed E. Phase I trials with ormaplatin (tetraplatin). IN (S Howell, ed) Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, Plenum Press, New York, pp 453-458, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06737-01 M

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Immunoreconstitutive therapy in patients with HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Robert Yarchoan, M.D., Chief, Retroviral Diseases Section, MB, COP, DCT, NCI

Other: Samuel Broder, M.D., Director, NCI
Bach-Yen Nguyen, M.D., Senior Clinical Investigator, MB, COP, DCT, NCI
James M. Pluda, M.D., Senior Clinical Investigator, MB, COP, DCT, NCI
Laura Shay, R.N., Medicine Branch, COP, DCT, NCI
Dan L. Longo, M.D., Frederick Cancer Research and Development Center, NCI
Gene M. Shearer, Ph.D., Cancer Immunology Branch, NCI

COOPERATING UNITS *(if any)*

Frederick Cancer Research and Development Center, NCI; Cancer Immunology, DCBD, NCI

LAB/BRANCH

Medicine Branch

SECTION

Retroviral Diseases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | ½ | ½ |

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.)*

T cell immunosuppression is a central defect in patients with HIV infection. There is evidence from autopsy studies that AIDS patients have substantial thymic damage. Also, there is evidence that HIV can infect cells in the thymus. It is thus possible that thymic damage is a limiting factor in the therapy of AIDS. There is some evidence that thymic involution in aged rats can be partially reversed by certain pituitary hormones (including growth hormone). Recently, Drs. Murphy, Durum, and Longo in the NCI have reported that recombinant human growth hormone (rhGH) could increase the number of CD4 T cells in the thymus and peripheral nodes in mice with severe combined immunodeficiency disease given human peripheral blood lymphocytes. Additional studies have indicated that recombinant human insulin-like growth hormone type 1 (rhIGF-1) can induce immunologic improvement in rodents, and that the two hormones were synergistic when given together. These results suggested that rhGH or rhIGF-1 might be useful in restoring immune function in patients with HIV infection if given with anti-retroviral therapy. Based on the above observations, we are exploring whether either or both of these hormones can induce immunologic improvements in patients with HIV infection. We have initiated a phase I trial in which rhGH, rhIGF-1, or both, will be administered to patients with symptomatic HIV infection in conjunction with antiretroviral therapy. We will closely monitor a variety of immunologic parameters.

PROJECT DESCRIPTION:

The focus of the efforts of the Retroviral Diseases Section (and previously the Laboratory of the Associate Director) has been predominantly directed towards developing effective antiretroviral therapy for HIV infection. However, it has been observed that while patients with AIDS generally have increases in their CD4 cells upon receiving with azidothymidine or dideoxyinosine, such advanced patients generally do not have full immunoreconstitution. Moreover, the CD4 increases are often transient. For these reasons, there is now a substantial interest in exploring immunoreconstitutive therapy in patients with HIV infection.

There is evidence from autopsy studies that AIDS patients have substantial thymic damage, and it is possible that this damage is a limiting factor in the therapy of AIDS. Growth hormone (GH) and insulin growth factor (IGF) (a polypeptide hormone structurally related to insulin, and a mediator of the effects of growth hormone) have been shown to have a pronounced effect on thymus growth and the number of CD4 and CD8 cells in a murine model. Recently, Drs. Murphy, Durum, and Longo in the NCI reported that 7 days after initiation of treatment of mice with severe combined immunodeficiency syndrome (SCID) with rhGH for 3 days, there was an increase in the percentage of CD4+ and of CD8+ cells (Durum, S. K., et al. The FASEB Journal 5: A1674). Two weeks after cessation of rhGH therapy, the thymus of rhGH-treated SCID mice displayed greater than 50% CD4/CD8 double positive cells. In a subsequent experiment, transplanted thymus cells were used to reconstitute SCID mice; rhGH accelerated the thymus and lymph node reconstitution by these transplanted cells (Murphy, W. J., et al. The FASEB Journal 5: A1669).

In another study with hypophysectomized rats, which have decreased IGF-1 levels associated with abnormal T- and B-cell responses, GH was able to restore the immunologic function. The simultaneous administration of rhGH and rhIGF-1 to hypophysectomized rats resulted in an increase in weight and lymphoid tissue that was greater than that seen with either agent administered alone. In rats made diabetic by streptozotocin, the cellularity of lymphoid organs is decreased, and impaired T-cell responses are found. Treatment of diabetic rats with rhIGF-1 increased the weight of the thymus, restored the histologic appearance to normal, and increased the number of double-staining W3/25 and Ox8 (CD4, CD8) cells. These studies suggest that growth hormone and insulin growth factor may provide therapeutic benefits in T-cell deficient states in humans.

Given the evidence that rhGH and rhIGF were able to induce immunologic improvements in rodents, we are exploring the possibility whether rhGH or rhIGF or both can induce immunologic improvements in patients with HIV infection. We are conducting a phase I study of the safety and immunologic effects of recombinant human insulin-like growth factor (rhIGF-1) and recombinant human growth hormone (rhGH) in patients with HIV-1 infection. Patients will be treated with either rhIGF-1, rhGH, or rhIGF-1 in combination with rhGH, for a period of up to 12 weeks. The clinical safety evaluations include those for hematology, coagulation profile, clinical chemistry, and urinalysis. The immunologic parameters monitored include mononuclear cell subset enumeration, quantitative immunoglobulins, functional T-cell studies, natural killer cell activity, and cytokine production. So far, 6 patients have been entered onto this study.

PUBLICATIONS

Yarchoan R, Mitsuya H, Broder, S. The immunology of HIV infection: Implications for therapy. AIDS Res. Hum. Retroviruses 1992; (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06738-01 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Differential phosphorylation of anti-HIV agents, AZT, ddC, and ddI, in PBM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. Hiroaki Mitsuya, M.D., Ph.D., Chief, Experimental Retrovirology Section, MB

Other: Takuma Shirasaka, M.D., Visiting Associate, ERS, MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Laboratory of Medicinal Chemistry, NCI: Wen-Yi Gao, David G. Johns; OD, NCI: Samuel Broder, M.D.

LAB/BRANCH
Medicine Branch

SECTION
Experimental Retrovirology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 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.)*

Rapid, reliable, and sensitive assay systems for testing antiviral activity of potential drugs are essential for the development of antiviral therapy of AIDS. Multiple *in vitro* assay systems have now been available and these assay systems should further accelerate the identification of new antiretroviral agents. However, a number of variables or factors influence the results of the *in vitro* antiviral testing. Antiviral data can greatly vary depending on the target cells employed. Viral doses, viral strains, time of drug addition, duration of culture, and endpoints used can also significantly influence the antiviral data.

Human peripheral blood mononuclear cells (PBM) have been frequently used as target cells following stimulation with phytohemagglutinin (PHA) for the study of activity of various drugs against HIV. PHA-stimulated PBM (PHA-PBM) may provide a milieu close to the in vivo situation and therefore may serve as one of the most suitable target cells for antiviral testing. However, it is as yet poorly understood whether the sensitivity of HIV-1 to drugs as assessed in PHA-PBM correlates with the virus-drug interactions in patients with AIDS.

Generally, HIV-1 replicates more abundantly and rapidly in activated cells (e.g. T-cells and monocytes/macrophages) than in resting, unstimulated cells. PHA, a T-cell mitogen, therefore has been used to activate PBM cells to ensure HIV-1 replication in *in vitro* drug assay. PHA stimulation initiates cellular DNA synthesis and increases intracellular dNTP pool sizes. PHA also activates enzymes that are directly involved in DNA synthesis. Among such enzymes, a group of cellular nucleoside kinases are responsible for 5'-phosphorylation of natural ribonucleosides and deoxynucleosides but also responsible for anabolic phosphorylation of dideoxynucleosides (ddN)(e.g. AZT, ddC, and ddI) to their corresponding 5'-triphosphates. It has been now known that different ddN require different cellular enzymes for their anabolic phosphorylation. For example, the initial step of AZT monophosphorylation is mediated by thymidine kinase, while that of ddC is catalyzed by deoxycytidine kinase. On the other hand, the monophosphorylation of ddI is mediated by 5'-nucleotidase. Following these initial steps of 5'-monophosphorylation, AZT, ddC, and ddI are ultimately 5'-triphosphorylated by different arrays of cellular kinases. Thus, each of these ddN must be considered to be different agents in its own right. It is then possible that PHA stimulation causes alterations of expression and functions of cellular nucleoside kinases and thereby alters the antiviral activity of ddN.

When comparatively evaluated on the basis of molarity by using phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells (PBM) as target cells and employing p24 Gag protein production as an endpoint. The mean concentrations that yielded 50% p24 Gag negative culture wells ($CN_{50}$) were 0.07 μM, 0.2 μM, and 6 μM for AZT, ddC, and ddI, respectively. Study of intracellular anabolic phosphorylation of nucleosides revealed that AZT was preferentially phosphorylated to its mono-, di-, and triphosphates with 110-, 30-, and 40-fold increases in PHA-PBM as compared to those in the counterpart resting PBM, whereas ddC was moderately phosphorylated and ddI and ddA were very poorly phosphorylated in PHA-PBM. The ratio of AZT-triphosphate (TP)/dTTP in PHA-PBM was 10- to 17-fold higher than that in resting PBM. In contrast, ratios of ddCTP/dCTP and ddATP/dATP were 3- to 8-fold and 13- to 18-fold lower in PHA-PBM as compared to those in resting PBM. The PHA-mediated induction of thymidine kinase activity was found to be most profound in PHA-PBM as compared to that in resting PBM. Comparative order of kinase activity induction following PHA stimulation was thymidine kinase >> uridine kinase > deoxycytidine kinase > adenosine kinase.

These PHA-induced changes of nucleoside metabolism should be responsible for the observed molarity-based, extremely potent anti-HIV activity of AZT in PHA-PBM as compared to ddC and ddI. The present data should have clinical relevances in the evaluation of antiviral activity of dideoxynucleosides against HIV-1.

## *Presentations*

Mitsuya H. Strategies for the Development of Anti-retroviral Drugs Against AIDS. Gordon Research Conference, March 15-20, 1992, Oxnard, California.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06739-01 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of transition-state mimetic 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., Ph.D., Chief, Experimental Retrovirology Section, MB

Other: Seiji Kageyama, Visiting Fellow, ERS, MB, COP, DCT, NCI
Takuma Shirasaka, Visiting Associate, ERS, MB, COP, DCT, NCI
Samuel Broder, M.D., Director, NCI

COOPERATING UNITS *(if any)*
Office of the Director, NCI

LAB/BRANCH
Medicine Branch

SECTION
Experimental Retrovirology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*

A number of potentially useful approaches for the therapy of acquired immunodeficiency syndrome (AIDS) and its related diseases have emerged. The use of family of 2',3'-dideoxynucleotides, such as 3'-azido-2',3'-dideoxythymidine (AZT or zidovudine), 2',3'-dideoxycytidine (ddC or zalcitabine), and 2',3'-dideoxyinosine (ddI or didanosine), which target the reverse transcriptase of human immunodeficiency virus type I (HIV-1), the causative agent of AIDS, is one such approach. HIV also codes a virus-specific aspartic protease which is essential for its replication. The HIV protease mediates crucial proteolytic processing of viral protein precursors at a late stage in the replication of the virus. Thus, HIV protease also represents a virus-specific target for the therapy of HIV infections.

The design of HIV-1 protease inhibitors based on the transition state mimetic concept has led to the generation of a variety of peptide derivatives highly active against the virus at least *in vitro*. These active agents contain a non-hydrolyzable, dipeptide isostere such as hydroxyethylene or hydroxyethylamine as an active moiety which mimics the putative transition state of the aspartic protease-catalyzed reaction. However, there are several hurdles in developing HIV protease inhibitors as drugs for therapy of AIDS. They include relatively short plasma half-life, poor oral bioavailability, and technical difficulty of scale-up synthesis.

In this study, we designed and synthesized a variety of tripeptide HIV protease inhibitors containing a unique non-hydrolyzable, dipeptide isostere, allophenylnorstatine (APNS) as an active moiety, and tested the activity of those compounds against a wide spectrum of HIV strains *in vitro*. One of the advantages of using APNS is in its great ease of preparation and handling as compared to the use of hydroxyethylene or hydroxyethylamine in the synthetic process. We have identified two compounds, KNI-227 and -272, which showed the most potent activity against the infectivity and cytopathic effect of a wide spectrum of HIV strains. As tested in target CD4+ ATH8 cells, the 50 % inhibitory concentrations ($IC_{50}$) of KNI-227 against HIV-$1_{LAI}$, HIV-$1_{RF}$, HIV-$1_{MN}$ and HIV-$2_{ROD}$ were 0.1, 0.02, 0.03, and 0.1 μM, while those of KNI-272, 0.1, 0.02, 0.04, and 0.1 μM, respectively. Both agents completely blocked the replication of both AZT-sensitive and -insensitive clinical isolates at 0.08 μM as tested in target phytohemagglutinin-activated peripheral blood mononuclear cells. The ratios of 50 % cytotoxic concentrations ($TC_{50}$)/$IC_{50}$ for KNI-227 and -272 were ~2,500 and >4,000, respectively. Both compounds were confirmed to block the posttranslational cleavage of the p55 precursor protein to generate the mature p24 Gag protein in HIV-1 infected cells. The octanol-water partition coefficients of KNI-227 and -272 were substantially high with log *p* values of 3.56 and 3.79, respectively. Degradation of KNI-227 and -272 in the presence of pepsin (1 mg/ml, pH 2.2) was negligible at 37°C for 24 hours.

Current data warrant further careful investigations of KNI-228 and -272 toward possible clinical application.

PUBLICATIONS

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06740-01 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of differential activity of an antisense oligomer against HIV-1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. Hiroaki Mitsuya, M.D., Ph.D., Chief, Experimental Retrovirology Section, MB

Other: Seiji Kageyama, M.D., Visiting Fellow, ERS, MB, COP, DCT, NCI
Takuma Shirasaka, M.D., Visiting Associate, ERS, MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
ABI Therapeutics, Foster City, CA: Gerry Zon; University of Nebraska, Omaha, NE: Patrick Iversen; Office of the Director, NCI, Samuel Broder, M.D.

LAB/BRANCH
Medicine Branch

SECTION
Experimental Retrovirology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 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.)*

In 1989, we reported that a nucleic acid-resistant phosphorothioate oligodeoxynucleotide with an antisense construct against the *rev* gene (α-*rev*) was active in suppressing the expression of HIV-$1_{IIIB}$ in chronically infected H9 cells (Matsukura et al. *Proc. Natl. Acad. Sci. USA.*, 86:4244-4248, 1989). In the present study, we asked whether this α-*rev* could exert comparable antiviral activity in a variety of chronically infected target cell lines using p24 Gag protein production as an endpoint. When H9 cells were stably infected with 9 different HIV-1-strains (3 laboratory strains: HIV-$1_{IIIB}$, HIV-$1_{MN}$, HIV-$1_{RF}$; 6 clinical isolates: HIV-$1_{ERS101}$, HIV-$1_{ERS201pre}$, HIV-$1_{ERS201post}$, HIV-$1_{ERS206}$, HIV-$1_{018pre}$, HIV-$1_{018post}$), α-*rev* could provide a substantial suppression (≥60% suppression) in the expression of only three of the virus strains, HIV-$1_{IIIB}$, HIV-$1_{201pre}$, and HIV-$1_{018post}$. When 6 different cell lines (H9, CEM, HUT102, MOLT4, U937, and G11) were stably infected with HIV-$1_{IIIB}$, HIV-$1_{IIIB}$ was sensitive to α-*rev* suppression only when harbored in H9 cells and CEM cells. The uptake and intracellular localization of fluorescein-labeled α-*rev* in each cell line did not correlate with the sensitivity of HIV-1 to the oligomer. The rates by which α-*rev* degraded intracellularly in each cell line also did not show detectable correlation. Levels of *rev* transcripts expressed in each cell line did not appear to correlate with the susceptibility of the virus to α-*rev*.

These data suggest that although antisense-based strategy is intriguing for therapy of AIDS, more in-depth studies on viral strain specificity and target cell specificity of antiviral activity of antisense oligomers are required.

## PUBLICATIONS

Mitsuya H, Yarchoan R, Kageyama S, and Broder S. Targeted therapy of human immunodeficiency virus-related disease. *FASEB J.* 1991;5:2369-2381.

Matsukura M, Mitsuya H, and Broder S. A new concept in AIDS treatment: an antisense approach and its current status toward clinical application In: Murphy JAH, ed. Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS. Wiley-Liss, 1991; pp. 159-178.

Mitsuya H. Overview: Development of inhibitors of reverse transcriptase and protease as therapeutics against HIV infection. *J. Enzyme Inhibition* 1992;6:1-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07209-04 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of anti-retroviral drugs for the treatment of HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

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G. Shearer, Ph.D., Senior Invest.,NCI
M. Clerici, M.D., Vis. Scient., NCI

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Medicine Branch

SECTION
Retroviral Diseases Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*

One of the principal projects of the Retroviral Diseases Section is the pre-clinical and early clinical development of antiretroviral therapy for the treatment of HIV infection. Since 1984, members of the section have been involved in the development of anti-viral therapy for AIDS and related disorders (until 1991, this section, as well as the Experimental Retrovirology Section were the Laboratory of the Associate Director under the directorship of Dr. Samuel Broder). Members of the section played pivotal roles in the development of azidothymidine (AZT, zidovudine), 2',3'-dideoxycytidine (ddC, zalcytabine), and 2',3'-dideoxyinosine (ddI, didanosine) as anti-HIV drugs for AIDS and in particular conducted the initial clinical trials of these agents as well as associated pre-clinical and associated laboratory work. In addition, members of the section have been involved in the study of suramin, soluble recombinant CD4 (rCD4), CD4-IgG immunoadhesin (rCD4-IgG), 2',3'-dideoxyadenosine (ddA), and pentosan polysulfate as potential AIDS therapies. The section is presently studying the negative enantiomer of 3'-thia-cytadine (3TC) as a potential anti-HIV agent in collaboration with Glaxo, Inc. and is planning studies of other agents, including an inhibitor of HIV protease. As will be discussed in a separate project, the section is also involved in the study of combination therapy of HIV infection. Finally, the section is involved in a study of the relationship between CD4 counts, the development of opportunistic infections and tumors, and mortality in patients on anti-retroviral therapy.

Project:

Soon after HIV was identified as the causative agent of HIV infection, the Laboratory of the Associate Director of the COP, then under the direction of Dr. Samuel Broder, initiated an effort to develop effective anti-retroviral therapies for AIDS and associated disorders caused by HIV. A pivotal step in this effort was the development by Dr. Mitsuya of an assay to effectively screen agents for anti-HIV activity. Early on, a number of members of the family of dideoxynucleosides were found to have potent anti-HIV activity. Among these compounds, azidothymidine (AZT, zidovudine), 2',3'-dideoxycytidine (ddC, zalcytabine), and 2',3'-dideoxyinosine (ddI, didanosine) were found to have potent anti-HIV activity without causing substantial cell toxicity. A phase I trial of AZT, initiated in July of 1985 by our group in collaboration with Duke University and Burroughs Wellcome Co., demonstrated for the first time that an anti-HIV agent could induce immunologic improvement in patients with AIDS or AIDS-related complex. This trial also found that hematologic toxicity was dose limiting in patients receiving AZT. This first clinical study led to a placebo-controlled Phase II study of AZT organized by Burroughs Wellcome Co. in which AZT was formally shown to reduce the mortality of patients with AIDS or advanced ARC. In March of 1987, AZT became the first drug approved by the U.S. Food and Drug Administration for the treatment of HIV infection.

We next turned our attention to another member of this class of drugs, ddC. A Phase I trial of ddC conducted by our group revealed that ddC also had clinical anti-HIV activity, but that at high doses, the limiting toxicities were painful peripheral neuropathy, rash, and aphthous stomatitis. We were struck by the different toxicities of AZT, and in 1987 initiated a trial of AZT alternating with ddC. Some patients had a sustained response to this regimen, and the overall toxicity was less than seen with either drug alone. Extramural studies have further explored the combination of AZT and ddC, and an FDA advisory committee recently recommended that this combination be approved for patients who were progressing despite AZT therapy.

The next dideoxynucleosides we tested were dideoxyadenosine (ddA) and ddI. A Phase I trial of 2',3'-dideoxyadenosine (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 October of 1991, the U.S. Food and Drug Administration approved ddI for patients with advanced HIV infection who could not tolerate AZT or who were failing AZT.

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.

During this period, we also explored the use of soluble recombinant CD4 (rCD4) and rCD4-IgG immunoadhesin in collaboration with Genentech, Inc. We found that both compounds were well tolerated in patients with HIV infection. The half-life of rCD4 was only about 1 hr, which made it difficult to sustain high levels. In contrast, rCD4-IgG had a substantially higher half life, and levels well over the in vitro inhibitory dose for HIV infection were sustained. In spite of this, we found no consistent clinical improvement or changes in HIV p24 antigen or CD4 counts. Interestingly, in collaboration with Drs. Mario Clerici and Gene Shearer, we found that patients receiving rCD4-IgG had an improvement in their T cell responses to alloantigens. The mechanism for this immunologic improvement is not defined at present.

We are presently studying a novel nucleoside analogue, the negative enantiomer of 3'-thiacytidine (3TC) in collaboration with Glaxo Inc. 3TC is a ddC analogue with a sulfur substitution in the 3'-position and an inversion of the sugar ring. There is in vitro data that 3TC causes less mitochondrial toxicity than ddC, and the hypothesis is that it may therefore cause less neurotoxicity. We have initiated a Phase I/II study of this compound in patients with AIDS or symptomatic HIV infection. This study will attempt to define the toxicity profile, the pharmacokinetics, and clinical activity of this drug. We are currently planning to test a protease inhibitor as an anti-HIV drug in the not too distant future.

Finally, we have retrospectively studied our patients on anti-viral therapy to discern the relationship between the CD4 count and the immediate hazard of dying. We have found that patients on AZT or on ddI almost die as long as their CD4 count remains above 50 cells/mm3. This observation suggests that a CD4 count of 50 cells/mm3 can be used as a surrogate marker for mortality in patients with HIV infection. This observation further suggests that maintaining the CD4 count above 50 cells/mm3 may enable a substantial prolongation of life in patients with HIV infection. We are currently exploring our clinical data base to explore the relationship between other opportunistic infections and the CD4 count.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07210-03 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

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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SECTION
Retroviral Diseases Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2 | 1½ | ½ |

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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.)*

Certain NHLs are included in the surveillance case definition of AIDS, and account for >3% of all AIDS-defining illnesses in the United States. Studies have previously identified several mechanisms for B cell activation in the setting of infection with HIV which may lead to development of NHL. We have followed 2 cohorts of patients receiving long-term antiretroviral therapy for the development of NHL: 55 patients receiving AZT-based therapy, of whom 8 have so far developed NHL, and 61 patients receiving ddI, of whom 4 have so far developed NHL. Combining the AZT-based treatment and the ddI patients into 1 cohort containing 116 patients yields a total of 12 high grade B cell lymphomas. For this combined cohort, the estimated probability of developing a NHL after 24 months of antiretroviral therapy was 8.4% (95% CI of 4.1 to 16.6%), increasing to 19.4% (95% CI of 10.9% to 32%) after 36 months. There was a statistically significant difference in the development of NHL in patients with <50 CD4 cells/mm$^3$ compared to those with >50 CD4 cells/mm$^3$ irrespective of time on antiretroviral therapy ($P_2$=0.015). Thus, patients with severe HIV infection who survive for prolonged periods, particularly with <50 CD4 cells/mm$^3$ are at significantly increased risk of developing NHL. We also examined the patients in the AZT-based cohort for a variety of entry parameters that may have been predictive for the subsequent development of NHL. Patients developing NHL had slightly higher entry IL-6 levels compared with those not developing NHL ($P_2$=0.048). We currently are collaborating with Dwight Kaufman, M.D. on a trial administering infusional chemotherapy with G-CSF and ddI to patients with AIDS-NHL. A previous trial, administering modified PrOMACE-CytaBOM chemotherapy with AZT and GM-CSF demonstrated that AZT could be administered simultaneously with combination chemotherapy using GM-CSF, and yielded a CR rate of approximately 40%.

Non-Hodgkin's lymphomas (NHL) have been noted to occur in association with HIV infection since very early in the epidemic. These tumors are typically high grade, B cell lymphomas occurring in extranodal sites, particularly the central nervous system (CNS). Such lymphomas frequently harbor monoclonal episomes of Epstein-Barr virus (EBV) and exhibit mutations, breakpoints, or rearrangements affecting the *c-myc* oncogene. In 1985, the Centers for Disease Control (CDC) expanded the case-surveillance definition of AIDS to include high-grade NHL as AIDS-defining illnesses in certain settings, and these tumors currently comprise approximately 3% of all AIDS-defining illnesses in adults reported to the CDC.

We are following several cohorts of patients with severe HIV infection receiving long-term antiretroviral therapy for the development of NHL. This includes 55 patients on protocols of azidothymidine (AZT, zidovudine) either as a single agent or in combination with acyclovir or 2',3'-dideoxycytidine (ddC) and 61 patients on a dideoxyinosine (ddI, didanosine) protocol. Eight of the 55 patients in the AZT-based treatment cohort developed NHL, yielding an estimated probability of developing NHL after 24 months of therapy of 12% (95% confidence interval (CI) of 4.7% to 27.1%), increasing to 29.2% (95% CI of 15.2% to 48.7%) after 36 months. Four patients in the ddI cohort developed NHL, yielding an estimated probability of developing NHL after 24 months of therapy of 6.2% (95% CI of 2.1% to 17%), increasing to 9.5% (95% CI of 3.6% to 22.8%) after 36 months. The difference between these two cohorts over the entire period of follow-up was not significant ($P_2$=0.13). Combining the two cohorts yielded 12 lymphomas in 116 patients for an estimated probability of developing NHL after 24 months of antiretroviral therapy of 8.4% (95% CI of 4.1% to 16.6%), increasing to 19.4% (95% CI of 10.9% to 32%) after 36 months.

We have evaluated the relationship between CD4 cells and the development of NHL in the AZT-based treatment cohort. The hazard rate for the development of NHL in this cohort were 0.164 lymphomas/patient-year (pt-yr) (95% CI of 0.075 to 0.306 lymphomas/pt-yr) in patients with < 50 CD4 cells/mm$^3$ versus 0 lymphomas/pt-yr (95% CI of 0 to 0.033 lymphomas/pt-yr) in patients with > 50 CD4 cells/mm$^3$ ($P_2$=0.0017). Examining a combined cohort consisting of patients on AZT-based and ddI therapy revealed that patients having > 50 CD4 cells/mm$^3$ developed 2 lymphomas during 125.9 pt-yrs of observation (0.016 lymphomas/pt-yr, 95% CI of 0.003 to 0.049 lymphomas/pt-yr), while those with < 50 CD4 cells/mm$^3$ developed 10 lymphomas during 102.4 pt-yrs of observation (0.098 lymphomas/pt-yr, 95% CI of 0.049 to 0.171 lymphomas/pt-yr) ($P_2$=0.0085). The effect of CD4 count on the hazard of developing NHL was still significant after adjusting for length of time on antiretroviral therapy ($P_2$=0.015).

Retrospective examination of the AZT-based treatment cohort for entry parameters that might be predictive for the subsequent development of NHL revealed that serum interleukin-6 (IL-6) levels at entry were somewhat higher in those patients who developed NHL than in those who did not ($P_2$=0.048). A number of other entry parameters, including serum immunoglobulin levels, soluble interleukin-2 receptors, the number of CD4 cells, the number of CD8 cells, and serum HIV p24 antigen, were not predictive for the subsequent development of NHL.

These data suggest that HIV-infected patients with profound immunodeficiency, especially those with less than 50 CD4 cells/mm$^3$, who survive for prolonged periods of time are at substantial risk of developing lymphomas. As therapies are developed to better treat HIV and its associated complications, opportunistic NHL may become a significant cause of mortality. Even so, it is possible that newer antiretroviral treatments and strategies that can maintain CD4 cells above 50 CD4 cells/mm$^3$ may prevent or delay the occurrence of these tumors. In addition, identification of parameters predictive for the development of NHL in HIV-infected patients may contribute insights into the pathogenesis of these tumors, as well as allow for closer surveillance and, thus, earlier diagnosis and treatment of lymphomas. We are continuing to study our intramural cohorts for the development of NHL and for factors associated with its pathogenesis. Also, in other projects, we are attempting to devise therapeutic strategies to maintain the CD4 count above 50 cells/mm$^3$ to see if this will reduce the incidence of NHL. In the laboratory, we are exploring factors that may be associated with increased IL-6 production.

In collaboration with Dwight Kaufman, M.D. in the ROB, we have conducted a trial of combination chemotherapy consisting of a modification of the PrOMACE-CytaBOM regimen with GM-CSF and AZT. A complete response rate of 40% was achieved. Overall, patients were able to tolerate AZT in conjunction with their chemotherapy with the addition of GM-CSF, although the AZT may have added to the myelotoxicity of the regimen. We currently are conducting a trial administering infusional chemotherapy, a modification of the EPOCH regimen, in conjunction with ddI and G-CSF to patients with HIV-associated NHL.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07211-03

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Regulation and control of HIV replication in monocyte/macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH

Medicine Branch

SECTION

Retroviral Diseases Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1½ | 1½ | 0 |

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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.)*

One of the principal laboratory projects in the Retroviral Diseases Section is the study of HIV replication in M/M. One goal of this effort is to develop anti-retroviral strategies directed at this population. We found that although dideoxynucleosides such as azidothymidine (AZT) are poorly phosphorylated in M/M, they still have potent anti-HIV activity. This is apparently because M/M have low levels of deoxynucleoside-5'-triphosphates. We also observed that certain cytokines, including granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) can enhance the replication of HIV in M/M, and that these have variable effects on the activity of dideoxynucleosides. More recent studies have explored the phenomenon of enhancement of HIV infection of M/M by anti-HIV antibodies. It had been hypothesized that this phenomenon may work by a CD4-independent mechanism. However, we have observed that enhancement of infection of M/M is blocked by anti-CD4 antibodies or by soluble CD4, indicating that it still involves CD4 as an essential viral receptor. We are now investigating the production of cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-a) by monocytes upon exposure to HIV. IL-6 producting in patients with HIV infection may be one factor leading to the development of non-Hodgkin's lymphoma (NHL). Finally, we are exploring novel strategies for inhibiting HIV replication in M/M.

While CD4+ T lymphocytes are believed to be the principal target cells for HIV replication, these are not the only cells known to be infected by HIV. In particular, infection of cells of the monocyte/macrophage (M/M) lineage by HIV is important in the pathogenesis of HIV-induced diseases. M/M-derived cells are the primary target for HIV infection in the brain. In addition, HIV-infected M/M cells can easily infect many T cells, and may thus serve to aid in the spread of HIV throughout the body.

Over the past several years, we have been exploring the regulating of HIV infection in M/M. Monocyte/macrophages were easily infected by the Ba-L strain of HIV, obtained from Drs. Popovic and Gartner. 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 de 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 ratio 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 anti-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 as 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 exploring the cytokines produced by M/M and their potential role in the pathogenesis of HIV-related conditions. This study was inspired in part by the previous observation that HIV-infected patients have high serum IL-6 levels and by our clinical observation that HIV-infected patients with relatively high serum interleukin-6 (IL-6) levels had a greater chance of developing non-Hodgkin's lymphoma (NHL). In contrast to previously published results, we have observed that M/M do not produce IL-6 or TNF-a when infected by HIV. We are now further exploring this and attempting to develop an in vitro model for the high serum IL-6 levels in HIV-infected patients. We unexpectedly found that GM-CSF induces production of IL-6 by M/M; this may explain the results of certain other investigators that HIV induces IL-6 (as certain HIV preparations may be contaminated by GM-CSF). Finally, we are now exploring the activity of a variety of cytokines on HIV replication in M/M and are exploring ways of regulating HIV infection in these cells and of selectively killing M/M which are infected with HIV.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07216-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of therapy for AIDS-related Kaposi's sarcoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Medicine Branch

SECTION
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INSTITUTE AND LOCATION
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| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
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| 1 | ½ | ½ |

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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.)*

Kaposi's sarcoma (KS) was one of the first AIDS-defining illnesses, and continues to be a source of substantial morbidity and mortality. Current therapeutic strategies and regimens for widespread or visceral disease are toxic and have not yielded significant prolongation of survival. Recent advances in the ability to culture KS spindle cells in vitro over the past several years have begun to lay the foundation for the consideration of novel approaches to the therapy of KS. In particular, evidence has emerged that a cascade of cytokines and other growth factors are important in the development of KS lesions. In addition, there is evidence that angiogenesis is important in this process. In the Retroviral Diseases Section, we have initiated a pre-clinical and clinical program to develop therapies for KS. Using the methodology initially developed by Dr. Gallo's laboratory, we have developed several spindle cell lines from patients with KS which grow in response to retroviral-conditioned media. We are now in the process of characterizing those lines. One important question to be addressed is whether these lines are unique to KS per se, or whether they are normal cells stimulated by cytokines. In preliminary experiments, we have learned that these lines are inhibited by pentosan polysulfate and by TNP-470 (an angiogenesis inhibitor initially developed by Dr. J. Folkman and Takada Co.) We will continue to study these and other in vitro models for KS and to use them to develop therapeutic strategies. We have recently completed a pilot study of pentosan polysulfate in patients with KS, and are in the process of initiating trials of all-trans retinoic acid (RA) (alone and in combination with alpha-interferon) and of TNP-470.

The reporting of Kaposi's sarcoma (KS), a heretofore uncommon tumor, in young, Caucasian, gay males in 1981 heralded the beginning of the acquired immunodeficiency disorder (AIDS) epidemic, now known to be caused by human immunodeficiency virus (HIV).

Strategies for the treatment of HIV-associated KS have, in general, been aimed at control. Palliation has generally been utilized for locally aggressive disease, while systemic therapies are reserved for more widespread disease or visceral involvement. However, systemic therapies generally are associated with myelosuppression, further immunosuppression, short duration of response, and have not been shown to result in a significant prolongation of survival. Thus, there is a need for more effective, less toxic therapies for the treatment of AIDS-KS.

Recent advances in the ability to culture KS spindle cells in vitro have lead to the discovery that cytokines associated with angiogenesis, such as basic fibroblast growth factor (bFGF), may play an important role in the pathogenesis of HIV-associated KS. Thus, there is an interest in agents that inhibit these cytokines, or angiogenesis per se, as possible novel therapies for HIV-associated KS. In the Retroviral Diseases Section, we have developed two spindle cell lines from the pleural fluid of a patient with AIDS-associated KS, following the methodology of Gallo, Nakamura, and Salahuddin. These lines proliferate in response to steroids and to conditioned media from HTLV-II-infected cells. We have also derived control cell lines from the pleural fluid of HIV-negative patients with other conditions, and are comparing these lines to the KS lines. A central and as yet unanswered question is as to whether these (and other) KS lines are in fact unique to KS, or whether they represent normal cells stimulated by various cytokines.

We are also exploring the effect of various novel compounds on the growth of these lines. Pentosan polysulfate (an inhibitor of basic fibroblast growth factor [FGF]) and TNP-470 (a fumagillin analogue developed by Dr. J. Folkman and Takada Co.) were both found to inhibit the proliferation of the KS lines in vitro. We plan to continue to study the cells of these lines, to explore their relationship to KS, and to use them to try to develop novel therapies for KS directed at inhibiting the steps leading to the development of KS lesions.

In the summer of 1990, we initiated a trial of pentosan polysulfate in patients with AIDS-associated KS. The rationale for this study was (1) the effect of pentosan on FGF-induced cell growth, (2) evidence that pentosan had weak anti-HIV activity in vitro, and (3) evidence from our lab that it inhibited the growth of a KS line in vitro. Sixteen HIV seropositive patients with biopsy proven KS and no evidence of visceral disease were administered pentosan via continuous venous infusion (CVI) for three to six weeks followed by subcutaneous (SC) administration three times per week on three separate dose levels: 1) 2 mg/kg/day by CVI followed by 2 mg/kg/dose SC. 2) 3 mg/kg/day by CVI followed by 3 mg/kg/dose SC. 3) 4 mg/kg/day by CVI followed by 4 mg/kg/dose SC. Also, 5 patients received intralesional injections of 1 mg of pentosan into 2 separate lesions three times per week for three weeks and then weekly. No patient had an objective clinical response to either systemic or intralesional pentosan administration; however, seven patients did have stable disease. No significant effect on CD4 cells or serum HIV p24 antigen was noted during pentosan administration. Dose limiting toxicities were reversible and consisted of reversible anticoagulation and thrombocytopenia. Pentosan polysulfate was well tolerated in this patient population. It is possible that with the administration of angiogenesis inhibitors, prevention of new lesions and stability but not regression of existing lesions is all that may be accomplished.

TNP-470 is an analogue of fumagillin, an antibiotic derived from the fungus Aspergillus fumigatus fresenius that was first described by Hanson and Elbe in 1949 and found to have amebicidal

properties. In 1990, Ingber, Folkman, and associates reported that fumagillin was able to inhibit endothelial cell proliferation in the presence of saturating levels of bFGF. They also found that administering fumagillin subcutaneously resulted in the inhibition of tumor-induced neovascularization in the mouse dorsal air sac. Subsequently, scientists at Takada Co., in collaboration with Dr. Folkman, synthesized numerous analogues of fumagillin, some of which retained the anti-angiogenic activity of fumagillin with less toxicity. These compounds, of which O-(chloracetylcarbamoyl)fumagillol or TNP-470 is one, represent a new class of angiostatic drugs they termed angioinhibins.

TNP-470 is an angioinhibin that has been shown to exhibit potent inhibition of endothelial cell growth. Ingber, Folkman, and colleagues found that TNP-470 administered SC every other day resulted in the inhibition of Lewis lung carcinoma, B16 melanoma, and other tumors inoculated into mice. Kusaka and associates found that TNP-470 could inhibit capillary growth by local administration and induce avascular zones in the chorioallantoic membrane (CAM) assay. In addition, TNP-470 was able to suppress the number and length of new blood vessels induced by bFGF in the rat corneal micropocket assay. Also, using the sponge assay in rats, they were able to show that systemic administration of TNP-470 inhibited angiogenesis induced by bFGF as well as unknown angiogenic factors involved in inflammation.

As noted above, there is evidence from our group that TNP-470 may have activity in vitro against spindle cells grown in culture, and there is apparently a 100-fold difference between the concentration of TNP-470 that inhibits spindle cells versus a T lymphocyte line (H9) or phytohemagglutinin-stimulated peripheral blood mononuclear cells. The relationship of such lines to clinical KS is uncertain and such results have to be interpreted with caution. Even so, the ability of TNP-470 to inhibit angiogenesis, plus its effects on spindle cell lines, support its clinical testing in patients with KS. We are about to begin a phase I trial administering TNP-470 to patients with HIV-associated KS.

Another class of drugs, the retinoids, have been used for many years to treat a variety of skin disorders. Also, due to their effects on epithelium, chemopreventive and therapeutic trials of retinoids against various epithelial tumors are being explored. All-trans-retinoic acid (retinoic acid, RA) is a retinol (vitamin A) metabolite formed by intestinal oxidation of b-carotene and from tissue metabolism of retinol and retinaldehyde. Recent anecdotal reports would seem to indicate that RA may have some activity against AIDS-KS. There is also evidence that the combination RA and interferon-alfa (INF-a) may result in better responses than are seen with either of these agents alone. Because of these properties of RA, we have recently initiated a pilot trial administering RA alone or in combination with INF-a to patients with HIV-associated KS.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07217-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

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)*

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LAB/BRANCH
Medicine Branch

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Retroviral Diseases Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4 | 1½ | 2½ |

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.)*

Over the past 7 years, several agents have been found to have clinical activity against HIV, and a number of other agents are in various stages of pre-clinical and early clinical development. However, the long-term activity of all the available agents is limited by incomplete activity, long-term toxicity, and the development of viral resistance. We are presently exploring whether the use of combinations of anti-HIV agents may address some or all of these limitations of therapy. Combinations of anti-HIV drugs can reduce toxicity, capitalize on drug synergy, delay the development of resistance, target various cells in the body, and target various organs. We have previously studied the combinations of AZT and acyclovir and an alternating regimen of AZT and ddC. We are now continuing a study of AZT with acyclovir alternating with ddI alternating with ddC. One central unresolved question is the relative advantages of giving alternating or simultaneous therapy with two (or more) agents. We have more recently started a study comparing a regimen of AZT alternating with ddI with one of AZT given simultaneously with ddI in patients with AIDS or symptomatic HIV infection who have not been heavily pre-treated. Preliminary results suggest that the CD4 rises are greater and more sustained with the simultaneous regimen. However, one of the principal advantages of alternating therapy is to reduce drug toxicity, and further study will be required to discern which is the better regimen.

In 1986, we initiated a trial of AZT and acyclovir in patients with AIDS or AIDS-related complex. The rationale for this was that acyclovir was found to be synergistic with AZT in vitro. 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 trial 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). GM-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. This small trial showed that GM-CSF could counteract the neutropenia associated with AZT therapy. Controlled studies, however, will be needed to determine whether this regimen is better than AZT used alone.

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 as 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 week). 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. Preliminary results suggest that the CD4 rises are somewhat greater with the simultaneous regimen than with the alternating regimen. However, toxicity may be found to be less with the alternating regimen, and it is not clear at this point which regimen is clinically superior. This study is still ongoing.

PUBLICATIONS

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Yarchoan R, Lietzau JA, Brawley O, et al. Therapy of AIDS or symptomatic HIV infection with alternating or simultaneous AZT/ddI regimens: Interim analysis of a randomized protocol. J. Cellular Biochemistry 1992 (abstract Suppl. 16E); 89.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07218-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Roles of DNA demethylation and cytotoxic effect in HIV expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
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Medicine Branch

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | ½ | ½ |

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.)*

One of the salient clinical features of human immunodeficiency virus type 1 (HIV-1) infection is the relatively long period of latency which is characterized by a low to undetectable expression of the virus, following an early acute phase of infection with viremia. However, the mechanisms responsible for the maintenance of this clinical latency have been as yet poorly understood. During the extended latency or asymptomatic period, only a few infected cells are detected in peripheral blood. This may be due to an efficient removal of HIV-1-expressing cells by the host's immune defense mechanisms at early stages of infection. However, yet unidentified mechanism(s) to suppress the expression of the virus may also be operative in infected individuals at the early stages of infection.

In this project, we specifically asked if the methylation status of HIV-1 proviral DNA was associated with the level of viral expression using 5-azacytidine (AZC), a nucleoside analog, in which a carbon atom at the 5 position is substituted with a nitrogen atom. We found that a persistently HIV-$1_{LAV1}$-infected T-cell line, ACH2, which otherwise produced a low level of HIV-1 *in vitro*, showed a profoundly high level of viral production upon exposure to 5-azacytidine (AZC) as assessed by syncytia formation, p24 Gag protein production, and synthesis of reverse transcriptase. Southern blot analyses using methylation-sensitive and -insensitive isoschizomer restriction enzymes revealed that the LTR region of HIV-1 in ACH2 cells had been extensively demethylated and its methylation profile was not affected by AZC treatment. However, the *tat/rev* and *env* regions had been heavily methylated and AZC treatment of ACH2 cells caused demethylation in these regions. The HIV-1 isolated from ACH2 cells (HIV-$1_{LAV1/ACH2}$) productively infected H9 cells, indicating that HIV-$1_{LAV1/ACH2}$ was a functionally complete virion, and that a cellular event(s) was linked to the suppression of viral expression in ACH2 cells. AZC-induced potentiation did not occur in four HIV-$1_{IIIB}$-infected cell lines including HIV-1-producing HIV-$1_{IIIB}$-infected H9 cells. All the LTR, *tat/rev* and *env* regions of the proviral DNA in the four HIV-$1_{IIIB}$-infected cell lines had been extensively demethylated, and AZC treatment did not alter their methylation patterns. These data suggest that DNA methylation may play a role in regulating HIV-1 expression at least under certain circumstances, although its relevance to *in vivo* situations remains to be further studied.

We also found that certain cytotoxic agents such as 5-fluorouracil, daunorubicin, and methotrexate potentiated the expression of HIV-1 in ACH2 cells although the magnitude of potentiation was less profound than that by AZC, suggesting that the inherent toxicity of AZC may, at least in part, be associated with the observed potentiation of HIV-1 expression in ACH2 cells. These data suggest that the use of anti-cancer agents may enhance the expression of HIV-1 in patients with AIDS-associated malignancies, and warrant further studies.

PUBLICATIONS:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07219-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Profiles of drug sensitivity of HIV-1 isolates in patients receiving antivirals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 2½ | ½ |

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 ability to provide effective long-term antiretroviral therapy using single agents for human immunodeficiency virus type 1 (HIV-1) infection became a complex issue when the isolation of HIV-1 strains that were less sensitive to 3'-azido-2',3'-dideoxythymidine (AZT or zidovudine) *in vitro* from patients with acquired immunodeficiency syndrome (AIDS) who received AZT therapy for more than 6 months was reported in 1989. Nucleotide sequence analysis of the reverse transcriptase-coding region from pairs of AZT-sensitive and -resistant HIV-1 isolates have revealed several mutations that result in amino acid substitutions in the viral reverse transcriptase. These amino acid substitutions include: (i) Asp at amino acid position-67 to Asn (designated Asp-67 -> Asn); (ii) Lys-70 -> Arg; (iii) Thr-215 -> Tyr or Thr-215 -> Phe; and (iv) Lys-219 -> Gln.

Besides AZT, two 2',3'-dideoxynucleosides, 2',3'-dideoxyinosine (ddI or didanosine) and 2',3'-dideoxycytidine (ddC), have recently been shown to have clinical activity in patients with HIV-1 infection, and these drugs have been administered for over 3 years to certain groups of patients with AIDS in the United States. It has recently been reported that patients who received long-term AZT therapy and then ddI therapy developed decreased sensitivity to ddI and that the emergence of HIV-1 variants with decreased sensitivity to ddI was associated with a reversion to a more AZT-sensitive phenotype. An HIV-1 variant with reduced sensitivity to AZT and ddI has also been isolated from a patient receiving 12 months of AZT therapy alone. These observations have increased interest in using multiple agents in combination for HIV-1 therapy. However, the behavior of HIV-1 at the genetic or phenotypic level upon exposure to multiple antiretroviral agents is as yet poorly understood.

In this project, we specifically asked how easily HIV-1 develops decreased sensitivity to three drugs: AZT, ddC, and ddI, when given as single drugs or in combination. We also asked whether the addition of ddC to AZT therapy could block the emergence of HIV-1 variants with decreased sensitivity in patients.

We first isolated HIV-1 strains from 9 patients before and after prolonged therapy with either an alternating regimen of AZT and ddC [AZT/ddC] or ddI alone. All strains obtained from 4 patients who received AZT/ddC for up to 41 months were highly resistant to AZT *in vitro*. Only one strain obtained following AZT/ddC therapy showed resistance to ddC in addition to AZT and had novel amino acid substitutions in the viral polymerase-encoding *pol* region, while three other strains had one or more of the four previously reported AZT-related mutations. In 5 HIV-1 strains obtained from patients who received ddI for up to 29 months, no appreciable decrease in the sensitivity to ddI was detected. Two strains isolated following ddI therapy had no significant amino acid mutations although 3 strains had a mutation reportedly associated with ddI administration. These data suggest that HIV-1 develops decreased sensitivity to AZT more readily than to ddC and ddI. Moreover, the present data suggest that an alternating regimen of AZT and ddC does not block the emergence of variants with decreased sensitivity to AZT. Although the current results do not provide a basis for concluding that AZT/ddC or ddI are inferior, equivalent, or superior to AZT as therapy of AIDS, these findings should provide data base and insights for devising means to avoid or retard the development of HIV-1 variants with decreased sensitivity to antiviral agents.

***PRESENTATION***

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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07220-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibition of HIV-1 replication by $C_2$ symmetric HIV protease inhibitors

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| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1½ | 1½ | 0 |

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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.)*


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, a number of 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 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-1 isolates. We also demonstrate and discuss the effects of combinations of $C_2$ symmetric HIV protease inhibitors and AZT or ddI, dideoxynucleoside analogues which are already in a clinical domain.

HIV strains used in this project included a laboratory strain (HIV-$1_{IIIB}$), a monocytotropic strain (HIV-$1_{Ba-L}$), and primary HIV-1 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-1 exposure. Analysis of drug interactions was performed using the COMBO program established by Dr. John Weinstein of NCI. 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 μM. In addition, they completely inhibited the replication of HIV-$1_{Ba-L}$ in M/M at 0.75 - 2 μM throughout 26 days of culture. A potent inhibitory activity against primary HIV-I isolates was also observed with $IC_{50}$ ranging from 0.09 to 0.22 μM for all three compounds when PHA-PBM were employed as target cells. Cellular toxicity was negligible at the highest concentrations used (up to 10 μM). When the protease inhibitors and dideoxynucleosides were used in combination against primary HIV-1 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-1 isolates, and they represent promising experimental antiviral agents for the therapy of HIV-1 infection.

## PUBLICATIONS

Kageyama S, Weinstein JN, Shirasaka T Kempf DJ, Norbeck DW, Plattner JJ, Erickson J, and Mitsuya H. In vitro inhibition of HIV-1 replication by $C_2$ symmetry-based HIV protease inhibitors as single agents or in combinations. *Antimicrob. Anticancer Chemother.* 1992;36: 926-933.

Mitsuya H., Yarchoan R., Kageyama S, and Broder S. Targeted therapy of human immunodeficiency virus-related disease. *FASEB J.* 1991;5:2369-2381.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07221-02 M

PERIOD COVERED: October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Quantitation of HIV-1 in HIV-1 infected individuals by PCR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Seiji Kageyama, M.D., Visiting Fellow, ERS, MB, COP, DCT, NCI
Takuma Shirasaka, M.D., Visiting Associate, ERS, MB, COP, DCT, NCI
Eiji Kojima, M.S., Guest Researcher, ERS, MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*: Retrovirology Diseases Section, MB, NCI: Robert Yarchoan, M.D., James M. Pluda, M.D., Kathy S. Wyvill; OD, NCI: Samuel Broder, M.D.; Advanced Biotechnologies, Inc., Columbia, Maryland: David T. Hoekzema

LAB/BRANCH: Medicine Branch

SECTION: Experimental Retrovirology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1½ | 1 | ½ |

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 immunodeficiency virus type 1 (HIV-1) is the 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, 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 viral load and immunosuppression are major determinants of the severity of HIV-1 related diseases. Moreover, it has been suggested that the level of viral load is related to the stage and progression of immunological deterioration. Thus, the measurement of viral load in patients may theoretically be useful for prognostic assessment. There are, however, no easily performed, reliable, sensitive and specific assay systems to determine the viral load at the present time. HIV-1-related antigen tests may be reliable for their specificity, but should still be considered in the developmental phase due to their limited sensitivity. For example, HIV-1 antigens such as p24 Gag protein are often undetectable in the 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 antibody-positive individuals depending upon patient populations or methods employed.

These findings and a study using *in situ* hybridization suggesting 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* at one point led to a notion that the level of HIV-1 expression is low and that the HIV-1 viremia status is difficult to monitor. If the levels of viral particles could be directly and quantitatively determined, such technologies might provide additional, and possibly more accurate information, in assessing the viremia status in HIV-1 infected individuals. Furthermore, such methods might also serve as useful clinical markers to help determine the activity of experimental antiretroviral drugs in early phases of clinical trials.

In this project, we have established a method to quantitate the amounts of HIV-1 particles in plasma from patients with HIV-1 infection by using polymerase chain reaction following reverse transcription of viral RNA (RNA-PCR). We have assessed the potential usefulness of this approach to monitor the changes of viral load in patients with AIDS or AIDS-related complex (ARC) receiving 2',3'-dideoxyinosine (ddI). Plasma samples were obtained from 77 patients with HIV-1 infection (49 AIDS/ARC and 28 asymptomatic seropositives). Among 49 patients with AIDS or ARC, 11 were enrolled in a phase I trial of ddI. Following ultracentrifugation of plasma, RNA was extracted from the pelleted virus and subjected to reverse transcription and PCR using *gag* and *env* primer pairs. The number of HIV-1 virus particles in each sample was determined using a standard curve obtained by PCR with known amounts of HIV-1 DNA.

The current plasma RNA-PCR technique quantitatively detected HIV-1 particles in plasma from 76 of 77 (98.7 %) HIV-1 infected individuals examined. The numbers of HIV-1 particles in plasma from patients with AIDS or ARC, 87,409 x/÷ 10 (vp/ml) (geometric mean x/÷ SD), were markedly higher than those in plasma from asymptomatic seropositive individuals, 958 x/÷ 34 ($p<0.0001$). Moreover, higher levels of plasma HIV-1 particle numbers were detected in individuals with lower CD4+ T cell counts as follows; 134,527 x/÷ 9, 18,435 x/÷ 9, and 555 x/÷ 51 in those with CD4+ T cell counts less than 200 (group A), between 200 and 500 (group B), and greater than 500 (group C), respectively (A vs. B, $p=0.0025$ and B vs. C, $p=0.0018$). Patients (n=10) who received oral ddI at doses $\geq$ 6.4 mg/kg/day for 8 to 14 weeks had a profound decrease in plasma HIV-1 particle numbers ($p=0.0051$). Patients (n=7) receiving ddI for 45 to 71 weeks also had a significant decrease ($p=0.018$).

These data suggest that the plasma HIV-1 particle numbers determined by the current RNA-PCR technique can provide a sensitive reflection of the level of viremia in patients with HIV-1 infection, and may serve as a marker to assess the effect of antiviral therapy, especially in patients with no detectable p24 antigenemia. However, it should be noted that more research is required to evaluate the usefulness of this technique in assessing the disease status and monitoring the activity of antiretroviral therapy. We have now improved this plasma RNA-PCR methodology by using radiolabelled-primers and multiple cycles of PCR reactions (e.g. 25 cycles and 35 cycles for the same set of the samples) so that the range of detection is widened. This improved method have been built in several clinical trials of experimental drugs for therapy of AIDS conducted in the Medicine Branch.

## PUBLICATIONS

Aoki-Sei S, Yarchoan R, Kageyama S, Hoekzema DT, Pluda JM, Wyvill KM, Broder S, and Mitsuya H. Plasma HIV-1 viremia in HIV-1 infected individuals assessed by polymerase chain reaction. *AIDS Res. Hum. Retroviruses*, 1992;8:1269-1276.

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: Andrieu J-M and P. Cramer P, ed. "*Implications for Prognosis and Drug Monitoring*" Paris: John Libbey Eurotext 1991; pp.161-170.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07222-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
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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Other: Mary C. O'Brien, General Fellow, MB, COP, DCT, NCI
Shizuko Aoki-Sei, Visiting Associate, MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
NCI, DCT, DTP: Harry Ford, David Cooney, David Johns: OD, NCI: Samuel Broder; NHLBI, CHB: Hiroyuki Fujii; Laboratory of Viral Carcinogenesis, NCI-FCRF: Dennis Gilbert, NIDDK, NIH: Korenman, J., Hoofnagle, J.H., Di Bisceglie, A.M.

LAB/BRANCH
Medicine Branch

SECTION
Experimental Retrovirology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | ½ | ½ |

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.)*

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. 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.



PHS 6040 (Rev: 5/92)

We asked whether experimental therapy for HBV infection, by targeting its reverse transcriptase using 2',3'-dideoxynucleosides, was possible. 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 this project, we asked if three dideoxynucleosides, 2',3'-dideoxyguanosine (ddG), 2',3'-dideoxyinosine (ddI), and 3'-azido-2',3'-dideoxythymidine (AZT), could be active against HBV replication in the actively HBV-producing human hepatoblastoma cell line, 2.2.15. We found that 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 in HBV-infected cells during culture. These data suggest that some 2',3'-dideoxynucleosides can exert a potent antiviral activity against HBV in vitro at least under certain circumstances.

We further asked whether prolonged treatment with ddG could block the replication of DHBV in chronically DHBV-infected Pekin ducks, an animal model for chronic hepatitis B infection. Eleven ducks with persistent DHBV infection were randomly assigned to treatment with either ddG (6 ducks) or saline (5 ducks). Ducks were subcutaneously administered 2.4 mg/kg of ddG, or an equivalent volume of saline, twice daily for 2 months and were observed for one month off treatment before sacrifice. Serum was obtained weekly and liver biopsies were performed before and during treatment and at autopsy in all animals. There was no detectable side effect during ddG administrationand there was no evidence of toxicity at autopsy. Serum DNA polymerase (DNAp) levels decreased markedly in all ducks treated with ddG by one week of therapy and remained at or near normal throughout the treatment period (ddG-treated vs. controls,

p<0.001). By dot blot hybridization, serum DHBV DNA levels showed a similar decrease over the 8 weeks of treatment (p<0.05). However, within a week of stopping ddG, serum DNAp and DHBV DNA levels returned to pretreatment values. This rebound may be related to the observation that intrahepatic DHBV DNA, measured by dot blot hybridization, did not decrease significantly in either group, during or after treatment. By Southern blot hybridization of intrahepatic DNA, replicative forms of DHBV DNA disappeared during treatment in 2/6 treated ducks, although full length linear DHBV DNA persisted. Thus, while the replication of DHBV DNA is inhibited, pre-existing forms of DHBV DNA do not appear to be affected by nucleoside analogues.

These findings indicate that although ddG is an effective inhibitor of DHBV replication, two month period of treatment with ddG, which can essentially completely suppress the replication of DHBV, is not sufficient for the elimination of the virus from chronically DHBV-infected Pekin ducks. Current data should have important clinical relevance in the development of antiviral therapy of HBV infection in humans.

## PUBLICATIONS

Aoki-Sei S, O'Brien MC, Ford H, Fujii H, Gilbert DA, Cooney DA, Broder S and Mitsuya H. *In vitro* inhibition of hepatitis B virus replication by 2',3'-dideoxynucleosides: inhibitors of reverse transcriptase. *J. Infect. Dis.* 1991;164:843-851.

Fried MW, Korenman JC, Di Bisceglie AM Park Y, Waggoner JC, Mitsuya H, Hartman NR., Yarchoan R, Broder S, and Hoofnagle JH. A short-term pilot study of 2',3'-dideoxyinosine for the treatment of chronic hepatitis B 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07223-02 M

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis and in vitro anti-HIV activity of lipophilic dideoxynucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
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Other: Takuma Shirasaka, Visiting Fellow, ERS, MB, COP, DCT, NCI
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COOPERATING UNITS *(if any)*
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LAB/BRANCH
Medicine Branch

SECTION
Experimental Retrovirology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*

2-Amino-6-halo-2',3'-dideoxypurine ribofuranosides (6-halo-ddGs) and 6-halo-ddPs (6-halo-ddIs) have been shown to suppress the infectivity, replication and cytopathic effect of HIV (Shirasaka et al. *Proc. Natl. Acad. Sci. USA.* 87:9426-9430, 1990). 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. These compounds have also shown a potent activity against HIV-2 and AZT-resistant HIV-1 variants *in vitro*. Several of the 6-halogen-containing ddPs have been 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-halogen-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.

INTRODUCTION

We have administered four compounds (6-F-ddG, 6-Cl-ddG, 6-Br-ddG, 6-I-ddG) intravenously or orally to mice and studied the pharmacokinetics and metabolic pathways of these compounds. We found that all four 6-halo-ddGs were orally bioavailable and a considerable level of the intact compounds penetrated into brain in mice. We found that all 6-halo-ddGs underwent not only hydrolysis at the 6-position mediated by adenosine deaminase but also conjugation with glutathione *in vivo.* The glutathione conjugation appears to be mediated by glutathione S-transferase and this conjugation apparently take place mainly in the liver.

More recently, several 6-halo compounds have been administered as a short intravenous infusion at doses of 1.5 g/M$^2$ (ddA, ddI) or 1.9 mMol/M$^2$(ddG, Cl-ddG, I-ddG). Drug concentration in plasma and CSF was measured with a paired ion HPLC method. ddA was almost instantaneously deaminated to ddI and similarly Cl- and I-ddG were rapidly dehalogenated to ddG. The ratio of the area under the drug concentration *vs.* time curve (AUC) in CSF to that in plasma for ddI, after intravenous administration of ddI or ddA, was 5%. The CSF to plasma ratio of ddG was 6.5 % when ddG was given, 23% for Cl-ddG and 17% for I-ddG. It was noted, however, that the actual drug exposure to ddG (AUC) in the CSF did not differ significantly for the 3 analogues (12 mM·hr for ddG, 18 mM·hr for Cl-ddG, 9 mM·hr for I-ddG) following administration of equimolar doses. The higher CSF:plasma ratios for the 6-halo-ddG drugs were the result of lower plasma AUC's, but not the result of higher CSF drug exposures. These findings suggest that tin order to achieve high concentrations of ddG, administration of high doses of 6-Cl-ddG may be required.

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 and may have a potential usefulness for treatment of HIV infection, in particular, HIV-caused neurological disorders, and further studies in the direction of clinical application are warranted.

## PUBLICATIONS

Shirasaka T, Watanabe K, Yoshioka H, Kojima E, Aoki S, Murakami K, and Mitsuya H. Lipophilic 6-halo-2', 3'-dideoxypurine nucleosides: potential antiretroviral agents targeting HIV-associated neurologic disorders. In: Kumar A, ed. *Advances in Molecular Biology and Targeted Treatment for AIDS*, Plenum Publishing Co., Washington, D.C: Plenum Publishg Co.,. 1991; pp. 323-333.

Anderson BD, Bake DC, Galinsky RE, Hoesterey BH, Morgan M, Murakami K, and Mitsuya H. Approaches toward the optimization of CNS uptake of anti-AIDS agents. *J. Controlled Release*, 1991;19:219-230.

Shirasaka T, O'Brien MC, and Mitsuya H. In vitro evaluation of experimental agents for anti-HIV activity. In: "*Techniques of HIV Research*", Current Protocols Publisher, New York 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 CM 06513 16 NMOB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

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| Others: | Bruce A. Chabner | Director, DCT | OD, DCT, NCI |
| | Donna Voeller | Chemist | NMOB, COP, DCT, NCI |
| | James C. Drake | Biologist | NMOB, COP, DCT, NCI |
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Biology and Therapeutics of Solid Tumors Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL | OTHER |
|---|---|---|
| 9.0 | 6.0 | 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.)*

This project is divided into two broad areas that include the development of strategies for the treatment of solid tumors 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 resistance to the antimetabolite class of agents. These studies have identified that the treatment of patients and malignant cells in in vitro model systems with fluoropyrimidines and antifolate agents results in an acute induction of thymidylate synthase and dihydrofolate reductase respectively. Since the level of intracellular thymidylate synthase is an important determinant of sensitivity to fluoropyrimidine agents, the acute induction of this enzyme following fluoropyrimidine exposure is a central cause of resistance to these agents both in vivo and in vitro. Interferon gamma has been shown to repress the acute thymidylate synthase induction resulting from fluoropyrimidines. This repression results in enhanced sensitivity of malignant cells. While the precise mechanism of interaction between interferon represents an ongoing investigative effort, we have identified an unprecedented mechanism of autoregulatory translational control as a means by which cells regulate the intracellular levels of thymidylate synthase. Given the importance of thymidylate synthase as a chemotherapeutic target, we have developed sensitive assays for the quantitation of this enzyme in cells and human tissues using a monoclonal antibody directed against thymidylate synthase. The use of monoclonal antibodies has resulted in ultrasensitive detection of thymidylate synthase, quantitation of enzyme free and bound by fluoropyrimidines and quantitation on a per cell basis in human tissues and cells. The availability of these sensitive assays will help delineate the role of thymidylate synthase as a prognosticator of survival and response. The investigations of therapies for opportunistic infections is focussed on the interactions of antifolate agents on the metabolic pathways in T. gondii, P. carinii and Mycobacteria (MTb, MAI). We are currently 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:

| | | |
|---|---|---|
| Edward Chu | Senior Investigator | NMOB, COP, DCT, NCI |
| Patrick Johnston | Senior Investigator | NMOB, COP, DCT, NCI |
| Chris Takimoto | Clinical Associate | NMOB, COP, DCT, NCI |
| Peter Brandon | Stay-in-School | NMOB, COP, DCT, NCI |
| Joe Fedorko | Microbiologist | NMOB, COP, DCT, NCI |

1. Thymidylate Synthase as a Therapeutic Target

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 one has 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. An 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. We have found that gamma-interferon is capable of repressing the acute induction of thymidylate synthase and results in enhanced sensitivity to the fluoropyrimidine agents. 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 increases following exposure to 5-FU. Pulse chase experiments have clearly demonstrated that the increase in protein levels following 5-FU exposure are the result of enhanced translational efficiency rather than a diminished rate of protein degradation. 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, we have found that the binding site occupancy of the enzyme is a central determinant in its ability to interact with the message. The identification of the protein-mRNA autoregulatory loop is unprecedented in eukaryotic systems and may provide a paradigm for the regulation of other critical chemotherapeutic targets.

2. Antifolate Projects

Our investigations 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 cell line dependent. Substrate depletion appears to be responsible for the majority of enzyme inhibition in the rat hepatoma cell 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 in the process of determining the reason for these apparent differences by applying a computerized model of the folate pathways developed by Dr. Paul Morrison. Dr. Morrison has identified the intracellular level of thymidylate synthase to be an important determinant in the degree of folate depletion manifest by cells following exposure to dihydrofolate reductase inhibitors. Dr. Morrison has found that as the level of thymidylate synthase is increased the degree of folate depletion is also increased following exposure to either methetrexate or a more lipid soluble antifolate, trimetrexate. These mathematical studies are consistent with the empiric observation of enhanced folate depletion following methetrexate exposure to the hepatoma cell lines compared to the breast cancer or colon cell lines in that the level of thymidylate synthase in the former line is approximately 15-20 fold higher than that in the two latter cell lines. We are currently in the process of studying the degree of folate depletion following methetrexate exposure in the wild type colon carcinoma cell lines H630 compared to the degree of folate depletion which occurs in a thymidylate synthase amplified H630 cell line.

Given the utility of the leucovorin/5-FU 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 leucovorin 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 central factor in the optimal use of leucovorin with 5-FU.

Given that 5-methyltetrahydrofolate rather than 5-formaltetrahydrofolate (leucovorin) is the physiologic folate found in the serum of humans we reasoned that the use of 5-methyltetrahydrofolate may be a better folate for enhancing the critical intracellular folate pools for optimal interaction with 5-FU. Studies recently completed in our laboratory demonstrated that the total intracellular level of folates following 5-methyltetrahydrofolate exposure was approximately two-fold lower than after an identical exposure to 5-formaltetrahydrofolate. In addition, we found that cells were unable to efficiently convert the 5-methyltetrahydrofolate to the most active folate required for tetrary complex formation with the fluoropyrimidine and thymidylate synthase, namely 5,10-methylenetetrahydrofolate. This folate was unmeasurable following exposure to 5-methyltetrahydrofolate whereas it was easily detectable following 5-formaltetrahydrofolate exposure. These studies suggest that despite the more physiologic nature of 5-methyletrahydrofolate it would appear that the currently used folate, that is, leucovorin, is a more preferable folate for use in therapies with fluoropyrimidines.

3. Thymidylate Synthase Quantitation

Because of the central importance of thymidylate synthase as a chemotherapeutic target, we have sought to develop a new and more sensitive method for its quantitation. Over the past two years, 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. These antibodies have been used to quantitate the level of thymidylate synthase in peripheral blood lymphocytes and malignant cells in culture using both an ELISA and Western immunoblot analysis. The ELISA assay coupled with a chemilluminescent detection system is capable of detecting thymidylate synthase levels as low as 30 attamoles. The Western immunoblot analytical technique is capable of measuring not only total enenzymatic activity but also can quantitate enzyme that is free from ligand binding as well as enzyme bound by fluoropyrimidines. Immunohistochemical techniques have also been developed for enzyme quantitation in formalin-fixed and paraffin embedded tissue. These various assays will be invaluable in the determination of the role of thymidylate synthase in the cellular biology of both gastrointestinal and breast carcinomas. Because of the easy applicability of these tests to human samples, both prospectively as well as retrospectively they will provide a convenient and precise mechanism for determining the prognostic significance of thymidylate synthase expression in survival and/or response to therapy in patients with gastrointestinal and breast malignancies. Furthermore, since thymidylate synthase is an "S-phase" sensitive enzyme, the monoclonal antibodies capable of quantitating this enzyme may serve as a means for identifying populations at high risk for developing either breast or gastrointestinal malignancies. The intrinsic proliferative rate of the gastrointestinal mucosa has been shown to be significantly increased in patients who developed colorectal malignancies. Prospective and retrospective studies to address these questions are currently ongoing.

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 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 as well as microbacterial organisms. This enzyme is expressed in relatively low levels and is unstable. Ongoing investigations include analysis of the physical characteristics, characterization of the kinetic interactions of the enzyme with its substrates, and interaction of the enzyme with various inhibitors. Presently, we are continuing investigations with purified enzyme harvested from the lungs of steroid treated rats. However, the P. carinii DHPS has recently been cloned and is in the process of being expressed at high levels in bacterial systems. The availability of this central enzyme will greatly facilitate the identification and development of more potent and less toxic inhibitors of these opportunistic organisms.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM 06579 09 NMOB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

| | | | |
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| Others: | Virginia Bertness | Biologist | NMOB, COP, DCT, NCI |
| | Kenneth Nakahara | Biologist | NMOB, COP, DCT, NCI |
| | Peter Aplan | Medical Staff Fellow | NMOB, COP, DCT, NCI |
| | Stanley Lipkowitz | Clinical Associate | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
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LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Acquired Gene Rearrangements Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7.0 | 5.0 | 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.)*

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 five 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

Additional Personnel Associated with Project:

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|---|---|---|
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| Mary Varterasian | Clinical Associate | NMOB, COP, DCT, NCI |

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 inroad 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 determine the functional role of NSCL-1 and NSCL-2, two newly identified SCL related basic domain helix-loop-helix genes that appear to play critical roles in neurogenesis.

Major Findings:

We have finished the complete structural characterization of the SCL NSCL-1 and NSCL-2 genes in terms of their genomic structure, mRNAs, encoded proteins, and 5' regulatory regions. 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 interrupted locus). We have now also finished the complete structural characterization of the SIL gene as well as investigation of its pattern of expression. 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.

We have developed an assay for at least one aspect of SCL function utilizing the murine erythroleukemia (MEL) system. MEL cells differentiate to globin producing normoblasts in response to a variety of chemical and biological agents. Increasing the level of expression of SCL increases spontaneous erythroid differentiation in the absence of chemical inducers. Inhibiting SCL expression by transfection with a variety of constructs ("antisense", "basic-domain-deletion") blocks erythroid differentiation in the presence of chemical inducers. Thus, SCL appears to be a positive regulator of erythroid differentiation.

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, NSCL-1 and NSCL-2. 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 cloned the genomic and cDNA versions of these genes from the mouse and the human and have accomplished their detailed elucidation. We are now attempting to develop a functional assay for these two genes utilizing in vitro hemogenesis, analogous to the SCL/MEL system described above.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM 06581 09 NMOB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

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| | Carol Kobrin | Staff Fellow | NMOB, COP, DCT, NCI |
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COOPERATING UNITS *(if any)*

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Molecular Biology of Differentiation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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 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 (MEL) cell line. By constructing and expressing mutant c-myb transgenes in MEL cells, we have demonstrated that the DNA binding and transactivation domains are necessary and sufficient for the c-myb mediated block. So far we are unable to determine whether these oncogenes block differentiation indirectly - by promoting proliferation - or directly - by disrupting the differentiation pathway. Using this model, our long term goal is to understand the molecular mechanisms which 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 that are expressed in most murine plasmacytomas but rarely in B lymphomas. We have identified two classes of genes having this property: 1) genes differentially expressed in plasmacytomas and normal plasma cells but not in B cells; and 2) genes differentially expressed in plasmacytomas but not normal plasma cells. Curiously, a number of genes in the former category are expressed in pre-B and plasma cells but not B cells, suggesting shared functional properties of the cells at either end of the B cell maturation pathway. Thus far we have not been able to demonstrate that either of the two genes in the second category are primary determinants of the malignant plasmacytoma phenotype. Our long term goal is to identify genes that not only mark but also determine the phenotypes of plasmacytomas and terminally differentiated normal 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.

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 ZnCl2 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 regulation of genes which might regulate the terminal differentiation process, e.g. Id genes (1,2, and 3), 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 (this approach is based on their experiments indicating that SCL is an essential mediator of chemically induced terminal differentiation of MEL cells). In addition, we have expressed a murine Id1 gene under control of a metallothionein promoter in MEL cells, but find no effect on the chemically induced terminal differentiation of these cells. This unexpected result needs to be explored further to determine whether or not Id1 protein expressed from the transgene is active in these cells.

2) We have constructed 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. A manuscript is being written to communicate these results. We are making c-myb antisense vectors which we will express either with or without potential "dominant negative" c-myb mutants (i.e. myb mutants containing the DNA binding domain but not the transcription activation domain) in an attempt to determine whether c-myb expression is necessary either to permit proliferation or to prevent 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. Preliminary results using an expression vector that encodes a myb/estrogen receptor suggest that myb directly increases c-myc expression in some cells. This result raises the possibility that c-myb blocks MEL differentiation by up-regulating c-myc expression, a possibility that we are attempting to investigate in the MEL system.

Our long term goal is to determine how c-myc and c-myb regulate proliferation and differentiation in normal and malignant hematopoietic cells.

B. Subtractive cDNA cloning to identify genes involved in plasma cell differentiation and/or tumorigenesis.

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. In an initial screen we identified 50 apparently unrelated genes, each of which expressed mRNAs in both the parental MPC11 and an unrelated plasmacytoma, but not in the A20 subtractive partner. Additional screening indicated that 7 of these 50 genes are expressed in most plasmacytomas but rarely in the seven B lymphomas examined. Provided below is a brief summary for five of these genes (PC70, EGP314, PC109, PC251, PC326) as well as two genes (PC315, PC166) that are expressed at a higher level in plasmactyomas than in the B lymphoma subtractive partner. Two of these genes (PC326, PC251) appear to relate to the neoplastic phenotype of plasmacytomas, whereas the others appear to represent markers of the normal, terminally differentiated plasma cell.

1) PC315 - 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) EGP314 (formerly PC289A) - This clone identifies the murine equivalent of an 314 amino acid pan-epithelial membrane glycoprotein that 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 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 two of three 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 luminal spaces, e.g. gut, lung, etc.

3) PC251 - this gene is a new member of the hematopoietic growth factor receptor family (i.e. distantly related to the IL-5, IL-3, and GMCSG receptors, with even more limited homology to the erythropoietin, IL2, and IL6 receptors. 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 all normal mouse tissues examined. It is also expressed by Balb/3T3, clone A31 cells but not by two retrovirally transformed clones of clone A31. Our working hypothesis is that PC251 resembles oncostatin-M, which is growth inhibitory to many cells but is growth stimulatory for plasmacytomas.

4) PC326 - this gene encodes an approximately 83,000 Dalton protein with 2 distinctive domains: 1) an amino-terminal acidic domain, including two highly acidic sequences that flank a unique, moderately acidic 20 amino acid sequence that is repeated 7.5 times in this protein; and 2) a different sequence of 40 amino acids that is homologous to a sequence repeated several times within members of the beta transducin gene family. PC326 is most like members of this family that contain a protein domain with a charged or mixed charged cluster in addition to the transducin repeats; most of the proteins in this part of the transducin gene family appear to regulate progression through the cell cycle, or transcription. Surprisingly, we cannot detect expression of this gene in normal tissues, with the exception of mouse testes. Also, with the exception of two myleoid leukemias, this gene is not expressed in other mouse cell lines that we have analyzed. We also have evidence that the expression of this gene is some how dysregulated in plasmacytomas. 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.

5) PC70 - this gene is placental alkaline phosphatase, and appears to be expressed in pre-B lymphoma and plasmacytomas but not B lymphomas. Data in the literature indicates that this gene is not expressed (or expressed at a low level) in normal B cells, whereas induction of differentiation (? to plasma cells) by LPS leads to increased expression of this gene. Thus this gene appears to be a marker of normal B cell differentiation, with expression limited to the early and late stages of B cell maturation.

6) PC109 - this gene is syndecan, a cell adhesion molecule that is expressed principally on epithelial and stromal cells. A search of the literature indicated that the protein had been shown to be expressed on pre-B, immature B cells, and plasma cells but not on mature B and other circulating B cells. It has been hypothesized that pre-B and plasma cells are located in environments in which they interact with stromal cells (bone marrow) or epithelial cells (e.g. gut epithelium).

7) PC166 - this gene demonstrates significant but limited homology to members of the XLR-1 and XLR-2 gene families, and thus we propose that this is a member of a newly defined XLR-3 gene family. All XLR genes are localized on the X chromosome, and in no case has it been possible to identify a human homologue, including our analyses using a PC166 (XLR-3) probe. PC166 is expressed at a high level in plasmacytomas and at a much lower level in some B lymphomas, but in none of the ten pre-B lymphomas we have examined. It is expressed at a low level in many murine tissues, including spleen cells. Its expression in spleen is up-regulated by in vivo stimulation with IL-6 or in vitro stimulation with LPS.

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 that 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 that are required for the malignant transformation to plasmacytoma cells.

C. Immunoglobulin somatic mutants in a novel murine B lymphoma.

We are studying an unusual highly differentiated murine B lymphoma that expresses mu and gamma immunoglobulin heavy chains, as well as kappa and lambda light chains within the same clone. We have sequenced cDNAs corresponding to all four of the immunoglobulin chains, and can make the following conclusions based on these preliminary results: 1) the mu and gamma heavy chain genes represent different VDJ rearrangements; 2) all of these chains contain somatic mutations compared to the corresponding germline genes; and 3) we have determined that among approximately a dozen clones and subclones examined, we can identify different V(D)J sequences in different clones, i.e.,

three different sequences for mu heavy chains, two different sequences for lambda light chains, two different sequences for kappa light chains, but only one sequence thus far for gamma heavy chains. Our results indicate that this B lymphoma cell line has undergone or is continuing to undergo high rates of somatic mutation of immunoglobulin variable region genes after we cloned the cell line. We are continuing to study these cells as a potential cell line model for somatic hypermutation of immunoglobulin variable regions.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM 06587 08 NMOB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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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| Others: | Peter Aplan | Medical Staff Fellow | NMOB, COP, DCT, NCI |
| | Stanley Lipkowitz | Clinical Associate | NMOB, COP, DCT, NCI |
| | Donald P. Lombardi | Biotechnology Fellow | NMOB, COP, DCT, NCI |
| | Verena Gobel | Visiting Fellow | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
Laboratory of Mammalian Genes and Development, NICHD (Kathleen Mahon, Ph.D.)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Acquired Gene Rearrangements Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER |
|---|---|---|
| 7.0 | 7.0 | 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.)*

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 one putative neurogenic gene NSCL-1 to human chromosome 1q21 and a second, NSCL-2, to human chromosome 1q12. 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

Additional Personnel Associated with Project:

| | | |
|---|---|---|
| Nita Seibel | Guest Researcher | NMOB, COP, DCT, NCI |
| Kenneth Nakahara | Biologist | NMOB, COP, DCT, NCI |

Objectives:

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 08 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 use the NSCL-1 and NSCL-2 genes as markers of neuroendocrine lineage and stage of neuronal development in neural crest and neuronally derived tumors.

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 risk of development of lymphoid malignancy. We had previously studied individuals with the genetic disease ataxia-telangiectasia, an illness of protein 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 of 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. We are further explaining the possibility of performing the assay on DNA extracted from a few drops of blood placed on filter disks. If it is possible to do this, the technique would be extendable to large population screening studies.

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 fushion mRNA that substitutes the 5' SIL untranslated region for the 5' SCL untranslated region. A full SCL protein is therefore still produced but in a dysregulated 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 development of a PCR based assay for following a subset of patients with T-cell ALL. This study is now underway in collaboration wit the Children's Cancer Study Group.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM 06594 07 NMOB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 | Chief, Lung Cancer Biology Section | NMOB, COP, DCT, NCI |
| Others: | Daniel C. Idhe | Deputy Director | OD, DCT, NCI |
| | Yoshi Ohsaki | Guest Researcher | NMOB, COP, DCT, NCI |
| | Han-Kwang Yang | Guest Researcher | NMOB, COP, DCT, NCI |
| | Gary Richardson | Visiting Associate | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

Endocrinology and Reproduction Research Branch, NICHHD (Hao-Chia Chen, Ph.D.)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Lung Cancer Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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 reviewed the serum sodium values of 263 lung cancer patients before the initiation of treatment. In contrast to none of the 130 non-small cell lung cancer (NSCLC) patients, 21 of 133 (16%) small cell lung cancer patients had hyponatremia ($p=0.0001$). Eleven of 21 patients with hyponatremia had tumor cell lines available and 9 expressed ANF mRNA, 7 expressed AVP mRNA, and 5 of 11 cell lines produced both ANF and AVP mRNA. All of the 11 cell lines produced ANF mRNA, AVP mRNA, or both. 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.

B. The atrial natriuretic factor A receptor mRNA has been detected on small cell lung cancer cells by PCR analysis of cDNA from the cell lines and RNase protection assay. Binding studies with iodinated ANF showed saturable binding but the number of sites were to low to accurately estimate. The cells respond to exogenously added atrial natriuretic factor with an increase in intracellular cGMP, similar to the normal receptors. Therefore, there appear to be functional ANF receptors on the surface of small cell lung cancer cells.

C. The serum and urine calcitonin values of 86 different individuals were studied by radioimmunoassay which included 20 nonsmokers, 17 smokers, and 49 patients with small cell lung cancer. The urine values were the most different in these three groups of individuals ($p<0.00001$). RNase protection assays (RPA) and RIAs of 11 tumor cell lines from these patients with small cell lung cancer showed some had elevated serum and urine calcitonin values but did not have ectopic calcitonin production in their tumor cell lines. Therefore it appears that elevated calcitonin levels in patients with lung cancer can come from a source other than the tumor cells.

PROJECT DESCRIPTION

Additional Personnel Associated with Project:

| | | |
|---|---|---|
| Michael Kelley | Clinical Associate | NMOB, COP, DCT, NCI |
| Andrew Gross | NCI-SRTP Fellow | NMOB, COP, DCT, NCI |

Molecular Genetic Events in Lung Cancer

Objectives:

1. Review the serum sodium values of patients with lung cancer and study their tumor cell lines 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 for expression of atrial natriuretic factor receptor mRNA, atrial natriuretic factor binding to its receptors, and intracellular cyclic GMP levels after atrial natriuretic factor addition.

3. Study serum and urine levels of calcitonin from nonsmokers, smokers, and patients with small cell lung cancer to compare the levels of calcitonin in the body fluids and study ectopic calcitonin production in their tumor cell lines.

Major Findings:

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 263 lung cancer patients treated at the NCI-Navy Medical Oncology Branch from November 1983 to November 1988. Twenty-one of 133 (16%) SCLC patients had hyponatremia (serum sodium $\leq$ 130 mmol/L), compared to 0/130 non-small cell lung cancer (NSCLC) patients ($p=0.0001$). Eighteen of 87 (21%) patients with extensive stage disease had hyponatremia compared to 3 of 46 (7%) patients with limited stage disease ($p=0.033$). Of the 21 SCLC patients with hyponatremia, 11 had tumor cell lines available for RNAse protection assays and radioimmunoassays for ANF and AVP. Nine expressed ANF mRNA, 7 expressed AVP mRNA, and 5 of 11 cell lines produced both ANF mRNA and AVP mRNA. All of the 11 cell lines produced ANF mRNA, AVP mRNA, or both. We selected 10 tumor cell lines from the 112 SCLC patients without hyponatremia to serve as controls. Eight of the 10 cell lines produced ANF mRNA and 2 produced AVP mRNA. We also selected 10 tumor cell lines from the 130 NSCLC patients. Two 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 30 of these 31 lung cancer cell lines. The radioimmunassays of the peptides, AVP and ANF correlated closely with the mRNA expression data ($p=0.00035$ and 0.0005 respectively). The AVP peptide hormone levels in the tumor cell lines was moderately associated with serum sodium levels ($p=0.0026; r^2=0.28$) in the patients from whom the tumor cell lines were established. In contrast, the ANF peptide levels were marginally associated with the serum sodium levels ($p=0.066; r^2=0.12$). 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 peptide, tumor cell lines from NSCLC patients do not commonly produce ANF mRNA or peptide and AVP mRNA and peptide.

Tumors and Tumor Cell Lines from Patients with Small Cell Lung Cancer Have Functional Atrial Natriuretic Factor A Receptors

Atrial natiuretic factor A receptors are present on small cell lung cancer cell lines and Hela cells. ANF A receptor mRNA was detected in 3 of 3 small cell lung cancer cell lines (NCI-H82, NCI-H660, and NCI-H1284) and Hela cells 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. Binding assays with iodinated ANF showed Hela cell had approximately 5000 sites per cell. The small cell lung cancer cells had saturable binding but the number of binding sites per cell was too low to accurately estimate. The intracellular cGMP increased in a dose response to increasing amounts of exogenously added ANF. We conclude that atrial natriuretic factor receptors are present on small cell lung cancer and have an appropriate physiologic response to the exogenous addition of atrial natriuretic factor. Studies are underway to determine if the addition of exogenous atrial natriuretic factor can inhibit the growth of the small cell lung cancer cells.

Calcitonin is a Marker of Pulmonary Injury and Small Cell Lung Cancer

The serum and urine values of 86 different individuals were studied by radioimmunoassay which included 20 nonsmokers, 17 smokers, and 49 patients with small cell lung cancer entered onto studies at the NCI-Navy MOB. The urine values were the most different in these three groups of individuals. The nonsmokers values were $55 \pm 4$ pg/mg creatinine, the smokers $168 \pm 34$, and the patients with small cell lung cancer $3102 \pm 1242$ ($p<0.00001$). Eleven tumor cell lines were available from these 49 patients with small cell lung cancer for RNase protection assays (RPA) and RIAs. Seven of 11 cell lines had calcitonin mRNA by RPA including 3 which did not have calcitonin immunoreactivity. Five of 11 cell lines had calcitonin immunoreactivity including 1 which did not have any calcitonin mRNA by RPA. It appears that urinary calcitonin is better for detecting differences in levels between nonsmokers, smokers, and patients with lung cancer. Some patients with lung cancer have elevated serum and urine levels of calcitonin when their tumor cell lines does not produce calcitonin. There observation will be applied to studying calcitonin production in patients with resected NSCLC to determine if elevated calcitonin can predict for development of second tumors.

PUBLICATIONS

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Ohsaki Y, Gazdar AF, Chen HC, Johnson BE. Magainin analogues have antitumor activity against human lung cancer cell lines. Cancer Res 1992;52:3534-38.

Winter SF, Carbone DP, Johnson BE, Takahashi T, Gazdar AF, and Minna JD. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. Cancer Res, in press.

Johnson BE, Kelley MJ. Overview of genetic and molecular events in the pathogenesis of lung cancer, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06596 06 NMOB

PERIOD COVERED: October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Treatment of Patients with Small Cell Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Bruce E. Johnson | Chief, Lung Cancer Biology Section | NMOB, COP, DCT, NCI |
| Others: | Daniel C. Ihde | Deputy Director | OD, DCT, NCI |
| | Gary Richardson | Visiting Associate | NMOB, COP, DCT, NCI |
| | Michael Kelley | Clinical Associate | NMOB, COP, DCT, NCI |
| | Herbert Oie | Microbiologist | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*: Head, Hematology/Oncology, NNMC (John Phares, M.D.); Radiation Oncology Branch, COP, DCT, NCI (Catherine Salem, M.D.); Biomarkers and Prevention Branch, EDCOP, DCPC, NCI (James L. Mulshine, M.D., R. Ilona Linnola, M.D.)

LAB/BRANCH: NCI-Navy Medical Oncology Branch

SECTION: Lung Cancer Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*


A. A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. Forty-four patients have been entered onto study and 29 of 41 (71%) patients who have completed therapy have achieved a complete remission. The projected median survival is 24 months with a median potential follow-up more than 3 years. Seventeen patients have undergone biopsy for attempts at in vitro drug sensitivity testing. Seven of the 17 (42%) have been treated with an in vitro determined combination and those patients have survived for a median 39 months.

B. The study of dose intensity using cisplatin and etoposide in patients with extensive stage studies continues. Eighty-three patients have been randomized to receive either VP-16 80 mg/m$^2$ on days 1-3 days with cisplatin 80 mg/m$^2$ administered on day 1 (43) or etoposide 80 mg/m$^2$ and cisplatin 27 mg/m$^2$ on days 1-5 (40) every 3 weeks for the first 2 cycles and receive standard chemotherapy after the first 6 weeks. Although there is increased hematologic toxicity in patients treated with higher dose etoposide cisplatin, there is not difference in complete response rate, overall response rate, or survival between the high and low dose arm. The trial has completed accrual. This prospective study of dose rate intensity of etoposide cisplatin in small cell lung cancer does not show any survival advantage to administering 67% more chemotherapy in the first six weeks of treatment.

C. A monoclonal antibody (2A11) directed against gastrin releasing peptide (bombesin) which functions as an autocrine growth factor in small cell lung cancer has been used to treat patients with small cell lung cancer. Twelve patients have been treated with the phase II dose of 25mgm/m$^2$. One of the ten patients (10%) who completed therapy has responded with a clinical complete response. The plans are to continue to trial.

PROJECT DESCRIPTION

Treatment of Patients with Lung Cancer

Objectives:

1. Determine the frequency with which adequate tumor tissue can safely be obtained from patients with small cell lung cancer 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 overall and complete response rate and survival of patients with extensive stage small cell lung cancer given VP/PLAT for the intial 12 weeks of treatment.

4. Determine if higher doses of VP/PLAT given during a six-week induction period will produce higher complete response rates and longer complete response duration of the same regimen in standard risk patients.

5. Determine the toxicity of 2A11 at the phase II dose level of 250 $mg/m^2$.

6. Determine the antitumor activity of 2A11 against patients with relapsed small cell lung cancer.

Methods:

1. Small cell lung cancer patients undergo staging.

2. Limited stage patients undergo surgical biopsy of tumor tissue.

3. Patients with limited stage small cell lung cancer undergo 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 extensive stage disease who have standard risk are randomized to receive high or standard dose etoposide cisplatin for the first six weeks of therapy.

5. Patients with both limited and extensive stage disease 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.

6 Patients with small cell lung cancer whose tumor has progressed after combination chemotherapy are treated with the monoclonal antibody 2A11 250 $mg/m^2$ three times weekly for 4 weeks.

7. Patients are followed for response to therapy, survival and toxicity.

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 Prolongation of Median Survival

Between 7/86 and 6/92, 44 previously untreated patients (pts) with LTD stage SCLC entered a

combined modality study. 31 were male, 13 female; 3 were PS 0, 38 PS 1, and 3 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). 43 pts have completed therapy and are evaluable for response. 33 had a CR (77%) and the remaining 11 a PR (23%). The median potential follow-up is now 40 months (range 1-43). The median survival is 24 months with an actuarial survival of greater than 90% at 1 year and 50% at 2 years. 17 of 44 pts (39%) underwent a biopsy procedure to obtain tumor tissue. 7 of those 17 pts (41% or 16% of the total) had adequate numbers 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.

Administration of Higher Dose of Etoposide and Cisplatin During the First Six Weeks of Therapy for Extensive Stage Small Cell Lung Cancer Does not Increase the Response Rate or Prolong Survival

Between 1983 and 1991, 83 patients with extensive stage small cell lung cancer were randomized to receive either standard dose VP-16 80 mg/m$^2$ on days 1-3 days with cisplatin 80 mg/m$^2$ administered on day 1 (43) or high dose etoposide 80 mg/m$^2$ and cisplatin 27 mg/m$^2$ on days 1-5 (40) every 3 weeks for the first 2 cycles and receive standard chemotherapy after the first 6 weeks. The patients in the high dose arm were projected to receive 67% more chemotherapy and the actual amount delivered was 44% higher than in the standard dose arm. The complete response rate to treatment was 25% in the high dose arm compared to 21% in the standard dose arm (p=0.86). The overall response rate was 85% in the high dose arm compared to 81% in the standard dose arm. The median potential follow-up is 55 months. The high dose arm patients have a median survival of 12 months compared to 11 months for the patients treated with standard dose etoposide cisplatin. The comparison of the survivals using the log rank test showed no difference between the two arms (p=0.93). The toxicity was greater in the high dose arm during the first 12 weeks. The median nadir white blood cell and platelet count was 1.6 and 53,000 in the high dose arm compared to 2.5 and 161 in the standard dose arm. The death related to myelosuppression was 5% in the high dose arm compared to 2% in the standard dose arm. This study shows administration of 44% more etoposide and cisplatin during the first 6 weeks of therapy for patients with extensive stage small cell lung cancer does not prolong survival but is associated with increased hematologic toxicity.

Monoclonal Antibody Treatment of Patients with Relapsed Small Cell Lung Cancer has Caused a Clinical Response in One of 10 Patients Treated

Between February of 1989 and June of 1992, 12 good performance status patients with small cell lung cancer who had previously received combination chemotherapy and subsequently progressed were treated with a murine monoclonal antibody directed against gastrin releasing peptide (bombesin). Three patients were women and 9 were men. The antibody was administered intravenously three times weekly for 4 weeks. There has been no detectable toxicity from the treatment and patients have not developed human anti-mouse antibody during treatment. One patient had a clinical complete response, 4 had stable disease, 5 had progressive disease, and 2 did not complete the 4 week course of treatment because of progressive disease. The plan is to obtain more of the antibody and treat more than 14 patients because of the clinical complete response observed in one patient.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM 07256 04 NMOB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
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| Others: | Albert Lin | Clinical Associate | NMOB, COP, DCT, NCI |
| | Greg Otterson | Clinical Associate | NMOB, COP, DCT, NCI |
| | Eiji Shimizu | Guest Researcher | NMOB, COP, DCT, NCI |
| | Robert Kratzke | Clinical Associate | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*
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LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Biology and Therapeutics of Solid Tumors Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*

We are investigating the role of tumor suppressor genes in the pathogenesis of human cancer. Our recent findings are as follows: 1) we have examined 130 lung cancer samples for inactivation of the retinoblastoma (RB) gene and have determined that 90-95% of small cell lung cancer and 15-20% of non-small cell lung cancer have homozygous mutations within this gene. We are completing a correlation of this data with clinical outcome and drug sensitivity testing to determine if RB testing offers prognostic information for patients with lung cancer; 2) using information obtained from our previous analyses of in vivo RB mutant proteins, we have constructed a series of in vitro mutations within the RB protein to define amino acid residues that are critical for RB function; 3) using RB fusion proteins as a reagent to molecularly clone RB-binding proteins, we have identified the interaction of the RB protein with a nuclear matrix product; 4) we have also studied the expression of another RB-binding protein (designated RBP-1) and have determined that it undergoes extensive alternative exon processing that modulates its interactions with the cell-cycle regulator, p34cdc2. We have also raised specific antisera against this 200kDa binding protein and are examining its biologic activity on cell growth. 5) we have successfully expressed RB protein in lung tumors and have observed suppression of tumorigenicity in nude mice. We have also observed that we can reverse the "tumor suppressor" effect with extracts enriched in extracellular matrix (ECM), and we are currently examining how ECM and RB pathways might interact. 6) in collaboration with Dr. Crystal (NHLBI), we have constructed a series of retroviral and adenoviral constructs containing the open reading frames of RB and p53. Finally, we plan to use these vectors to determine the feasibility of in vivo tumor suppression in animal models.

Additional Personnel Associated with Project:

| | | |
|---|---|---|
| Amy Coxon | Biologist | NMOB, COP, DCT, NCI |
| Patrick Johnston | Senior Investigator | NMOB, COP, DCT, NCI |

Mechanisms of oncogene action in tumorigenesis

We have completed an extensive analysis of RB protein expression in lung cancer and have determined that 90-95% of small cell lung tumors have acquired inactivating mutations within this tumor suppressor gene (Shimizu, Kratzke, Kaye). Mutational inactivation of the RB gene, therefore, appears to be an essential step in the pathogenesis of small cell lung cancer. To understand the implications of this finding we wish to address two important questions:

A. What is the role of the RB gene in normal cellular physiology?

B. Can we revert tumorigenicity of lung cancer by the reintroduction of tumor suppressor genes?

Question A:

The functional activity of the RB tumor suppressor product appears to be dependent on the acquisition of a specific conformation of RB that allows protein binding to a set of viral peptides and to a heterogeneous group of cellular proteins[1]. We have examined the effects of single amino acid substitutions flanking regions of RB that we had identified as important for protein binding from our previous work with in vivo RB mutants in lung cancer. To date we have not identified amino acid residues with intrinsic binding activity, but our findings do suggest that the RB protein is present in a tightly folded pattern as small polar or non-polar amino acid substitutions in these regions have no effect on protein binding activity while substitutions with bulkier R-group side chains result in both loss of protein binding and defective in vivo phosphorylation (Kratzke, Horowitz, Kaye). We have also conducted experiments to identify and characterize the new family of RB-binding proteins. These molecules are believed to play a key role in modulating or regulating RB tumor suppressor activity. Using recombinant RB fusion protein attached to sepharose beads, we have recently demonstrated specific binding to a nuclear matrix protein and we are presently determining the significance of this interaction. In addition, we have raised specific antisera to another RB-binding protein, RBP-1, and determined by immunoblotting and immunohistochemistry that it is a 200 kDa nuclear protein. We have cloned most of the open reading frame for this gene and observed that it undergoes extensive alternative splicing clustered in a small internal exon that contains multiple $p34^{cdc2}$ sites. We are currently investigating the biologic role of RBP-1 in cell growth pathways (Otterson, Lin, Johnson, Kaye).

Question B:

We have transfected the RB gene into lung cancer and observed suppression of soft agar cloning and tumorigenicity in nude mice. The suppression of soft agar cloning and tumorigenicity, however, was completely reversed by the addition of an extract enriched in extracellular matrix (ECM) to the transfected lung tumor cells just prior to testing. Tumors from cells pre-treated with ECM were harvested from mice and shown to still express functional human RB protein. We are presently investigating the nature of the ECM interaction with RB tumor suppressor pathways. (Kratzke, Shimizu, Kaye). In addition, expression of wild-type p53 has been demonstrated to markedly suppress the growth of a wide range of tumor lines, including lung cancer cell lines. In collaboration with Drs. Brody and Crystal of NHLBI, we have constructed a series of retroviral and adenoviral vectors containing either the RB or p53 genes to test the ability of of these recombinant vectors to exert tumor suppression on in vivo murine models (Kratzke, Otterson, Kaye).

PUBLICATIONS

Kaelin WG, Pallas DC, DeCaprio JA, Kaye FJ, Livingston DM. Cell 1991;64:521-32.

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Kaelin WG, Pallas DC, DeCaprio JA, Kaye FJ, Livingston DM. The Molecular Basis of Human Cancer. New York: Cold Spring Harbor Press, 1992.

Otterson GA, Lin AY, Kaye FJ. Genetic etiology of lung cancer. Oncology, in press.

Kratzke RA, Shimizu E, Kaye FJ. Molecular genetics of lung cancer. In: Benz C and Liu E, eds. Oncogenes II. Academic Publishers, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 07257 04 NMOB

PERIOD COVERED: October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Max, a Positive and Negative Regulator of Myc in Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Shoshana Segal | Assoc Prof Med NCI-USUHS | NMOB, COP, DCT, NCI |
| Others: | Constance Cultraro | Biologist | NMOB, COP, DCT, NCI |
| | Barbara Dunn | Clinical Associate | NMOB, COP, DCT, NCI |
| | Tiziana Cogliati | Visiting Fellow | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH: NCI-Navy Medical Oncology Branch

SECTION: Molecular Biology of Differentiation Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 4.0 | 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 recent findings which demonstrate specific interactions between Myc and Max resulting in a transcriptionally active complex, we investigated the regulation, expression, and role of the max gene in cell growth and differentiation of murine erythroleukemia (MEL) cells.

PROJECT DESCRIPTION

Max, a Positive and Negative Regulator of Myc in Cell Growth and Differentiation

Objectives:

1. To study the expression and regulation of max in inducer mediated differentiation of MEL cells.

2. To study the effect of a transfected max gene on cell growth and differentiation of MEL cells.

3. To mutate the regulatory regions of the max gene, use the mutants in transfection experiments, and study Myc/Max or Max/Max interactions and the effect of the dimeric forms on cell growth and differentiation of MEL cells.

A. Regulated expression of max in HMBA induced differentiation of MEL cells.

MEL cells express high levels of the c-myc protooncogene, however, shortly after the addition of inducer (HMBA) a sharp decline in c-myc mRNA occurs which is followed by a cessation of cell growth and terminal differentiation. In these cells, max expression mimics this pattern perfectly, demonstrating co-regulation of the two genes.

B. The effect of wild type and basic mutant max on cell growth and differentiation of MEL cells.

Max protein forms DNA binding dimers with itself and Myc family proteins. To elucidate the role of max in regulating cell proliferation and differentiation, we transfected MEL cells with wild type (wt) human max and a basic region mutant which abolishes specific DNA binding. Transfected cells expressing wt-max grow slowly and are delayed in inducer-mediated differentiation. Bm-max transfectants exhibit growth retardation, accumulation in G0/G1, and spontaneous differentiation. Our findings are consistent with a model in which a large excess of wt-Max enhances the formation of Max-Max growth suppressor complexes while elevated bm-Max deprives the cell of growth promoting Myc-Max heterodimers in a dominant-negative manner, presumably by inactivating endogenous Myc and Max.

C. Identification of proteins interacting with Myc in MEL cells.

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. We also constructed an expression cDNA library using poly-A selected RNA from MEL cells. We were able to identify a number of proteins which are being characterized.

PUBLICATIONS

Unger T, Nau MM, Segal S, Minna JD. p53: A transdominant regulator of transcription whose function is ablated by mutations occurring in human cancer. EMBO J 1992;11:1383-90.

Bar-Ner M, Messing LT, Cultraro CM, Birrer MJ, Segal S. Regions within the c-Myc protein that are necessary for transformation are also required for inhibition of differentiation of murine erythroleukemia cells. Cell Growth & Diff 1992;3:183-90.

Bar-Ner M, Messing LM, Segal S. Inhibition of murine erythroleukemia cell differentiation by normal and partially deleted c-myc genes. Immunobiology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 CM 07258 04 NMOB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 | Asst Prof Med USUHS | NMOB, COP, DCT, NCI |
| Others: | W. Michael Kuehl | Head, Molecular Biology of Differentiation Section | NMOB, COP, DCT, NCI |
| | Ross Turner | Biologist | NMOB, COP, DCT, NCI |
| | Dat Nguyen | Guest Researcher | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

Clinical Pharmacology Branch, DCT, NCI, NIH (Edward Sausville)

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Biology and Therapeutics of Solid Tumors Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 3.0 | 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.)*

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

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 chemotherapeutic 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.

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 fresh sezary cells from patients have shown that these cells represent a mature T-helper phenotype, expressing the CD4 antigen and lacking the TAC antigen, or IL2 receptor. The cells demonstrate a moderate but variable response to compartment are largely nonproliferating, 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.

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 analyzed the tumor suppressor p53 gene exon 4 to 8 in lymph node tissue using RT-PCR-SSCP analysis to detect point mutations and have found mutations in one-third of the patients studied. Sequencing revealed that these were base pair changes and deletions. Earlier stage tissues from these patients will be analyzed in order to study the evolution of these mutations.

Development of New Therapies for MF

Over the past year we have attempted to evolve new therapies in the laboratory 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 dose interferon was based on demonstrates 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-2 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 IL-2-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.

We have recently identified synergistic growth promoting activity of a new cytokine with IL-2 in many patients with Sezary syndrome and are developing a strategy to use these receptors in targeted therapies.

Proposed Course

We will be focusing an identification of membrane receptors and ligands which can be used for targeted therapy. We will be more fully exploring the expression of IL-2 receptor subunits by Sezany cells.

PUBLICATIONS

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Foss F, Ihde D, Brenneman D. Phase II study of pentostatin and recombinant interferon alfa 2A in MF/SS. J Clin Oncol, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 CM 07260 01 NMOB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Preclinical and Clinical Pharmacology/Experimental Therapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jean Grem | Medical Officer (Research) | NMOB, COP, DCT, NCI |
| Others: | Carmen J. Allegra | Chief, NCI-Navy Medical Oncology Branch | NMOB, COP, DCT, NCI |
| | J. Michael Hamilton | Chief, Biology & Therapeutics of Solid Tumors Section | NMOB, COP, DCT, NCI |
| | Pamela Daychild | Chemist | NMOB, COP, DCT, NCI |
| | Lorrin Yee | Senior Investigator | NMOB, COP, DCT, NCI |
| | Pedro Politi | Visiting Associate (MSF) | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

Pediatric Oncology Branch, COP, DCT, NCI; Clinical Pharmacology Branch, COP, DCT, NCI; Laboratory of Medicinal Chemistry, DTP, NCI; Laboratory of Biologic Chemistry, DTP, NCI

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Biology and Therapeutics of Solid Tumors Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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.)*

Colorectal cancer has proven refractory to most chemotherapeutic agents; the best available therapy is 5-fluorouracil (5-FU) modulated with leucovorin. A composite analysis of nine randomized trials indicates that the addition of leucovorin to 5-FU approximately doubles the response rate from 13% to 25%. The majority of responses are partial and not durable. Thus, although biochemical modulation of 5-FU with leucovorin has met with some success, innovative strategies are crucially needed to improve the prognosis of patients with colorectal cancer. Our current investigations focus on two areas. The first is the identification of new agents with potential activity against adenocarcinomas of the gastrointestinal tract. Of particular interest are new drugs which display potent in vitro activity (IC50 for a 24 hour exposure $\leq 10\ \mu M$) and/or in vivo efficacy against human colorectal carcinoma cell lines. Studies designed to elucidate the optimal schedule of administration and mechanism of action of such agents are vital to facilitate their rational clinical use. The second line of investigation includes the interaction of other agents with 5-FU in an attempt to define optimal doses and sequences of drug combinations for potential clinical use. Finally, we are implementing Phase I clinical trials which incorporate biochemical or molecular endpoints in addition to clinical endpoints as a reflection of the biologic activity of the particular agent. The ultimate goal is to develop new agents and drug combinations which may be useful in the treatment of patients with gastrointestinal and breast malignancies.

1. New Therapeutic Agents for Solid Tumors

Efforts are continuing in the search for new agents useful in 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 as well as with other agents. CPEC has been shown to be a potent inhibitor of cytidylate synthetase. In addition to its activity as an enzyme inhibitor, we have demonstrated 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 currently undergoing a Phase I clinical investigation (see below).

A new folate analog, D1694, is capable of potent inhibition of thymidylate synthase with inhibition constants in the nanomolar range for the polyglutamated metabolite. We are presently investigating the interaction of this new analog with human thymidylate synthase and its effects on the interaction of anti-thymidylate synthase antibodies with the enzyme. A Phase I clinical trial with D1694 is currently ongoing (see below).

Pyrazoloacridine (PZA) is currently under Phase I clinical testing. Although this agent belongs to a class of DNA binders, little is known about its precise mechanism of action. Because colorectal cancers are particularly refractory to drugs such as doxorubicin and other natural products which are exported from cells via the P-170 glycoprotein membrane pump, we are particularly interested in new agents which have activity against multidrug resistant cell lines. We have found that PZA is cytotoxic against a panel of human cancer cell lines. Remarkably, PZA is potent with one hour pulse exposures. The IC50 values obtained by short duration exposure are within 10-fold the IC50 values obtained with continuous exposure. This observation suggests that the drug interacts with its intracellular target rapidly. These facts plus the published lack of cross-resistance of PZA to other multidrug resistant cell lines, has driven our investigations to characterize the mechanism of action of this potentially important compound and to consider implementation of a clinical trial.

2. Dihydrofolate Reductase as a Chemotherapeutic Target in Colorectal Carcinoma Cell Lines

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 thorough 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. The acute induction of dihydrofolate reductase following antifolate exposure appears to be similar to that described for thymidylate synthase following fluoropyrimidine exposure. Several previous investigations have demonstrated that the acute induction of dihydrofolate reductase following antifol exposure is the result of enhanced translational efficiency rather than decreased protein degradation. Additional studies aimed at elucidating the potential causal role in resistance of this dynamic phenomenon and the molecular mechanism response for the intracellular control of enzyme expression are the topics of future investigations.

3. Clinical Trials

The current 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 trials is based on the combination of 5-FU with leucovorin plus an additional manipulation designed to further enhance the synergy of the basic combination. In addition, several investigations have been initiated as Phase I trials of new therapeutic agents.

MB 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 two-fold 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 et al. has shown that the use of interferon with 5-FU results in a high response rate in patients with previously untreated colorectal cancer (60%). These results have been supported 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 closed in early 1991. We found that these agents could be administered with acceptable toxicity. Of particular importance was the lack of necrologic 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 II trial testing this combination in a homogeneous population of untreated patients with advanced and measurable colorectal carcinoma has been completed and is in the process of being analyzed.

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. 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. Thus far, we have found that a dose of 250 mg/$m^2$ of PALA given 24 hrs. prior to the initiation of the 5-FU leucovorin infusion

results in little or no inhibition of the target enzyme in peripheral blood mononuclear cells. Conversely, a dose of 1266 mg/$m^2$ of PALA results in significant enzyme inhibition in a large percentage of patients without compromise in the delivery of 5-FU.

Monoclonal Antibody Studies Carmen Allegra - Principal Investigator

The focus of this trial will be to determine the MTD of the 131I-Coli anticea conjugate and to examine the tissue-to-tumor ratio of the radiolabel through imaging studies and timed tissue biopsies. An ongoing clinical trial has demonstrated that the antibody localizes to the tumor of all patients studied to date and can be safely administered up to a dose of 50 mCi/$m^2$. Identification of am MTD and final analysis of the pharmacodynamics of the antibody await future analysis.

Cyclopentenyl Cytosine Jean Grem - Principal Investigator

This antimetabolite has entered Phase I testing and a maximum tolerated dose is in the process of being identified. In addition to the usual endpoints of MTD and toxicity, this trial has also incorporated a variety of nonclinical endpoints including a pharmacokinetic analysis of CPE-C and an examination of target enzyme inhibition in tumor tissue, peripheral blood lymphocytes and bone marrow cells. In addition, through collaboration with the Laboratory of Dr. David Johns, we are quantitating the level of CPE-C triphosphate in the peripheral blood lymphocytes of treated patients.

D1694 Carmen Allegra - Principal Investigator

D1694 is a potent folate-analog inhibitor of thymidylate synthase. A Phase I trial is currently ongoing and an MTD is being identified. In addition to clinical endpoints, this trial also includes a pharmacokinetic analysis of D1694 as well as an investigation of the extent and duration of thymidylate synthase enzyme inhibition in peripheral blood mononuclear cells and tumor tissues taken from treated patients. These latter studies will be conducted using the monoclonal antibody technology developed in our laboratories.

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 to 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 along with surgery.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 CM 07261 01 NMOB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Transcriptional Regulatory Factors and Their Role in Malignant Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Carmen J. Allegra | Chief, NCI-Navy Medical Oncology Branch | NMOB, COP, DCT, NCI |
| Others: | John Wright | Senior Investigator | NMOB, COP, DCT, NCI |
| | Marion Nau | Chemist | NMOB, COP, DCT, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Biology and Therapeutics of Solid Tumors Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

| TOTAL STAFF 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 zinc finger transcriptional regulatory factors constitute a family of early response genes. The recent cloning and characterization of four of the genes in this family by this laboratory has stimulated an investigation of the role of these factors in the malignant phenotype. Presumably, the dysregulation of factors critical to cellular proliferation such as the zinc finger transcriptional factors could lead to unbridled malignant proliferation. Recent investigations from this laboratory have demonstrated that these early response genes are expressed in a constitutive fashion in T-cells transfected with either of the human retroviruses HTLV-1 or HTLV-2. Also of interest are preliminary studies that have demonstrated that the 225 zinc finger gene as well as other members of this gene family are also constitutively expressed in a variety of human malignant cell lines and tissues. The role of this constitutive expression in human malignant cells either in the pathogenic process or as a potential therapeutic target are the topic of ongoing and future investigations.

Activation of quiescent cells by mitogens, growth factors or hormones initiates a program sequence of inter-regulated transcriptional events that culminates in cell proliferation and/or a differentiated phenotype. The early events following cell stimulation involve the coordinate activation of primary response genes, many of which encode nuclear transcriptional regulatory factors that presumably direct the subsequent genetic program critical to cell division. In light of their central role linking cell membrane receptors to the compliment of genes that compromise the specific response to endogenous stimuli, the early response genes with transcriptional regulatory activity constitute a potential target for disrupting the tightly controlled signaling pathways activated by proliferative and differentiative stimuli. Dysregulation of these genes conceivably may lead to oncogenic transformation. Recent studies have identified several novel early response genes that comprise a family of transcriptional regulatory factors related by the presence of a nucleic acid binding region with 3 tandum zinc fingers. The zinc finger proteins 225, 592, and 133 were isolated from a cDNA library constructed by the activation of human peripheral T-lymphocytes with phytohemagglutinin and phorbol myristate acetate in the presence of the protein synthesis inhibitor cycloheximide. The zinc finger domains of these genes show striking homology and mediate sequence-specific interactions with DNA, consistent with their transcriptional regulatory function. Our previous studies established that T-lymphocytes transformed with human retroviruses HTLV-I or HTLV-II constitutively expressed 225, in contrast to the transient expression of this gene following mitogen stimulation of T-cells. Additional work has indicated a strong correlation between the expression of HTLV-I pX region gene products, and specifically Tax, a viral transactivating factor, and expression of the 225 zinc finger gene. Subsequently, preliminary studies have shown that members of this gene family also are constitutively expressed by other human malignant cell lines including solid tumors of human origin. The major objective of our research program is a molecular analysis of this group of zinc finger transcriptional regulatory factors in order to understand the contribution of each gene to the process of malignant transformation. Specifically, this analysis will focus predominantly on the role of these genes in gastrointestinal tumor although other solid tumor types will also be considered. Critical to an understanding of their role in malignant transformation will be a determination of whether these genes are dysregulated in solid tumors and formulating a mechanistic explanation for the development of this apparent expression. Primary to these goals also will be a study of how altered expression of these genes may effect the malignant phenotype. The emphasis in these investigations will be on target genes induced by these nuclear factors such as proto-oncogenes, cytokines or growth factors whose constitutive production would establish an autocrine feedback loop leading to continuous proliferation. Finally, we will attempt to demonstrate the oncogenic potential of these genes by transformation of cell cultures in vivo and in vitro using transgenic models.

PUBLICATIONS

Wright J, Wagner D, Hagengruber C, Blaese RM, Waldmann TA, Fleisher, TA. Characterization of common variable immunodeficiency: Identification of a subset of patients with distinctive immunophenotypic and clinical features. Blood (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06813-10 PB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cell & Molecular Genetic Analysis of Pediatric Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

| | | | |
|---|---|---|---|
| PI: | Carol J. Thiele | Senior Investigator | PB, NCI |
| Others: | Kazue Matsumoto | Chemist | PB, NCI |
| | Fabrizio Ensoli | Visiting Fellow | PB, NCI |
| | Enrico Lucarelli | Visiting Fellow | PB, NCI |
| | Aurelio Cafaro | Volunteer | PB, NCI |
| | Sharon Sickafuse | Biologist | PB, NCI |

COOPERATING UNITS (if any)
NCI (Drs. Rosenburg, Steeg, Kaplan); Univ. of CA, San Francisco (Dr. Israel); Univ. of Florence, (Drs. Maggi, Vanelli); Institute Regina Elena, Italy, (Dr. Gaetano); Univ. of CA, Los Angeles, (Dr. Cohen)

LAB/BRANCH
Pediatric Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5 | 3.5 | 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.)

We use human pediatric tumors and their normal cellular counterparts as model systems to study the molecular events associated with the development of malignant tumors as well as mechanisms mediating HIV-induced neuropathy in children. The focus of the laboratory is pediatric peripheral neuroectodermal tumors including neuroblastoma and the Ewing's sarcoma family of tumors (ESFT). Since neuroblastoma (NB) can be induced to differentiate *in vitro*, it is an excellent system in which to study the molecular mechanisms regulating growth and differentiation in tumor cells. Our current focus is to: 1) identify chemicals and biologic response modifiers that control cell growth and/or induce differentiation in tumor cells; 2) use recombinant DNA technology to identify the molecular mechanisms mediating the signal transduction pathways; and 3) clone the genes important for the regulation of these processes. Once critical genes involved in regulation of neuroectodermal cell growth and differentiation have been identified, we are able to use a normal human fetal olfactory neuroblast cell line that grows and can be induced to differentiate *in vitro* to address questions regarding the ability of dysregulation of these genes to alter development or tumorigenicity. Past studies have contributed to clinical protocols utilizing retinoic acid and tumor infiltrating lymphocyte therapy protocols utilizing interferon gamma in pediatric tumors.

The ability of NB to be induced into normal neuronal cells and our development of a normal human fetal olfactory neuroblast cell line that can be induced to differentiate, serve as *in vitro* models to study HIV infection of neuronal cells, and effects of HIV infected monocytes and glial cells on normal neuronal development and physiology.

Ultimately, our goal is to develop new strategies and novel therapeutics based on understanding the specific alterations in pediatric malignancies and related diseases.

1. Evaluation of neuroblastoma cell growth: An understanding of the mechanisms controlling cell proliferation is critical to delineating mechanisms of growth dysregulation in cancer. Furthermore control of cell growth is usually a prerequisite for terminal differentiation. We reported decreased $p34^{cdc2}$ levels and RB phosphorylation in the retinoic acid growth arrested and differentiated cell line KCNR while a cell line that fails to growth arrest and differentiate, SKNAS, doesn't regulate these genes. Current studies have extended these results to include the $G_1$ associated protein kinases cdk2 and cyclins C, D, E. We have found that transcription of $G_1$-related cyclins yet not $G_2$ cyclins is dramatically decreased in RA treated cells. In contrast signals transduced via increases in cAMP and presumably activation of protein kinase A increase IGF-II mRNA expression and do not alter cell cycle profiles nor regulation of $G_1$ cyclins.

In collaboration with Drs. Maggi and Serio at the Endocrinology Unit of the University of Florence, we have evaluated the effects of somatostatin (SS) on NB signal transduction pathways. We have characterized SS receptors on NB cells, finding low affinity SS receptors on all NB cell lines and high affinity SS receptors on a subset of NB cells lines with a relatively immature chromaffin phenotype. Biologic activity of SS only occurs on NB cells possessing high affinity SS receptors. Maximum growth inhibition is only 20% of control, thus it would not be useful as an anti-proliferative agent. We have identified a novel SS mediated signal transduction pathway and are evaluating if this is peculiar to the NB tumor cell. The ability of SS to image tumor cells with relatively immature cell phenotype indicates that it may have clinical utility as imaging with MIBG is more efficacious in more differentiated NB cells.

2. Evaluation of resistance to retinoic acid: Retinoic acid (RA) has been approved for clinical trials and as part of our ongoing studies we are evaluating 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 a correlation between expression of IGF-II and resistance to the growth inhibiting effects of RA. 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 RA resistant cell lines and characterized them in order to understand the nature of the development of resistance to RA. We have found increased expression of FOS and JUN mRNA. The complex of FOS and JUN proteins constitutes the transcription factor AP1 which is known to inhibit transcription regulated by retinoic acid receptors. Current studies are focused on determining genes directly stimulated by IGF-II and evaluating ability of IGF-II to block signals controlling growth and inducing differentiation. Despite the fact that RA resistant cell lines grow in vitro, soft agar cloning and tumor formation studies in nude mice indicate that they have a reduced tumorigenic phenotype.

3. Differentiation of neuroectodermal cells:

A. Neuroblastoma --

1. RA induced differentiation of NB continues to be our paradigm for development of agents with therapeutic potential.

2. As concentration gradients of RA are important in embryologic development, we have initiated a study to characterize the response of cells to various concentrations of RA. We have noted that at $10^{-9}$ M RA, NB cells show initial stages of morphologic differentiation but fail to growth arrest and remain markedly tumorigenic in comparison to $10^{-6}$ M RA which induced cell growth arrest, terminal differentiation and suppression of tumorigenicity. With this system we are cloning genes expressed that control suppression of tumorigenicity and will be able to subtract genes expressed at $10^{-9}$ M RA which are associated with morphologic alterations.

3. We have extended our studies in collaboration with David Kaplan to investigate the role of neurotrophins and their receptors in differentiation of NB cells in vitro. We have demonstrated that TGF$\beta$ mRNA and protein increase in RA treated NB cells that growth arrest and differentiate but fail to do so in resistant line.

4. We have found that agents raising intracellular cAMP and presumably activating protein kinase A are not sufficient to induce cell growth arrest and terminal differentiation. We have determined that these agents induce an intermediate chromaffin cell phenotype.

5. Screening studies continue to evaluate novel drugs with activities of therapeutic interest on NB cells. Completed studies have shown cytosine arabinosides and interferon gamma have growth arresting properties.

B. Ewing's Sarcoma Family of Tumors (ESFT): The prognosis of children with metastatic ESFT continues to be poor. We have a program to evaluate biologics with growth inhibiting activities on ESFT. To date we have found that agents such as dibutyrl cAMP inhibit but do not arrest the growth of ESFT cell lines. This occurs in 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. We have completed a study in which cytosine arabinoside (ARA-C) is active on all ESFT cell lines tested with a mean $IC_{50}$ of $10^{-8}$ M. We have found ARA-C causes ESFT cells to accumulate in $G_2$/M of the cell cycle and preliminary data indicate cells die via apoptosis. In addition to these studies, we have investigated the anti-proliferative effects of ICRF-187, a heavy metal chelator currently undergoing clinical 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. In contrast to NB, RA has no growth inhibiting activity but combinations of RA and TGF$\beta$, TNF$\alpha$, or interferon $\alpha$ are being evaluated. We

have identified ETS-1 gene expression in ESFT cells and are developing a PCR based assay to type ESFT with t(11, 22) since the gene on chromosome 11 involved in the translocation is related to ETS-1.

C. Normal Human Fetal Olfactory Neuroblast Cell Line: In collaboration with Dr. Vanelli (Department of Pathology, University of Florence), we have developed a normal neuroectodermal cell line derived from fetal olfactory neuroblasts. This cell line is 'invaluable' in addressing questions related to mechanisms of differentiation and tumorigenicity in neuroectodermal cells. We have found that this cell line can be growth arrested and induced to differentiate in response to RA and express olfactory marker protein. Since amplification of NMYC has been associated with tumor progression, we have transfected NMYC and overexpressed it in this record cell line. Current studies are in progress to evaluate contribution of NMYC to tumorigenicity.

4. HIV Associated Neuropathy: The acquired immune deficiency syndrome is a neuroimmune disease. The encephalopathy and resulting neurologic disorders are a major problem in the HIV-1 infected pediatric population. To study the mechanism(s) involved in neural function impairment, we have developed in vitro model systems. The first model system employs neuroectodermal tumor cell lines while the second is a normal fetal olfactory neuroblast cell line adapted to in vitro culture. We have found HIV viral LTR is permissive for expression (transient CAT assays) in these cells and is higher in Schwannian cell type as compared to neuronal cell types. Treatment of cells with nerve growth factor enhances viral transcription while treatment with RA neither stimulates HIV-LTR transcription nor significantly alters TAT induced HIV-LTR transcription. HIV-LTR can be expressed in normal olfactory neuroblast and studies are in progress to evaluate neurotrophic, cytokine and hormone effects on transcription. Viral infection of neural cells has been difficult to demonstrate in vivo, however, HIV-1 has been shown capable of infecting neuroectodermal tumor cells in vitro, through a non-CD4 receptor mediated mechanism. We are evaluating the potential of various isolates of HIV-1, characterized by different cellular tropisms, to infect normal neuroblasts either by direct addition of viruses to the cell or by culture with infected primary blood cells and accessory (glial) cells. We have been unable to infect normal fetal neuroblasts using a condition which lead to infection of neuroectodermal tumor cells using a lymphotrophic HIV isolate.

5. Antibody Enhancement of HIV Infection: Current emphasis on HIV-vaccine development necessitates studies to critically evaluate antibody enhanced HIV infection of cells. To study this a macrophage cell line lacking $CD_4$ receptors was generated utilizing chemical mutagenesis. The mutant cell line still retains other phenotypic properties of macrophages as well as phagocytic capabilities and receptors for Fc portion of immunoglobulins. The mutant cell line is capable of expressing HIV-LTR transcription in transient CAT assays. Lymphotrophic and macrophagotopic HIV isolates and anti-HIV antibodies are being employed to study the phenomenon of antibody enhancement of HIV infection.

Publications:

Kidowaki T, Thiele CJ, Kleinman HD, Israel MA. Matrix proteins induce neuroblastoma cell differentiation without altering cell growth. Pathobiology 1991;59:316-23.

Gaetano C, Matsumoto K, Thiele CJ. Retinoic acid negatively regulates $p34^{cdc2}$ expression during human neuroblastoma differentiation. Cell Growth Diff 1991;2:487-93.

Sacchi N, Wendtner CM, Raynaud SD, Thiele CJ, Papas TS. Single-cell detection of ETS1 transcripts in both lymphoid and neuroectodermal cells. Oncogene 1991;6(11):2149-54.

Thiele CJ. Biology of pediatric peripheral neuroectodermal tumors. Cancer Met Rev 1991;10:311-9.

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Maggi M, Finetti G, Pioli P, Zurli C, Lanzillotti R, Cioni A, Brocchi A, Camboni MG, Thiele CJ, Serio, M. Somatostatin, VIP, and retinoic acid interactions in a human neuroblastoma cell line. Clin Chem & Enzy Comm 1992, in press.

Thiele CJ, Helman LJ. New insights into the causes of cancer. In: Pizzo PA, Horowitz ME, eds. Pediatric Clinics of North America: Solid Tumors. Philadelphia: WB Saunders, 1991; 38(2):201-21.

Thiele CJ, Gaetano C. Regulation of cell proliferation and differentiation in the human tumor cells: The neuroblastoma cell model. In: Tonini G-P, Thiele CJ, Sansone R, eds. Molecular Genetics of Solid Pediatric Tumors. Basic Concepts and Recent Advances. London: Harwood Academic Publishers, 1991, pp. 207-20.

Thiele CJ, Helman LJ. Differentiating agents, growth factors, and novel molecular approaches to the treatment of pediatric cancer. In: Pizzo PA, Poplack DG eds. Principles and Practice of Pediatric Oncology, 2nd ed. Philadelphia: JB Lippincott Co., 1992, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 CM 06830-22 PB

PERIOD COVERED
October 1, 1991 - September 30, 1992

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<br>Chief, Pediatric Branch | Head, Infectious Disease Section | PB, NCI |
| Others: | Thomas J. Walsh | Senior Investigator | PB, NCI |
| | Karina Butler | Senior Staff Fellow | PB, NCI |
| | Emile (Pim) Brouwers | Visiting Scientist | PB, NCI |
| | Robert Husson | Medical Officer | |

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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 22 | 27 | 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 Infectious Disease Section (IDS) has three major areas of investigation. First are clinical and preclinical studies to improve the diagnosis, management and prevention of infectious complications that occur in immunocompromised hosts. Second is to elucidate the pathophysiology and to improve the treatment of invasive mycoses that are associated with cancer and AIDS. Third is to elucidate the immunopathogenesis and neuropathology that occur in children with AIDS and to develop effective treatment strategies. Studies in each of these three areas combine preclinical research along with comprehensive clinical trials. In each area, considerable progress has been made in advancing the status of knowledge.

Professional Personnel (Continued):

| | | |
|---|---|---|
| Alison Freifeld | Medical Officer | PB, NCI |
| Linda Lewis | Medical Officer | PB, NCI |
| Emmanuel Roilides | Visiting Scientist | PB, NCI |
| Gareth Tudor-Williams | Visiting Scientist | PB, NCI |
| Stephen Chanock | Senior Staff Fellow | PB, NCI |
| James Lee | Senior Staff Fellow | PB, NCI |
| Steven L. Zeichner | Senior Staff Fellow | PB, NCI |
| Brigitta Mueller | Visiting Associate | PB, NCI |
| Shizuko Sei | Visiting Associate | PB, NCI |
| Tore Abrahamsen | Special Volunteer (Norwegian Cancer Society) | PB, NCI |
| Barry Anderson | Clinical Associate | PB, NCI |
| Peter Francis | Medical Staff Fellow | PB, NCI |
| Maria Allende | Visiting Fellow | PB, NCI |
| Miriam Weinberger | Visiting Fellow | PB, NCI |
| Maureen Farley | Nurse Specialist (Research) | PB, NCI |
| Janet Gress | Nurse Specialist (Research) | PB, NCI |
| Freda Jacobsen | Nurse Specialist (Research) | PB, NCI |
| Donna Marchigiana | Nurse Specialist (Research) | PB, NCI |
| Doris Marshall | Nurse Specialist (Research) | PB, NCI |
| Susan Sandelli | Nurse Specialist (Research) | PB, NCI |
| Vicki Stocker | Nurse Specialist (Research) | PB, NCI |
| Mary Wells | Nurse Specialist (Research) | PB, NCI |
| Patricia Whitcomb | Nurse Specialist (Research) | PB, NCI |
| Colleen Higham | Nurse Practitioner | PB, NCI |
| Karen Montrella | Nurse Practitioner | PB, NCI |
| Susan Mertins | Biologist | PB, NCI |
| Robert Schaufele | Biologist | PB, NCI |
| Howard Moss | Psychologist | PB, NCI |
| Pam Wolters | Psychologist | PB, NCI |

Accomplishments and Results:

A. Diagnosis, Management and Prevention of Infectious Complications in Immunocompromised Hosts: Clinical and Preclinical Studies

1. We are currently analyzing the data from this study, which spanned 5 years and includes over 500 episodes of fever and neutropenia. We aim to compare the efficacy of imipenem and ceftazidime within the context of two different sets of endpoint criteria --

those put forth by theImmunocompromised Host Society and those used traditionally by the National Cancer Institute -- in order to determine whether these different criteria will bring forth variations in the final interpretation of data. In addition, we plan to do a comparative cost analysis.

2. The recent development of potent, highly bioavailable oral antibiotics which provide a broad antibacterial spectrum has allowed us to initiate a study exploring the use of such oral agents as empiric therapy for neutropenic patients who become febrile. We have begun a double-blind, placebo-controlled, randomized comparison of oral ciprofloxacin plus amoxicillin/clavulanate with intravenous ceftazidime, our current standard therapy. The study population is limited to febrile neutropenic cancer patients who are clinically stable on presentation and are anticipated to have less than 10 days of neutropenia from the time of their initial fever. The two regimens will be evaluated with regard to their ability to provide safe, effective initial antibiotic therapy for febrile neutropenic patients, and specifically to determine whether the numbers of modifications of each primary antibiotic regimen will vary, reflecting any differences in the antimicrobial coverage provided. If the oral regimen proves to be comparable to standard intravenous therapy, there is the potential for neutropenic patients to be treated as outpatients during all or part of their febrile episode, with a large projected savings in hospitalization costs and an improvement in quality of life for the patients. This study is anticipated to accrue over 200 patients in a 2 to 3 year span, in order to produce statistically evaluable data.

3. In an effort to define the prevalence of common respiratory viral infections in our inpatient population of immunocompromised children, we plan to survey those who present with symptoms by performing a set of respiratory viral cultures on nasal wash material obtained from these patients. By routine nasal wash and culture of all symptomatic children, we will be able to determine the extent to which each of these viruses contributes to morbidity, and to perhaps identify children who were initially symptomatic but who are subsequently chronically shedding these viruses following clinical resolution. Thus far, we have standardized and taught the nasal wash procedure to nursing staff and made available a "nasal wash kit" to facilitate their performing the procedure. We will be prospectively collecting data in the near future.

4. In collaboration with Dr. Stephen Straus in the NIAID, we are exploring the efficacy of BV-Ara-U, a new orally available agent for the treatment of herpes zoster infections. BV-Ara-U is approximately 100 times more potent than acyclovir against herpes zoster *in vitro*, and we are testing its ability to hasten the resolution of localized zoster in immunocompromised patients, as compared to oral acyclovir. Current studies are limited to adult populations. We anticipate having the opportunity to ultimately evaluate the pharmacokinetics and efficacy of this drug in immunocompromised children.

5. The Fever V study also addresses the vital issues of prevention and treatment of invasive fungal infections. In order to reduce the frequency of invasive fungal infections in high-risk cancer patients (those who are considered to have an anticipated duration of neutropenia of > 10 days ["long neutropenia"]), we are investigating the utility of early empirical therapy of fluconazole in a randomized, double blind placebo-controlled clinical trial in children with standardized utilization of empirical amphotericin B for those patients who remain persistently febrile after 7 days of administration of study drug. Based upon the same principles of early empirical antifungal therapy in children, we are also initiating a randomized, double blind placebo-controlled clinical trial of

saperconazole, a broad spectrum antifungal triazole with activity against aspergillosis, in high-risk adults with prolonged granulocytopenia. This study will be preceded initially by a phase I-II trial for assessment of safety, tolerance, and plasma pharmacokinetics. Lipid formulations of amphotericin B are employed in patients with proven invasive fungal infections in phase I-II protocols.

6. To compare the frequency of infectious episodes or other problems occurring with an externalized catheter (Hickman/Broviac) versus a subcutaneously implanted device (Port-a-Cath) in cancer patients we performed a prospective, randomized study in 100 cancer patients (age 5 to 74 years). There were no differences between the two study groups regarding incidence of documented infections, mechanical or thrombotic complications.

7. We have shown that both IFN-gamma and GM-CSF can enhance the fungicidal activity of elutriated monocytes against *Aspergillus fumigatus* hyphae in vitro. The same cytokines can restore the fungicidal activity of dexamethasone-suppressed elutriated monocytes. The role of M-CSF in the fungicidal activity of human elutriated monocytes as well as monocyte-derived macrophages against *Aspergillus* is being studied currently.

8. 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.

9. 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.

10. We have successfully reconstituted the superoxide production capacity of lymphocytes from patients with chronic granulomatous disease by stable transfection of a shuttle vector which expresses the wild type cDNA. We have done this in two forms of CGD, both autosomal recessive, p47-phox and p67-phox deficient.

11. With the development of this new system for whole cell reconstitution, we have constructed a series of mutants to study the structural and functional characteristics of the gene products. Initially, we have deleted the SH3 domains of the p67 and recently, the p47. We have also constructed a series of mutants based on theoretical sites of phosphorylation. Lastly, we have substituted the SH3 domains from homologous regions derived from src-like sequences.

12. We have cloned, sequenced and analyzed the 2.5 kb upstream region of the p47-phox gene, a myeloid specific gene integral to the NADPH-oxidase system. We have identified several sequences that are homologous to a loose consensus sequence for interferon-gamma responsiveness.

13. We have begun to map the cis-elements of the p47-phox 5' upstream region by constructing a series of deletion mutants in reporter gene constructs. Initial studies suggest several regions are necessary for transcription in myeloid cell lines.

14. We characterized the molecular defect of several new patients with p47-phox autosomal recessive CGD and described new mutations.

15. We have been investigating the immunosuppressive effects of recombinant gp41 and gp120 proteins of HIV on several functions of peripheral polymorphonuclear cells, specifically, superoxide generation, chemotaxis, and bactericidal activity.

16. We have finished characterizing the cDNA of a late interferon-gamma responsive element. We have found that this cDNA is expressed hours after stimulation with interferon-gamma and is expressed in cells that contribute to an immunologic response, B and T lymphocytes, myeloid cells, mast cells, megakaryocytes and vascular endothelial cells. We have recently isolated the genomic clones and plan to map the structure of the gene including the start of transcription and intron/exon borders.

17. We have coordinated a study of the bactericidal and fungicidal in vitro activity of polymorphonuclear cells isolated from patients receiving intravenous M-CSF. To complement this, we have looked at the regulation of several myeloid specific genes which are integral to microbicidal activity.

18. We have observed that ascorbic acid is significantly taken up by monocyte cell lines, specifically in response to stimulation with cytokines. We have begun a long-term cloning project to identify and characterize the transporter for ascorbic acid by oocyte injection and the subsequent screening of a constructed cDNA library.

19. Using a monocyte cell line, we have studied the effect of numerous cytokines on intracellular triphosphorylation of AZT.

20. In order to further understand the potential anti-infective effects of human macrophage colony stimulating factor (hM-CSF), We are currently studying the ex vivo antifungal and antibacterial activity, superoxide activity, and phagocytic capacity of elutriated monocytes from surgical oncology patients who are receiving hM-CSF in a phase-1 study.

21. 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.

22. In order to understand the *in vivo* effects of cytokines thought to be important in immunomodulation of host effector functions, we evaluated the bactericidal capacity of elutriated monocytes derived from patients with cancer prior to and during infusion of macrophage colony stimulating factor (MCSF). Analysis of 4 patients indicates that monocytes 3 days post infusion are better able to limit the growth of *S. aureus in vitro* when compared to baseline values.

23. Because increased frequency of bacterial infections was noted in patients treated with the antineoplastic agent suramin, we studied the effects of suramin on neutrophil function in vitro. Pretreatment with suramin impaired the phagocytic and bactericidal

activity of normal neutrophils against *Staphylococcus aureus* but not the oxidative burst and the phagocytosis of *Candida albicans*. Pretreatment with G-CSF appeared to improve the suramin-induced bactericidal defect.

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. Experimental antifungal studies completed in our laboratory are planned in rabbit models of acute, subacute, and chronic disseminated candidiasis provided the scientific rationale for design of an ongoing multicenter clinical trial to test the concept of early empirical antifungal therapy with fluconazole and for the first phase I-II trial of a systemic antifungal triazole (fluconazole) in children.

2. We recently completed the first phase I-II pharmacokinetic study of fluconazole in children with cancer. This study found that fluconazole was safe and well-tolerated but had a shorter mean plasma half-life than that of adults, thus warranting the underscoring the need for separate clinical trials of fluconazole in children with cancer.

3. In order to be able to monitor these patients receiving fluconazole for the emergence of resistance, we completed a multi-center trial for the standardization of methods for in vitro susceptibility of fluconazole.

4. Applying these same concepts in high-risk adults with prolonged granulocytopenia, a phase I-II trial and a randomized, double blind placebo-controlled clinical trial of saperconazole (a broad spectrum antifungal triazole with activity against candidiasis and aspergillosis) is being initiated.

5. We demonstrated that cilofungin, the model compound of the class 1,3-b-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.

6. We also 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.

7. We found 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.

8. 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.

9. 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.

10. Pursuant to these immunohistochemical findings, we developed a new animal model of esophageal and gastrointestinal candidiasis and found that methylprednisolone and non-absorbable antibacterial antibiotics, alone and in combination, increase the intensity and duration of mucosal colonization by *Candida albicans*. Studies further revealed that when cytotoxic chemotherapy due to cytosine arabinoside was administered to such colonized rabbits that invasion of the gastrointestinal tract led to dissemination of Candida to multiple tissues. These *in vivo* systems of esophageal and gastrointestinal candidiasis, which pertain to problems of patients with HIV-infection, as well as granulocytopenia, are currently being studied for investigation of new antifungal strategies, recombinant cytokine therapy, and diagnostic methods.

11. *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 (DTI), we developed models of disseminated and gastrointestinal infection in persistently granulocytopenic rabbits. Antigenemia cross-reactive with cryptococcal polysaccharide (described in cases of DTI) were reproduced. We further demonstrated the immunohistological origin of cryptococcal antigenemia in disseminated *Trichosporon* infection as arising from cell wall and matrix of *Trichosporon beigelii*. DTI developed in rabbits with *T. beigelii* gastrointestinal colonization following cytotoxic chemotherapy.

12. We have also demonstrated in this model 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 *Trichosporon beigelii* infection. Antigenemia declined during the course of antifungal therapy. These studies have afforded new understanding of this and other emerging resistant fungal pathogens.

13. In order to further understand the pathogenesis and molecular epidemiology of *Trichosporon* infection, we further identified and characterized the biochemical and physiological factors that 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. This amplified fragment is now being employed in new strategies for PCR-based diagnostic methods.

14. In view of the complexity of nomenclature of new and emerging invasive fungal infections in immunocompromised patients, the Infectious Diseases Section contributed to an internationally convened consensus panel for the development of uniform terminology in describing disease processes due to these organisms.

15. 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. We further 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.

16. We also developed, characterized, and implemented a rapid enzymatic assay for the detection of d-arabinitol in high risk cancer patients for the early diagnosis and therapeutic monitoring of invasive candidiasis in a multi-center clinical study coordinated by the Pediatric Branch. The data indicate that this enzymatic system may permit the rapid daily monitoring of high risk patients of an entire oncology unit. Correlative studies in our rabbit models of disseminated candidiasis revealed a threshold effect in infected tissues, thus further elucidating the pathophysiological basis of expression of d-arabinitol in serum. These in vivo and clinical studies provide a foundation for a new approach to diagnosis and therapeutic monitoring of this disseminated candidiasis in cancer patients.

17. We have identified several oligonucleotide sequences, including those from C14-demethylase, aspartyl proteinase, and ribosomal RNA, of *Candida* and other species of pathogenic fungi for the development of polymerase chain reaction methods suitable for detection of small quantities of these organisms or their nucleic acids in blood, bronchoalveolar lavage specimens, and other normally sterile body fluids.

18. 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.

19. Little is known about the daily dosage, total dose, duration, and dose intensity of amphotericin B, the mainstay of systemic antifungal therapy. We therefore completed a series of *in vivo* studies to investigate the pharmacodynamic properties both amphotericin B and amphotericin B lipid complex (ABLC) in our rabbit model of chronic disseminated (hepatosplenic) candidiasis. These findings indicate that among the three parameters, duration, daily dosage, and total dose, extended duration and high daily dosage, rather than total dose, are the important determinants of antifungal response by amphotericin B and ABLC.

20. We developed the concept of "reticuloendothelial loading" of ABLC in our rabbit model of chronic disseminated candidiasis, demonstrating [1] that high doses of ABLC (5 mg/kg/d) achieved high concentrations in the reticuloendothelial tissues (liver and spleen),. [2] that these concentrations persisted long after discontinuation of ABLC, [3] and that lesions in liver, spleen, and other tissues continued to resolve after discontinuation of ABLC.

21. These pharmacodynamic studies of ABLC in chronic disseminated (hepatosplenic) candidiasis have led to a nationwide protocol of ABLC, wherein a child with hepatosplenic candidiasis may be referred from anywhere in the United States to receive either 3 or 6 weeks of ABLC, thus potentially reducing the length of treatment of this infection from a approximately 6 months to more than 1 year of conventional therapy to 1 to 2 months.

22. We recently reviewed all cases of hepatic abscesses at the NCI and reported that bacterial and fungal hepatic abscesses tend to be distinguishable on the basis of epidemiologic and radiologic features but that biopsy of suspected fungal lesions is clearly warranted in order to be establish a definitive diagnosis of candidiasis and to exclude other processes, particularly the primary neoplastic disease.

23. In order to better understand the management of catheter-associated fungemia, we studied 155 consecutive episodes of this infection at the NCI. The data indicated that virtually all cases of catheter-associated fungemia in cancer patients were clinically significant, require prompt therapy with amphotericin B, and are optimally managed by removal of the vascular catheter.

24. We recently described the association of pulmonary cryptococcosis simulating metastatic soft tissue sarcoma in children being monitored by CT scans for potential neoplastic relapse. Excisional biopsy of new nodules is warranted to exclude pulmonary cryptococcosis and other opportunistic mycoses causing nodular asymptomatic lung lesions.

25. 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.

26. 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.

27. 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) ABLC. The positive outcome of this patient, who received the first compassionate release of this compound in the United States, led to a nationwide program for compassionate release of this agent in selected mycoses.

28. A subsequent study in our rabbit model of invasive pulmonary aspergillosis investigated a cholesterol sulfate based liposomal formulation of amphotericin B that is under consideration for clinical trials against invasive aspergillosis in the United States. This study clearly demonstrated a highly significant optimal safety and efficacy dosing regimen at 5 mg/kg/d, which may facilitate determination of the appropriate dose in these future trials.

29. We found that the *Aspergillus* metabolite, d-mannitol, as measured by mass spectroscopy-gas-liquid chromatography (MS-GLC) and gas-liquid chromatography-flame ionization detector (GLC-FID) was present in serum and bronchoalveolar lavage specimens obtained from persistently granulocytopenic rabbits with primary pulmonary aspergillosis.

30. 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.

31. 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.

32. To evaluate the potential therapeutic role of interferon-g (IFN-g) in prevention of disseminated candidiasis, we studied the *in vivo* and *ex vivo* effects of recombinant rabbit IFN-g in normal and methylprednisolone-treated rabbits with disseminated infection due to *Candida albicans*. IFN-g enhanced $O_2^-$ production in normal and

corticosteroid-treated rabbits *ex vivo* and augmented *in vivo* clearance of *C. albicans* from reticuloendothelial tissues in normal rabbits, but not in corticosteroid-treated rabbits.

33. The effects 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* were studied and compared with those 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. These findings suggest a potential beneficial role of the two cytokines in host defenses against *Candida.*

34. The effects of G-CSF and IFN-g on the hyphal killing capacity of normal PMNs against nonopsonized hyphae of *C. albicans* were studied and compared with those against non-albicans *Candida* species (*C. tropicalis* and *C. parapsilosis*). G-CSF and IFN-g enhanced the killing of *C. albicans* and *C. parapsilosis* whereas the killing of *C. tropicalis* was marginally affected. This study illustrated the differences in modulatory effects of cytokines that exist among different species of *Candida.*

35. 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. The implications of this finding are important for the prevention and treatment of invasive aspergillosis in immunocompromised cancer patients. In other experiments, we found that both hydrocortisone and dexamethasone inhibit the antihyphal capacity of normal PMNs but pretreatment of the PMNs with G-CSF or IFN-g appear to prevent this steroid-induced defect. The combination of the two cytokines together showed greater effect than each of them separately.

36. Because transfusions of large numbers of elutriated monocytes may be beneficial for neutropenic patients with invasive aspergillosis, we studied the in vitro effects of GM-CSF and IFN-g on the fungicidal activity of human elutriated monocytes against *Aspergillus fumigatus* hyphae. The two cytokines augmented the fungicidal activity of those cells and the combination of them in low concentrations was more effective than each of them in the same concentrations. Similar experiments with dexamethasone-treated monocytes showed that GM-CSF and IFN-g eliminated the dexamethasone-induced suppression of the fungicidal activity. The role of M-CSF in the fungicidal activity of human elutriated monocytes as well as monocyte-derived macrophages against *Aspergillus* is being studied currently.

37. The effects of G-CSF and IFN-γ on the hyphal killing capacity of normal PMNs against nonopsonized hyphae of *C. albicans* were studied and compared with those against non-albicans *Candida* species (*C. tropicalis* and *C. parapsilosis*). G-CSF and IFN-γ enhanced the killing of *C. albicans* and *C. parapsilosis* whereas the killing of *C. tropicalis* was marginally affected. This study illustrated that differences in modulatory effects of cytokines exist among different species of *Candida.*

38. We have shown that both IFN-gamma and GM-CSF can enhance the fungicidal activity of elutriated monocytes against *Aspergillus fumigatus* hyphae in vitro. The same cytokines can restore the fungicidal activity of dexamethasone-suppressed elutriated monocytes. The role of M-CSF in the fungicidal activity of human elutriated monocytes as well as monocyte-derived macrophages against *Aspergillus* is being studied currently.

39. To evaluate the potential therapeutic role of interferon-γ (IFN-γ) in prevention of disseminated candidiasis, we studied the *in vivo* and *ex vivo* effects of recombinant rabbit IFN-γ in normal and methylprednisolone-treated rabbits with disseminated infection due to *Candida albicans*. IFN-γ enhanced $O_2^-$ production in normal and corticosteroid-treated rabbits *ex vivo* and augmented *in vivo* clearance of *C. albicans* from reticuloendothelial tissues in normal rabbits, but not in corticosteroid-treated rabbits. Studies investigating the effects of IFN-γ in the prevention and treatment of primary pulmonary aspergillosis are currently being planned to further establish an understanding of these agents as a guide to potential clinical trials.

40. Pulmonary alveolar macrophages are the primary host defenses against inhaled conidia of *Aspergillus spp*. We are currently studying the fungicidal activity of rabbit alveolar macrophages not only against conidia but also against hyphae of *Aspergillus fumigatus* and the immunomodulatory role of the cytokine M-CSF on these functions. The effects of cytotoxic chemotherapeutic agents on the fungicidal activities of rabbit alveolar macrophages are also being studied.

41. Cyclosporine A is a potent immunosuppressive agent with specific T cell effects. We are currently studying its effects on the oxidative burst and the fungicidal activity of human PMNs, elutriated monocytes and monocyte-derived macrophages against *Aspergillus fumigatus* hyphae in vitro.

42. In collaboration with investigators from the Surgery Branch, we are currently evaluate the effects of in vivo administration of M-CSF to patients with colon cancer on the oxidative burst and the fungicidal activities of elutriated monocytes against blastoconidia and pseudohyphae of *Candida albicans* as well as hyphae of *Aspergillus fumigatus*. The preliminary results show that the cytokine enhances the fungicidal activities of the patients' monocytes as tested ex vivo.

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 nearly 300 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, 32 patients have been enrolled.

3. We initiated a Phase I/II protocol to evaluate the effect of subcutaneously administered granulocyte colony-stimulating factor (G-CSF) in pediatric patients who developed an absolute neutrophil count below $0.8 \times 10^9$/L while receiving AZT despite dosage reductions to 120 mg/m$^2$ every six hours. With doses of G-CSF ranging from 1 to 20 mcg/kg/d, 17 of 19 patients were able to tolerate 120 - 180 mg/m$^2$ of AZT every six hours. We conclude that G-CSF therapy enables patients who have had AZT related neutropenia to receive therapeutic doses of AZT. Since this initial evaluation an additional 13 patients have been treated with G-CSF according to this protocol. We are also studying the effect of human erythropoietin on overcoming AZT-induced anemia. To date 14 patients have been treated and we continue to assess the role and value of this treatment modality.

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, 114 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/m$^2$/day. 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. Median CD4 cell count increased from 218/mm$^3$ at baseline to 327/mm$^3$ at 24 weeks (P=-0.001). Patients with baseline CD4 cell counts greater than 100/mm$^3$ 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. Pancreatitis occurred in eight patients. Optic atrophy has been observed in four patients. Two patients stopped drug altogether and the atrophy has remained stable and has not affected their sight. One patient continued drug and another stopped drug and recently re-started. Both remain stable and their sight remains unaffected. All patients are monitored frequently for this.

5. "A randomized study to evaluate the safety and efficacy of AZT versus ddC/AZT in children with symptomatic HIV infection" had been initiated. This study will be performed in close collaboration with Dr. Jose Santos, Hospital Infantil de Mexico, Mexico City. We plan to enroll most patients in Mexico City. The protocol compares AZT 180 mg/$m^2$ q6 hrs po (Arm A) with AZT 180 mg/$m^2$ q 6 hrs po x 21 days, followed by ddC 0.03 mg/kg q 6 hrs po x 1 week (Arm B). A third arm combines AZT 120 mg/$m^2$ q 6 hrs po with ddC 0.01 mg/kg q 6 hrs po daily (Arm C). We plan to enroll approximately 50 patients into each arm.

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 66 children have been enrolled at doses ranging from 60 to 180 mg/m2 every 6 hours of AZT, and 60 to 180 mg/$m^2$ 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). This combination has been well-tolerated over the full range of doses, without evidence of new short or long term toxicities or enhancement of known toxicities, with over 20 children having been on study for over one year. 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 at all dose levels at both the 12 and 24 week major evaluation points. These data suggest that this combination is active *in vivo*.

7. In April, 1992 we initiated a Phase I/II investigation of 2'-deoxy-3'-thiacytidine (3TC), a new nucleoside analog antiretroviral agent. This agent is most similar to ddC but exhibits less cytotoxicity *in vitro* than either ddC or AZT while retaining good antiviral activity. The study is designed as a two-armed trial of 3TC in previously untreated, relatively well HIV-infected children (Group A) and in more severely ill children who have experienced either disease progression or unacceptable toxicity on other therapeutic regimens (Group B). This dose-escalating study will investigate safety, pharmacokinetic profile, optimal dosing and preliminary efficacy of the drug in pediatric patients. To date similar adult trials have shown an excellent safety profile but have not yet identified either the maximal tolerated dose or the most efficacious dose. At present we have enrolled 6 children in the lowest dose level, 3 in each group and all have tolerated drug well; none have reached the 12 week evaluation point as yet.

8. Within the next 3 months we hope to start a pilot study of the safety, growth and immunological effects of recombinant human insulin-like growth factor (rhIGF-1) and recombinant human growth hormone, nutropin (rhGH) in children with HIV-1 infection and growth retardation.

9. All cases of *Mycobacterium avium-intracellular* infection in our HIV patient population were reviewed and clinical and laboratory characteristics of infected children were determined. Nineteen 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 mean CD4% 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.

10. We completed a Phase I-II dose escalation trial of oral clarithromycin for pediatric patients with disseminated *Mycobacterium avium* complex infection. This study was being conducted in collaboration with the Children's Hospital of Los Angeles. A total of 25 patients were enrolled at doses of 7.5, 15, and 30 mg/kg/day of clarithromycin. A decrease in low frequency hearing in a single patient is the only significant possible toxicity observed to date. Preliminary analysis indicates that clarithromycin suspension does not appear to affect AZT or ddI pharmacokinetics in children.

11. Short term improvements in energy levels, appetite and decreased fever were observed in most patients, however recurrence of symptoms has occurred after several weeks in most patients at all dose levels. Significant decreases in mycobacteremia were observed only at the highest dose level. Tolerance and toxicities of this agent, as well as the durability of clinical response, remain to be determined. The effective management of HIV-infected children with *Mycobacterium avium* infection will require multi-drug regiments, earlier treatment or prophylaxis, and prevention through better immune preservation. Analysis of quantitative mycobacterial cultures, clarithromycin susceptibility patterns and correlation with symptoms is currently underway.

12. 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. Small decreases in sensitivity to ddI in HIV from patients being treated with this agent, however, were observed. Selective PCR was used to demonstrate the presence of a known mutation related to AZT resistance in 5 to 7 patients who received long-term AZT/ddC therapy, and a known mutation related to ddI resistance in 5 of 7 patients who received long-term ddI therapy. We have begun to use the selective PCR technique to evaluate the occurrence of resistance mutations in HIV from patients receiving simultaneous combination therapy with AZT and ddI.

13. We have attempted to assess the efficacy of antiretroviral therapy by following the changes of viral load in blood as determined by quantitative viral culture using 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. Currently, we are planning to employ quantitative polymerase chain reaction (PCR) technology to determine the level of plasma viremia in these patients.

14. Using PCR technology, we have also quantitatively evaluated the amount of HIV viral DNA in tissues obtained from autopsy of 14 patients with AIDS or ARC. Among 13 organs tested, lymph nodes contained the highest numbers of HIV-1 DNA (copies/mg

total DNA) followed by spleen, colon, skin, lung, etc. We then examined the level of HIV-1 RNA and DNA in PBMC's and biopsied lymphoid tissues by PCR in combination with reverse transcription (RNA PCR). We found that the numbers of HIV RNA were substantially greater than those of HIV DNA in lymphoid tissues. These data suggested that lymphoid tissues represent one of the main sites for active infection as well as replication of HIV-1 in patients with AIDS or ARC.

15. In addition, we have developed a quantitative HIV-1 RNA assay for small (4 ml) whole blood samples using a direct competitive PCR strategy. This detects both virion RNA and cellular mRNA. The utility of this for monitoring viral burden will be compared to p24 antigen detection (standard and with immune-complex disruption), age-adjusted CD4 counts and the PCR plasma viremia assay.

16. 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.

17. 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.

18. 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.

19. We produced the first comprehensive, fine-scale map of regulatory sequences present in the HIV LTR. This map identified several previously unknown LTR regulatory sequences active in unstimulated and stimulated lymphocytic cells. The identification of these additional regulatory sites should provide useful insights into basic HIV biology as well as suggest additional targets for possible future therapies.

20. We showed that some of the well-characterized HIV LTR regulatory sequences like NF-kB and TATA behave differently in unstimulated, stimulated, and particularly in tat-expressing lymphocytic cells. For example, we showed that the two NF-kB sites are not equivalent in that the 3' site appears to be functionally much more important than the 5' site in the presence of tat.

21. We made a contribution to understanding the function of tat by showing that the sequences close to the transcription start site are required for maximal expression in the presence of tat, while sequences 5' to the NF-kB sites provide no contribution to wild type LTR activity in the presence of tat, providing support for the "flip-back" model for tat-mediated transactivation.

22. We identified additional sequences at the extreme 5' end of the HIV LTR that are required for maximal expression and transcription complex formation in HeLa cells.

23. We collaborated in studies that identified sequences in the R region of the HIV LTR between the TATA element and the transcription start site, 5' to the AAUAAA, that are required for wild type levels of HIV RNA polyadenylation. This result helps to explain why HIV produces a full-length RNA instead of the very short transcript that would be produced if the polyadenylation signal in the 5' LTR were active.

24. HIV makes use of the transcriptional apparatus of its host cell. Since the regulation of gene expression changes dramatically during development and differentiation, largely as the result of changes in the regulation of transcription, we performed experiments designed to examine the effects of differentiation-dependent changes in the regulation of cellular gene expression on HIV gene expression.

25. Through these experiments we hoped to understand some of the unique features of HIV infection in developing infants and children. We used our comprehensive collection of HIV LTR linker-scanning mutants in a human embryonal carcinoma (EC) cell model to look for LTR regulatory elements that function differently during the course of differentiation. We found that the HIV LTR contains several previously uncharacterized regulatory sequences active in the differentiated EC cells, but not in lymphocytic cells. Some of the regulatory sequences active in the differentiated EC cells behave differently depending on the cell's state of differentiation. These differentiation-dependent regulatory sequences include some previously well-characterized sequences like NF-kB, as well as several previously unknown sequences. The existence of regulatory sequences active in differentiated cells, but not in lymphocytic cells suggests that the ability of the virus to replicate in cells other than lymphocytes and monocytes may be important for virus survival and transmission. Such findings also suggest that LTR sequences, as well as the better characterized envelope sequences, may contribute to HIV tropism. Finally, the existence of differentiation-responsive HIV LTR regulatory elements may help to explain some of the distinctive features of pediatric AIDS.

26. We have demonstrated a relation between levels of CSF Quinolinic acid (an excitatory neurotoxin) and neuropsychological function: CSF Quinolinic Acid may function as a marker for HIV associated encephalopathy. In addition, we observed decrease in CSF Quinolinic Acid with a concurrent increase in general cognitive function after anti-retroviral treatment: thus CSF Quinolinic Acid may function as a measure of therapeutic response.

27. We have demonstrated the specific vulnerability of expressive language to the effects of HIV infection in children. A significant discrepancy between expressive and receptive language was found both in encephalopathic and non-encephalopathic children. The

magnitude of this discrepancy was related to the degree of CT-brain scan abnormality in the encephalopathic children. In addition, poorer immune status was associated with more impaired language functioning.

28. We have demonstrated that the level of psychological functioning in symptomatic HIV infected children prior to anti-retroviral treatment is related to the integrity of the CNS as visualized by CT-brain scans. CT brain-scans showed a lack of lateralized abnormalities, which seems to support the hypothesis, based on an analysis of intellectual profiles, that HIV encephalopathy in children has a global, stage like character, with stepwise decline in function.

29. Bacterial infections are 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.

30. Invasive fungal infections and in particular invasive aspergillosis have emerged as serious infections in HIV-infected patients. PMNs from HIV-infected children with low CD4 count (<25% of normal median) were shown to have significantly impaired fungicidal capacity against *Aspergillus fumigatus* hyphae. This defect was acquired by normal PMNs after in vitro incubation with sera from HIV-infected patients as compared to sera from healthy donors. In vitro incubation of defective PMNs with G-CSF improved the fungicidal impairment. These findings may explain the increased susceptibility of these patients to invasive aspergillosis, and suggest a potential therapeutic role of G-CSF and potentially other cytokines.

31. In studies of human monocyte-derived macrophages from children with HIV infection we found that these cells do not ingest or inhibit the germination of *Aspergillus* conidia as well as normal controls. This finding suggests that HIV-infected patients are at increased risk to develop invasive aspergillosis as compared to control healthy population.

32. In order to understand the pathogenesis of recurrent bacterial infections in children infected with HIV, we are investigating the effects of recombinant HIV proteins (gp120) on normal neutrophil function. The results indicate that gp41 inhibits the neutrophil migration and bactericidal activities. This mimics abnormalities previously observed in the patient population. When gp120 is substituted in these *in vitro* assays, the reverse effects are found, i.e., enhanced directed cell migration and antimicrobial effects against *Staphylococcus aureus*. Preliminary findings suggest that important serum components such as immunoglobulin and complement may effect enhanced bactericidal activities. These results may have implications on the role of the dysgammaglobulinemia and its contribution in establishing serious bacterial infections. If subunit vaccines enter clinical trials, these results suggest that short term neutrophil defects may occur.

33. To further establish the mechanism of action of HIV proteins on neutrophil function, normal neutrophils were incubated with HIV recombinant proteins, gp41 and gp120 during stimulation (with standard activators at maximal concentration) in *in vitro* assay

that measures extracellular release of superoxide, a presumed toxic antimicrobial agent. As predicted by the previous findings, gp41 suppressed release of toxic oxygen products; gp120 enhanced the release. The peptides alone had similarly altered spontaneous release of superoxide.

34. To determine if the *in vitro* neutrophil defects could be replicated with patient sera, several dilutions of patient sera were preincubated with normal neutrophils prior to evaluation in the functional assays. Reduced bactericidal activities were noted (5/6 sera tested). If a monoclonal antibody to epitopes on gp41 was included in the patient sera, a return to normal function was noted. Most patient sera (9/13) limited direct migration of normal neutrophils.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 CM 06840-17 PB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 |
| Others: | | | |
| | F. Balis | Senior Investigator | PB, NCI |
| | P. Adamson | General Fellow | PB, NCI |
| | S. Berg | Biotechnology Fellow | PB, NCI |
| | B. Mueller | Clinical Associate | PB, NCI |

COOPERATING UNITS *(if any)*

Medicine Branch, NCI (A. Fojo, K. Cowan, L. Neckers); Navy, NCI (L. Kirsch); Children's Cancer Study Group (G. Reaman); Children's Hospital of Philadelphia (C. Felix)

LAB/BRANCH Pediatric Branch

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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 subsequent, high risk protocol was devised to improve the prognosis for these and other poor risk patients. The results of that study indicated that the therapy was highly effective in preventing both systemic and central nervous system relapses while avoiding the use of cranial radiation. A new protocol for high risk patients is attempting to demonstrate that dose intensification, accomplished through the concomitant use of G-CSF, will improve the prognosis for this patient group. In this study molecular analysis (pcr) will be performed to evaluate minimal residual disease. A major, multi-institutional pharmacologic monitoring protocol which has studied the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL has recently been completed and the results are being analyzed. Study of the clinical pharmacology of the thiopurines, mercaptopurine and thioguanine, has led to the development of new therapeutic approaches with these agents. Several new intrathecal chemotherapy approaches have been developed to provide new therapy for patients with central nervous system leukemia. Collaborative studies are evaluating the role of the P53 gene, a candidate tumor suppressor gene, 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 65% for the entire study group. With a median duration on study of approximately 10 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 demonstrated that alternative CNS preventive therapy is feasible and as efficacious as cranial radiation and IT MTX and served to focus attention on the importance of avoiding neurotoxic regimens using cranial radiation. This study led to the development of our more recent current clinical trials discussed below.

2. NCI 83-P/CCG 134P

The major aim of this pilot protocol was to demonstrate that high risk patients can be effectively treated on a regimen that uses CNS preventive therapy devoid of cranial radiation. Overall, 120 patients were entered on study; 96% achieved complete remission. With a median potential followup of 5.3 years, the event free survival (at 3 years) is between 55 and 60%. The occurrence of isolated CNS relapse in only three patients

indicates 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 randomized average risk patients to one of two forms of CNS preventive therapy - either high dose methotrexate infusions or intrathecal methotrexate alone. One hundred seventy-six patients were randomized on study. With a median potential followup of 5.5 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. NCI-CCG 1911: 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).

This new ALL treatment protocol was inititiated in January 1992. 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 new protocol, we are evaluating 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 is being carried out collaboratively with selected institutions of the Childrens Cancer Study Group (CCG).

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 monitoring of oral 6-MP and methotrexate with relapse rate and remission duration in children with low and average risk ALL treated at participating CCSG institutions. Plasma and RBC samples have been collected from 89 patients and analyzed for both 6-MP and MTX. Each patient was studied on up to four separate occasions during the course of maintenance therapy. The pharmacokinetic data has been entered into a database to which the clinical data will also be added. We have analyzed the "population" pharmacokinetics of these two agents, and have confirmed the wide inter-patient variability in plasma MTX and 6-MP concentrations following oral administration under standardized conditions. We 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 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. Limited sampling strategies will be designed for MTX to simplify monitoring of the AUC. 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. Now that patient accrual is complete and sufficient follow-up is available, the final pharmacokinetic analysis and clinical correlations will be completed in the next few months.

2. Laboratory Studies of 6-Mercaptopurine

Investigations into the intracellular metabolism of thiopurines have revealed a wide array of effects on purine metabolism, but the primary mechanism of cytotoxicity for both 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) is best correlated with incorporation of drug as the thioguanylate metabolite into DNA. Recently, several investigators studying murine leukemia cell lines have reported observing paradoxical cytotoxicity with thiopurines, defined as a decrease in cytotoxicity with increasing drug concentration. This paradoxical cytotoxicity of thiopurines has usually been attributed to dose-dependent perturbations in the cell cycle. A more plausible mechanism is dose–dependent intracellular desulfuration of thiopurine into the naturally occurring purine base. A series of investigations were performed to determine whether 6-MP paradoxical cytotoxicity occurred in human leukemia cell lines, and to determine whether dose-dependent generation of hypoxanthine underlies this phenomenon. Paradoxical cytotoxicity was observed in two human leukemia cell lines, but only when 6-MP concentrations exceeded 100 μM. Hypoxanthine was shown to antagonize the cytotoxicity of 6-MP in a dose-dependent fashion, with concentrations as low as 10 μM partially reversing the effects of 500 μM 6-MP. Hypoxanthine concentrations in cell culture media were found to be in this concentration range when human leukemia cell lines were exposed to high concentrations of 6-MP. The extent of incorporation of thiol versus non-thiol derivatives of 6-MP into DNA was analyzed by performing parallel

experiments with $^{14}C$ and $^{35}S$ labeled drug. With 5 μM concentrations of 6-MP, a large fraction of drug was incorporated into DNA with its thiol group intact. With increasing drug concentrations however, the degree of thionucleotide incorporation remained essentially unchanged, but the amount incorporated as the desulfurated metabolite (adenine or guanine) increased, such that with 500 μM 6-MP concentration less than 10% of the drug incorporated did so with the thiol group intact. Cell cycle data obtained indicated that the observed perturbations in cell cycle were not the cause of the paradox, but reflected the relative amounts of thiopurine nucleotide and hypoxanthine formed at varying concentrations of 6-MP. These results suggest that thiopurines may be vulnerable to a unique mechanism of detoxification, in which a malignant cell can eliminate a cytotoxic drug and simultaneously generate a potent "self-rescue" agent.

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 have investigatied 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. Studies using PCR techniques have demonstrated that 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 are being addressed using similar methodology in our 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. 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. In a continuing collaborative study with Dr. C. Felix bone marrow and peripheral blood lymphoblasts of patients with B-cell precursor ALL, have been studied to evaluate the status of the p53 gene. The data obtained to date 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 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. For more details of these molecular studies see the related references in the bibliography.

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. A Phase I study evaluatuing a novel approach with thioguanine is discussed below. For a more detailed listing and discussion of this and other new agent studies the reader is referred to the *Clinical Pharmacology Project Report.*

Phase I Trial of Intravenous 6-Thioguanine

6-thioguanine (6-TG) has been used for the treatment of cancer for more than 30 years, but has undergone only limited testing in pediatric cancer patients. 6-TG requires fewer metabolic activating steps to produce cytotoxicity than does the more widely used thiopurine, 6-MP. *In vitro* cytotoxicity studies with human lymphoblastic leukemia cells and cell lines were performed to compare the concentrations and duration of exposure of 6-MP and 6-TG required for cytotoxicity. Cytotoxicity was assessed in 3 human leukemia cell lines. These studies indicated that 6-TG has a distinct cytocidal advantage over 6-MP against lymphoblastic leukemia cells. With 6-TG, cytotoxicity occurred with 10-fold lower concentrations and was achieved with a considerably shorter duration of exposure. Review of published studies indicated that 6-TG has not previously been administered on a schedule which achieves and maintains the desired cytocidal plasma concentrations. Therefore, a pediatric phase I trial is being performed to determine the rate of continuous IV infusion of 6-TG required to achieve steady state plasma drug concentrations of approximately 1 μM (the maximum cytotoxic concentration). Following determination of the required dose rate, the maximum tolerated duration of exposure will be determined by increasing the duration of infusion in cohorts of patients by increments of 12 hours, starting with a 24 hour infusion.

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*..

3. Studies of 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. We are performing a pharnacokinetic and pharmacodynamic study of PEG-L-Aparaginase which is being administered to patients with and without demonstrated hypersensitivity to native L-asparaginase. The aim of this study is to determine whether differences exist in the half-life of PEG-L-Aparaginase among these different patient populations and to determine the optimal schedule(s) of PEG-L-Aparaginase administration.

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 has been 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 06880-15 PB

PERIOD COVERED

October 1, 1991, to September 30, 1992

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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| TOTAL STAFF 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. Preclinical, Phase I andpharmacokinetic studies of a variety of new agents including All-trans retinoic acid, Topotecan, Pyrazoloacridine, Taxotere, Taxol, Piroxantrone, Cyclopentenylcytosine (CPE-C) and Amifostine are in progress. In addition, Phase I, Phase II and correlative pharmacologic studies evaluating new approaches or new formulations of older chemotherapeutic agents including thiotepa, intravenous 6-thioguanine and PEG-L-asparaginase are being pursued. Phase II studies of agents previously studied both preclinically and in Phase I trials in this section are also in progress (e.g.Fazarabine, ICRF-187). Several collaborative studies in which this section provides pharmacologic support for important clinical studies in other COP Branches are being pursued. 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. Clinical studies of intrathecal AZQ, intrathecal 6-MP, and intrathecal mafosfamide, all novel 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. Two new trials evaluating the role of systemically and intrathecally administered Carboxypeptidase-$G_2$ have begun. As part of the Pediatric Branch AIDS research effort, the PETS Section is studying the clinical pharmacology of antiretroviral agents in children. This effort 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 pediatric cancers. 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 also participate in the design of clinical trials of antiretroviral agents in children and perform 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 of Thiotepa

We 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 clinical Phase I study in pediatric cancer patients demonstrated that intravenous thiotepa can be safely administered at more than a twofold higher dose than that previously recommended. Based on these findings we initiated a Phase II trial of thiotepa in pediatric solid tumors in conjunction with the CCSG (see #10). In addition, our pre-clinical studies and Phase I clinical trial demonstrated that substantial amounts of both thiotepa and its metabolite Tepa are present in the CNS following intravenous administrationand suggesting that systemically administered thiotepa may be a valuable agent for the treatment of CNS malignancies. *In vitro* studies demonstrated activity of thiotepa and tepa against human medulloblastoma and glioma cell lines at drug concentrations achievable at the dose identified as safe ($65mg/m^2$) in our phase I trial. Based on these findings we performed a multi-institutional Phase II trial of intravenous Thiotepa in pediatric brain tumors.60 patients were entered on this study in a variety of pathologic categories. Partial responses were noted in 3/13 patients with PNET CNS tumors and stable disease was observed in 9 patients in the remaining disease categories. These data confirm the activity of thiotepa against pediatric brain tumors. Our laboratory data and this clinical experience, however, suggest that the clinical effectiveness of thiotepa might be optimized if this agent is used at significantly higher doses ( a strategy potentially feasible using peripheral stem cell transfusions or hematopoetic growth factors). Future clinical trials of this type are contemplated.

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 $t_{1/2}a$ of 8.8 min, a mean t1/2b 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 this agent. Based on these findings an adult phase I study which incorporates a pharmacologically directed dose escalation strategy has been initiated. Preliminary pharmacokinetic results from this adult phase I study are similar to those predicted from our preclinical pharmacokinetic studies in our nonhuman primate model. A pharmacologically guided pediatric phase I trial has been approved by the IRB and will be initiated as soon as biologic activity has been observed in the adult phase I study.

Mechanism of Resistance to Cyclopentenyl Cytosine (CPE-C) in Molt-4 Lymphoblasts

Cyclopentenyl cytosine (CPE-C), a carbocyclic analogue of cytidine, has preclinical antineoplastic activity against ara-C resistant murine leukemias and a broad spectrum of human tumor xenografts. CPE-C is a prodrug which requires intracellular phosphorylation to CPE-CTP which depletes endogenous CTP pools. The initial step in this activation process is catalyzed by uridine/cytidine kinase. We studied the mechanism of resistance to CPE-C 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 parent Molt-4 cells (Molt-4$^{WT}$) was 0.5 μM after a 24 hr drug exposure. In contrast, cytotoxicity was not observed at concentrations as high as 100 μM in the Molt-4$^R$ cells. Following a brief exposure to 1 μM CPE-C, equivalent amounts of parent drug could be detected intracellularly in the resistant and sensitive cell lines. However, CPE-CTP formation was markedly reduced in the resistant cell line. Measurement of the activity of anabolic and catabolic enzymes in the Molt-4$^{WT}$ and Molt-4$^R$ cells revealed equivalent activities of alkaline and acid phosphatases as well as cytidine and dCMP deaminase but there was a significant reduction in uridine/cytidine kinase activity in Molt-4$^R$ cells. Endogenous ribonucleotide

pools and CPE-CTP pools were measured in the absence and presence of CPE-C. CTP pools were markedly reduced in Molt-$4^{WT}$ cells following exposure to CPE-C. However, CTP pools in Molt-$4^{R}$ cells exposed to 100 μM CPE-C were 2 times greater than in the untreated Molt-$4^{WT}$ cells. At high concentrations of CPE-C (10 and 100 μM), Molt-$4^{R}$ cells were able to generate amounts of CPE-CTP equivalent to that seen in Molt-$4^{WT}$ cells exposed to 1 μM CPE-C (a cytotoxic concentration of drug in Molt-$4^{WT}$ cells), but no cytotoxic effect was seen in in Molt-$4^{R}$ cells. Therefore, in addition to decreased uridine/cytidine kinase activity in this Molt-4 leukemia cell line, a second mechanism of resistance that is the result of alterations in CTP synthetase activity also appears to be operative. Elucidation of the mechanism of resistance *in vitro* may provide insight into the mechanism of action of the drug and potential mechanisms of resistance *in vivo*.

Modulation of the Cytotoxic Effect of Cyclopentenyl Cytosine by Its Primary Metabolite, Cyclopentenyl Uridine

Cyclopentenyl cytosine (CPE-C), a synthetic cytidine analogue with significant preclinical antitumor activity against both solid tumor xenografts and ara-C resistant murine leukemia cell lines, will soon enter phase I clinical trials. Unlike ara-C which is activated by deoxycytidine kinase, the enzyme responsible for the phosphorylation of CPE-C is uridine/cytidine kinase. Preclinical pharmacokinetic studies of CPE-C in nonhuman primates revealed that the primary route of elimination in this species was deamination to CPE-U, an inhibitor of uridine/cytidine kinase. Since CPE-C is likely to be deaminated in humans, we investigated the modulating effect of CPE-U on the *in vitro* cytotoxicity of CPE-C in Molt-4 lymphoblasts. Concurrent exposure of cells to cytotoxic concentrations of CPE-C and 50 μM CPE-U resulted in rescue of 50% of cells and exposure to CPE-U concentrations in excess of 100 μM resulted in the rescue of greater than 90% of cells. Progressive attenuation of the rescue effect was observed with delayed administration of CPE-U and no cells were rescued when addition of CPE-C was delayed for more than 2 hr. At the intracellular level it was observed that the formation of the cytotoxic metabolite, CPE-CTP was blocked by increasing concentrations of CPE-U presumably secondary to inhibition of uridine/cytidine kinase by CPE-U. Although CPE-U can modulate the cytotoxic effects of CPE-C *in vitro*, the minimum CPE-U levels that are required for modulation coupled with the available preclinical pharmacokinetic data from nonhuman primates suggests that this modulation is not likely to impact on the anti-tumor effects of CPE-C in humans.

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.

Phase I trial of Pyrazoloacridine (PZA )

Based on the preclinical pharmacology studies decribed above, a Phase I trial of PZA in children is underway. In the first part of this trial, the maximum tolerated dose of PZA given as a 1 hour infusion will be determined. Subsequently, the MTD of a 24 hour infusion will determined.

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

Although the potential for piroxantrone-related neurologic toxicity is a concern, the CSF penetration of piroxantrone in man is unknown. 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 was used to determine the plasma and CSF pharmacokinetics of this agent following intravenous administration. In this model, the CSF penetration was poor despite plasma concentratons in the predicted range. 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. Six animals have been studied. The PEG-L-asparaginase plasma half-life was $6 \pm 1$ days. In all 6, plasma L-asparagine was undetectable for at least two weeks after PEG-L-asparaginase administration and remained undetectable as long as PEG-L-asparaginase levels were > 0.1 IU/ml. CSF L-asparagine depletion, however, was more variable and did not correlate directly with the plasma PEG-L-asparaginase concentration. There was no difference in PEG-L-

asparaginase pharmacokinetics or L-asparagine depletion between the two enzyme preparations.

Pharmacokinetic and Pharmacodynamic study of PEG-L-Aparaginase

A major obstacle to L-asaparaginase therapy is the development, in a significant number of patients of allergic reactions to this foreign protein. The PEG-modified enzyme is less immunogenic and has a longer plasma half-life than the native enzyme. We are performing a collaborative, multi-institutional pharnacokinetic and pharmacodynamic study of PEG-L-Aparaginase which is being administered to patients with and without demonstrated hypersensitivity to native L-asparaginase. The aim of this study is to determine whether differences exist in the half-life of PEG-L-Aparaginase among these different patient populations and to determine the optimal schedule(s) of PEG-L-Aparaginase administration.

6. Preclinical and Clinical Studies with All-*trans* Retinoic Acid (all-*trans*-RA)

Phase I Trial of All-*trans* Retinoic Acid

All-*trans*-RA is an agent which has demonstrated activity *in vitro* as a tumor differentiating agent. *In vivo* all-*trans*-RA has demonstrated significant activity in patients with acute promyelocytic leukemia. Whereas there have been several studies of the pharmacokinetics of 13-*cis*-RA, there is little information regarding the pharmacokinetics of all-*trans*-RA in humans and none in children. We recently initiated and subsequently completed a Phase 1 trial of all-*trans*-RA in pediatric patients with refractory malignancies. All-*trans*-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$^2$. Pseudotumor cerebri was the dose limiting toxicity. Complete responses were observed in two patients with multiply relapsed acute promyelocytic leukemia. Pharmacokinetic studies demonstrated that the plasma half-life of all-*trans*-RA was approximately 45 minutes, significantly shorter than the 12 to 24 hour half-life for 13-*cis*-RA. Furthermore, in the seven patients studied on day 1 and again on day 28 of therapy, a marked decrease in the plasma AUC of all-*trans*-RA was observed. The metabolism of all-*trans*-RA appears to differ from that of 13-*cis*-RA, as the 4-oxo-all-*trans*-RA metabolite was detected only in low concentrations, suggesting that other metabolic pathways must be active.

Pharmacokinetic Studies of Intravenous All-*trans*-RA in the Non-human Primate

In order to gain further insight into the pharmacokinetics and metabolism of all-*trans*-RA, an intravenous form of the drug was prepared and studied in the Rhesus monkey model. Administration of the drug intravenously eliminated the variability in bioavailability associated with oral administration.Groups of three animals received IV bolus doses of all-*trans*-RA of 20, 50 and 100 mg/m$^2$. Plasma disappearance of all-*trans*-RA was characterized

by 3 distinct phases: a brief, initial exponential decline, followed by a plateau in the disappearance curve, the duration of which was dose-dependent, and finally a terminal exponential decay. This profile is consistent with a capacity-limited (saturable) elimination process. The first-order (terminal) half-life for all-*trans*-RA averaged 19 minutes, and the mean clearances were 77, 52 and 59 ml/min for the 20, 50 and 100 mg/m$^2$ dose groups, respectively. The mean $K_m$ for the capacity-limited process was 3.2 ± 1.9 μM. Peak plasma concentrations following oral administration of 45 mg/m$^2$ of all-*trans*-RA in humans approach this concentration, suggesting that the dose-dependent pharmacokinetics of all-*trans*-RA described here may occur within the clinically used dosage range.

Time Course of Induction of All-*trans*-RA metabolism

The decrease in plasma concentrations of all-*trans*-RA observed with chronic dosing in the pediatric phase I trial could be related to induction of catabolic enzymes. The time course of this change in the pharmacokinetic profile of all-*trans*-RA was therefore studied in the Rhesus monkey by administering 8 consecutive daily 50 mg/m$^2$ all-*trans*-RA IV doses. An increase in plasma clearance was noted as early as day 3 of drug administration, with further increases by days 5 and 8. Up-regulation of tissue CRABP (cytosolic retinoic acid binding protein) levels, determined from serial skin punch-biopsies, was simultaneously studied. CRABP may act as an intracellular sink for all-*trans*-RA and limit its ability to bind nuclear receptors. CRABP was rapidly induced and peaked at 1119 ± 88 fmol RA/mg protein (three-fold baseline value) within 3 days of chronic all-*trans*-RA administration. Animals were then studied following a 7 day rest period without all-*trans*-RA. Plasma drug clearance returned to near baseline values, while tissue CRABP levels, although diminished, remained approximately two-fold elevated at 813 ± 264 fmol RA/mg protein. These studies demonstrate that all-*trans*-RA is metabolized by an inducible capacity-limited process, which accounts for decreasing plasma drug concentrations observed following chronic administration of all-*trans*-RA. The more rapidly induced changes in CRABP levels may serve to further diminish the amount of all-*trans*-RA that is available to bind nuclear receptors. An intermittent schedule for all-*trans*-RA administration that allows for recovery of metabolism and down-regulation of CRABP may prove more effective than current regimens of daily drug administration.

Phase I Trial of all-*trans*-RA and Interferon-α2a (IFN-α2a)

A body of preclinical data has provided a strong rationale for evaluating the combination of IFN-α with retinoic acid. The two drugs have different mechanisms of action and, when used in combination, show enhanced activity in both adult and pediatric tumor cell lines. Two recent clinical trials, using 13-*cis*-RA and IFN-α2a, in adult patients with heavily pretreated squamous cell carcinoma of the skin and of the cervix, demonstrated response rates of 68 and 50%, respectively. Among the retinoids currently available for clinical study, all-*trans*-retinoic acid (all-*trans*-RA) appears to be the most potent inducer of cellular differentiation. In the pediatric phase I trial of all-*trans*-RA, dose limiting toxicity was observed during the first week of therapy in patients treated at the 80 mg/m$^2$/day dose level. Pharmacokinetic studies have demonstrated that the metabolism of

all-*trans*-RA is rapidly induced, occurring as soon as after two doses of drug. The preclinical studies in the non-human primate suggest that metabolism can return toward baseline levels if all-*trans*-RA is administered on an intermittent schedule. The current study will attempt to maintain more consistent plasma drug concentrations over time by administering drug t.i.d. on an intermittent schedule of three consecutive days per week, repeated weekly. The combination of the antiproliferative and differentiation inducing effect of retinoids together with the antiproliferative, immunostimulatory and differentiation-potentiating effects of IFN-α warrant clinical investigation of this combination for the treatment of refractory pediatric malignancies.

7. Preclinical and Clinical Studies of Topotecan

Phase I Study of Topotecan

Topotecan [(S)-9-Dimethylaminomethyl-10-hydroxycamptothecin hydrochloride, SK&F 104864-A, NSC 609699] a water soluble analog of camptothecin, inhibits topoisomerase I, the enzyme that relaxes supercoiled DNA by creating transient single strand breaks through which another DNA strand can pass during DNA replication, RNA transcription, and other DNA functions. These enzyme-bridged breaks are then resealed by the topoisomerase I enzyme. Topoisomerase I inhibitors, like topotecan, stabilize the covalent complex between topoisomerase I and DNA resulting in enzyme-linked DNA single-strand breaks that cannot be religated in the presence of drug, leading to cytotoxicity.

During the late 1960's phase I trials of the parent drug sodium camptothecin demonstrated objective antineoplastic activity against gastrointestinal adenocarcinoma, melanoma, non small cell lung cancer and acute myelogenous leukemia. However, further clinical development of sodium camptothecin was limited due to unpredictable and severe myelosuppression, gastrointestinal toxicity and hemorrhagic cystitis. The subsequent elucidation of the novel mechanism of action of camptothecin and the development of water soluble analogs, such as topotecan, rekindled clinical interest in this class of compounds.

We recently completed a phase I and pharmacokinetic study in pediatric patients with refractory malignancies to determine the maximum tolerated dose and dose limiting toxicities, the incidence and severity of other toxicities, and the pharmacokinetics of topotecan in children. Twenty-nine patients received 42 courses of intravenous topotecan administered as a 24 hr continuous infusion every 21 days at doses ranging from 2.0 $mg/m^2$ to 7.5 $mg/m^2$. Dose-related hematologic toxicity was the dose-limiting toxicity. Leukopenia, neutropenia, and thrombocytopenia occurred sporadically at the 3.0 to 5.5 $mg/m^2$ dose levels, but at 7.5 $mg/m^2$ 4 of 5 patients experienced dose-limiting thrombocytopenia (grade 4) and 2 of 5 had dose-limiting neutropenia (grade 4). No other dose-limiting toxicities were observed. Nausea and vomiting were mild and occurred in less than 20% and 10% of patients, respectively. Grade 2 hematuria occurred in 1 patient. No objective responses were observed. Pharmacokinetic studies revealed a linear relationship between the steady-state topotecan concentration and dose. The mean steady state concentration at the MTD was $18.2 \pm 3.7$ nmol/L and the $CL_{TB}$ was $28.3 \pm 6.5$ $L/hr \cdot m^2$. Elimination was bi-exponential with a $t_{1/2}a$ of $14.4 \pm 1.8$ min and a $t_{1/2}b$ of $2.9 \pm 1.1$

hr. In conclusion, thrombocytopenia is the dose-limiting toxicity of topotecan in children receiving topotecan administered as a 24 hr continuous infusion. The recommended starting dose for phase II pediatric trials is 5.5 mg/m$^2$. Although this dose exceeds the MTD identified in heavily pre-treated adult patients receiving topotecan on the same schedule, it is less than the MTD for minimally pretreated adult patients. Therefore, dose escalation to 7.5 mg/m$^2$ in phase II pediatric trials should be considered for patients that tolerate treatment well at the 5.5 mg/m$^2$ dose.

Preclinical Studies of Topotecan

We studied the plasma and CSF pharmacokinetics of topotecan following intravenous administration of topotecan (10 mg/m$^2$ over 10 minutes) in 3 nonhuman primates with indwelling Ommaya reservoirs. Plasma pharmacokinetic parameters were similar to those previously reported from human clinical trials. The mean $Cl_{TB}$ was 77.3 $\pm$ 15.7 L/hr/m$^2$, the VDss was 88.6 $\pm$ 33.2 L, and the $t_{1/2}a$ and $t_{1/2}b$ were 21.6 $\pm$ 4.8 min and 1.3 $\pm$ .1 hours, respectively. The mean CSF:plasma ratio was 0.32. The excellent CNS penetration of this agent that has a novel mechanism of action makes it an ideal candidate for further study in pediatric patients with CNS malignancies. A phase II study that incorporates a phase II window for patients with poor prognosis tumors (e.g. anaplastic glioma, brain stem glioma, glioblastoma multiforme and other histologic variants) approach in which therapy will be administered and therapeutic response evaluated in patients who are previously untreated (except for surgery) and prior to radiation therapy will be incorporated into this study. In addition, an attempt to identify biological predictors of treatment response to topoisomerase I inhibitors by the measurement of topoisomerase I protein expression and activity in brain tumor biopsy samples will be made.

Phase II Study of Topotecan in Pediatric Patients with Advanced Neoplastic Disease

Topotecan, a water soluble camptothecin analog, is an inhibitor of topoisomerase I. The lactone form (active form) of the drug stabilizes a covalent complex between topoisomerase I an DNA leading to enzyme-linked DNA cleavage (DNA single-strand breaks) and subsequent interference with DNA replication and RNA transcription.
Topotecan has recently been evaluated in a NCI/CCSG phase I pediatric trial. There are several compelling reasons for proceeding to a phase II evaluation of topotecan in pediatric patients with recurrent or refractory sarcomas of soft tissue or bone and neuroblastomas. First, since the long-term survival of pediatric patients with the above recurrent solid tumors is relatively poor, agents with novel mechanisms of action should be evaluated further in this patient population. Second, topotecan has been shown to have significant preclinical antitumor activity against a wide variety of murine tumors, multi-drug resistant p388 leukemia cell lines and against xenografts derived from childhood tumors. Houghton, *et al* recently reported observing complete regressions in each of 6 rhabdomyosarcoma xenografts (with 3 of 6 lines demonstrating no regrowth during a 7 month observation period ) following treatment with topotecan. In addition,

significant growth inhibitory activity was observed in 2 of 3 osteosarcoma xenografts with complete regression in a third xenograft after topotecan administration.

Although the primary objective of this study is to determine the antitumor activity of topotecan in children with progressive or refractory sarcomas of soft tissue and bone and neuroblastomas, a secondary objective is to attempt to increase the dose intensity of topotecan administered to patients enrolled on this study by intrapatient dose escalation. The rationale for further escalation of the dose includes: a) the strong correlates between response and dose intensity in both adult and pediatric malignancies b) the MTD defined for non-heavily pretreated adult patients has generally exceeded the MTD defined in heavily pretreated patients (the MTD for heavily pre-treated adult patients at M.D. Anderson on the 24 hour continuous infusion schedule was 4.5 mg/m2, while the MTD for non-heavily pre-treated patients was 12.5 mg/m2 [personal communication - L. Cazenave] and the MTD for the adult 24 hour continuous infusion phase I trial in Amsterdam was 8.4 mg/$m^2$ [ten Bokkel Juinink, 1992] which also exceeds the MTD of 5.5 mg/$m^2$ defined in our phase I pediatric trial. Since the pediatric patients enrolling on this phase II trial will be minimally to moderately pretreated (compared to the very heavily pretreated patients enrolled in our phase I pediatric trial) attempts should be made to maximize their drug exposure in the absence of dose-limiting toxicity.

Intrathecal Topotecan

There are currently a limited number of chemotherapeutic agents that can be administered intrathecally for the treatment of meningeal malignancies. The feasibility of administering topotecan by the intrathecal route is currently being evaluated in our nonhuman primate model. Three animals have received intrathecal topotecan without acute neurologic or systemic toxicity. These preliminary experiments have shown that CSF topotecan levels decline biexponentially following bolus administration. Additional studies to evaluate the long term toxicity of weekly intralumbar injections of topotecan are in progress.

8. 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 and MTD of amifostine (WR2721) in pediatric patients with refractory malignancies, (2) determine the maximum tolerated dose of melphalan when administered in conjunction with amifostine and to (3) study the pharmacokinetics of amifostine in pediatric patients. Seventeen patients have been entered to date. The amifostine dose has been escalated from 750 mg/m$^2$ to 2700 mg/m$^2$, which is approximately 3 times the adult recommended dose. Toxicities observed have not been dose limiting, and include transient non-dose related hypotension, nausea and vomiting, flushing and anxiety. The melphalan dose was escalated when a cohort of 3 patients pre-treated with 1650 mg/m$^2$ amifostine did not experience dose-limiting hematologic toxicity following a dose of 35 mg/m$^2$ of melphalan. Currently patients are being studied at the 2700 mg/m$^2$ amifostine + 45 mg/m$^2$ melphalan dose. If hematologic DLT is not observed, the melphalan dose will continue to be escalated.

9. Additional Phase I Trials

In addition to those studies mentioned above, a protocol has been prepared to conduct a Phase I trials of Taxotere, a semisynthetic analogue of taxol. A trial of Intravenous 6-Thioguanine is also beginning. Trials evaluating both a new thymidylate synthetase inhibitor and the combination of GM-CSF and IL-3 are also planned.

Phase I Study of Taxotere in Pediatric Patients with Advanced Neoplastic Disease

Taxotere, a semisynthetic analogue of taxol, is an antimitotic agent which induces microtubule polymerization resulting in nonfunctional microtubules. The antitumor activity of taxol, the naturally occurring analogue of taxotere, has become evident in phase I and II human trials. In preclinical studies, taxotere has demonstrated a broad spectrum of antitumor activity against both murine leukemias and solid tumor xenografts. These studies have demonstrated that taxotere has equivalent or superior *in vivo* activity versus taxol in some murine tumor models. Furthermore, the supply of taxotere is significantly more stable and predictable than that of the parent compound, taxol. There purpose of this study is to determine the maximum tolerated dose and the dose-limiting toxicity of this agent with a novel mechanism of action in pediatric patients with malignancies refractory to standard therapy. Taxotere will be administered to patients every 3 weeks as a 1 hour intravenous infusion using a starting dose equivalent to 80% of the adult MTD. Patient accrual to this study will begin in the very near future.

Phase I Trial of Intravenous 6-Thioguanine

6-mercaptopurine (6-MP) is the purine analogue most commonly used for the treatment of pediatric malignancies. 6-thioguanine (6-TG) has also been used for the treatment of cancer for more than 30 years, but has undergone only limited testing in pediatric cancer patients. There are, however, potential advantages to using 6-TG

over 6-MP. 6-TG is initially converted intracellularly to its monophosphate nucleotide form (TGMP) by hypoxanthine-guanine ribosyl transferase (HGPRT). This activated metabolite can then be incorporated into DNA as a fraudulent nucleotide, which appears to be the critical step for cytotoxicity. 6-MP also exerts its cytotoxicity primarily via incorporation into DNA. However, following incubation with 6-MP, only 6-TG residues are found in DNA suggesting that 6-MP metabolites must undergo additional conversion to 6-TG metabolites to effect cytotoxicity. Therefore, 6-TG requires fewer metabolic activating steps to produce cytotoxicity. *In vitro* cytotoxicity studies with human lymphoblastic leukemia cells and cell lines were performed to compare the concentrations and duration of exposure of 6-MP and 6-TG required for cytotoxicity. Cytotoxicity was assessed in 3 human leukemia cell lines (MOLT-4, CCRF-CEM, Wilson) using the MTT assay, and in ALL patient samples (prepared by Ficoll-Hypaque separation and frozen for viability) using the fluorescein diacetate (FDA) assay. Following a 48-hour drug exposure, the $IC_{50}$'s for 6-TG in cell lines (0.06, 0.04, 0.04 μM, respectively) were significantly lower than those for 6-MP (0.65, 0.9 and 1.1 μM) ($p < .05$). To achieve a 50% cell kill with 6-MP in MOLT-4 cells, a minimum of 12 hours of exposure to concentrations between 1 and 10 μM was required, whereas for 6-TG only 4 hours of exposure to concentrations between 0.1 and 1 μM were required. In lymphoblastic leukemic patient cell samples exposed to drug for 48 hours, the geometric mean $IC_{50}$ for 6-TG (17 μM) was significantly lower ($p=0.002$) than for 6-MP (255 μM). These studies indicate that 6-TG has a distinct advantage over 6-MP against lymphoblastic leukemia cells. With 6-TG, cytotoxicity occurred with 10-fold lower concentrations and was achieved with a considerably shorter duration of exposure. A pediatric phase I trial is therefore being performed to determine the rate of continuous IV infusion of 6-TG required to achieve steady state plasma drug concentrations of approximately 1 μM (the maximum cytotoxic concentration). Following determination of the required dose rate, the maximum tolerated duration of exposure will be determined by increasing the duration of infusion in cohorts of patients by increments of 12 hours, starting with a 24 hour infusion.

10. 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 Trial of Thiotepa

As discussed earlier, a phase II trials of thiotepa for pediatric solid tumors is being completed as a collaborative sffort with the CCSG. No definitive data is yet available from this study.

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 is in progress within the CCSG.

8. Other Pharmacologic Studies

Studies with Systemic Carboxypeptidase-$G_2$ ($CPDG_2$)

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 were therefore determined in the Rhesus monkey. Groups of animals received a 300 mg/m$^2$ loading dose of MTX followed by a 60 mg/m$^2$/hour infusion over 18 hours. One group received $CPDG_2$ at the end of the infusion and the other group served as control. Two additional animals with high titers of anti-$CPDG_2$ antibody were also studied. During infusion, the steady-state MTX plasma concentration was 11.3 ± 4.8 μM. Without $CPDG_2$, the post-infusion plasma MTX concentration remained above 0.1 μM for more than 6 hrs. After administration of 50 units/kg $CPDG_2$, plasma MTX concentrations fell to non-toxic levels (< 0.05 μM) within 30 minutes. The initial half-life of MTX decreased from 5.8 ± 2.1 minutes to 0.7 ± 0.02 minutes following enzyme administration. The post-infusion area under the plasma concentration time curve (AUC) of MTX was 301 ± 171 μM•min without $CPDG_2$, compared to 19.6 ± 6.1 μM•min with $CPDG_2$. Immunogenicity studies performed indicated that although animals developed anti-$CPDG_2$ antibody, none manifested allergic symptoms. The effectiveness of $CPDG_2$ was diminished but not eliminated in animals with high titers of antibody. $CPDG_2$ is capable of rapidly decreasing plasma MTX concentrations to non-toxic levels. Administration of $CPDG_2$ appears safe, well tolerated, and may be useful as an alternative to LV rescue. A nationwide protocol, organized through CTEP, will determine the ability of $CPDG_2$, in combination with thymidine, to rescue patients who develop life-threatening MTX toxicity secondary to renal dysfunction. The pharmacokinetics of

MTX following $CPDG_2$ rescue, and the immune response of patients to the enzyme, will be determined.

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 $266 \pm 54$ ml/min with a distribution half-life of $10 \pm 7$ minutes and an elimination half-life of $114 \pm 22$ minutes. The mean AUC is $14600 \pm 3600$ 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, interferon-γ, PALA, and GM-CSF. Recent analysis of the 5-FU/interferon study demonstrated a relationship between plasma 5-FU concentration and toxicity following a bolus dose schedule and we demonstrated that α-interferon delays 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. Similarly, on the trial of PALA, 5-FU, and leucovorin, in which 5-FU was administered as a continuous infusion over 72 hours, steady state plasma concentrations of 5-FU exceeding 10μM were associated with a higher incidence of grade 3 and 4 toxicity. These studies may provide a basis for the use of therapeutic drug monitoring of 5-FU.

Continuous Infusion Taxol

In collaboration with the Medicine Branch, we are investigating the pharmacokinetics of taxol adminstered as a 96 hour infusion. To date there are no published studies of this schedule. Pharmacodynamic correlations among clearance, plasma levels, and toxicity and response will be sought. In addition, the possibility of non-linear clearance with prolonged infusion will be explored.

Taxol/Adriamycin

In collaboration with the Medicine Branch, we are performing a pharmacokinetic study of the combination of taxol and adriamycin. Cohorts of patients will be enrolled adn treated with these agents separately and in combination, with patients acting as their own controls. Both taxol and adriamycin pharmacokinetics will be studied in order to ascertain possible alterations in clearance of either agent with coadministration.

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 39 patients with refractory meningeal malignancy have been entered onto this protocol. Complete responses have been achieved in 14 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 5.0 mg biweekly dose level. Several patients at this dose level have experienced non-dose limiting grade 2 headaches that appear to be related to the dose rate of mafosfamide administration. We are currently studying the effect of decreasing the dose rate and delivering the drug at a constant infusion rate over a fixed interval of 20 minutes. Preliminary results indicate that these minor modifications in rate of drug administration have obviated this side effect. An additional dose escalation to 6.5 mg biweekly is anticipated if the

findings of reduced toxicity with decreased dose rate of administration are confirmed in another cohort of patients at the 5.0 mg 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 been initiated, and pharmacokinetic studies in the first two patients 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 - $G_2$

Methotrexate is the most widely used intrathecal antineoplastic agent. Standard intrathecal doses of 6 to 12 mg are well tolerated, but accidental IT overdose, usually the result of inadvertent IT injection of a higher dose intended for systemic administration, can produce severe and frequently lethal toxicity. Current therapeutic recommendations for patients who receive IT MTX doses greater than 100 mg include (1) immediate lumbar puncture to drain CSF, (2) emergency ventriculostomy placement followed by ventriculo-lumbar perfusion, (3) administration of systemic corticosteroids and (4) administration of systemic leucovorin to prevent systemic MTX toxicity. Despite these interventions, the outcome is often fatal. Although IT MTX overdose occurs infrequently, its life threatening nature emphasizes the need for a better antidote. The carboxypeptidase G class of enzymes hydrolyzes the C-terminal glutamate residue from folic

acid and classical antifolates, such as MTX. 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. $CPDG_2$ will rapidly hydrolyze MTX to the inactive metabolites 4-deoxy-4-amino-$N^{10}$-methylpteroic acid (DAMPA) and glutamate. In experiments performed in the Rhesus monkey, IT $CPDG_2$ successfully rescued groups of monkeys following IT administration of MTX in doses equivalent to 250 and 500 mg in humans. The CSF MTX concentration determined 15 minutes following $CPDG_2$ (27.3±4 μM and 262.4±75.9 μM for the 250 and 500 mg equivalent dose groups, respectively) was even lower than the 947 μM lumbar CSF concentration found 15 minutes after administration of standard IT MTX doses (equivalent to 12 mg in humans), confirming the rapidity and effectiveness of rescue provided by this approach. Administration of the enzyme alone produced an asymptomatic CSF pleocytosis. A cooperative pediatric group trial with the CCSG, POG and the Pediatric Branch, NCI is underway to determine the effectiveness of $CPDG_2$ rescue in patients who develop life-threatening MTX neurotoxicity following accidental IT MTX overdose. The CSF pharmacokinetics of MTX following $CPDG_2$ rescue will also be studied.

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 involved at several levels in the development of new antiretroviral agents. Both preclinical and clinical studies have been conducted with the intent of 1) evaluauting the effect of biologic agents like interferon and the colony stimulating factors on the biochemical pharmacology of the dideoxynucleosides; 2) defining the central nervous system pharmacology of existing and proposed antiretroviral agents in order to predict their potential clinical efficacy against AIDS dementia complex; and 3) studying the pharmacokinetics of antiretroviral agents in patients in order to determine the optimal route and schedule of administration and to establish correlations between pharmacokinetic parameters and both treatment response and toxicity. In addition, preliminary efforts are underway to develop a cell and virus-free drug screening technique to identify inhibitors of the viral enzyme, integrase.

1. Biochemical pharmacology of dideoxynucleosides

The purpose of these studies is to evaluate the phosphorylation (activation) of the nucleoside analogs AZT, ddI, and ddC in the target cells of HIV infection. Previous studies evaluated the influence of the ddC on AZT phosphorylation to AZTTP in CD4 positive lymphoblasts and found no

effect. Current studies are concentrating on the influence of cytokines and other biologic agents on AZT phosphorylation. To date, GM-CSF, IL-3, interferon-$\alpha$ and $\gamma$ and several other interleukins have been systematically evaluated in a monocytic cell line, and there has been no influence on the conversion of AZT to its active intracellular metabolite. Future studies will evaluate the interaction between thymidylate synthase inhibitors and AZT activation.

2. Central Nervous System Pharmacology of Antiretroviral Agents

Using the existing nonhuman primate model we have studied the CSF penetration of a wide variety of antiretroviral compounds as part of their preclinical pharmacologic evaluation. This includes more than 12 dideoxynucleosides (including a series of halogenated compounds) which have been evaluated in an attempt to define molecular characteristics that promote better penetration. To date it appears that the nucleobase is the primary determinant of 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 (ddT), which had a CSF to plasma ratio of 30%, and azidodideoxycytidine (AZC) 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. hydrogen) 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 as evidenced by the limited penetration of AZC and the excellent penetration of ddT. 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, ddG, and a series of halogenated ddG analogs. The penetration of ddI was limited (5%) and the penetration of ddG, a less water-soluble drug was higher (15%). The series of halogenated dideoxynucleosides (from Dr. Mitsuya) which were developed to enhance lipophilicity and optimize CNS penetration, were rapidly dehalogenated to ddG in vivo, and therefore, do not appear to offer an advantage. Newer agents such as protease inhibitors and "Uniroyal Jr" which was highly active in the NCI drug screen are also being studied.

We have initiated studies in our nonhuman primate model evaluating factors that influence the penetration of the currently available drugs AZT and ddI. Probenicid administered concurrently with AZT appears to enhance CSF penetration of AZT independent of its effects on AZT systemic clearance, The effect of probeicid on efflux of AZT from the CSF is also being studied to define the mechanism of probenicid action. A collaborative study with the group at the University of Washington to evaluate the effect of AZT and ddI on each other's CSF penetration is also underway. These studies may provide a rationale for clinical trials of drug combinations.

Prior to the initiation of an intrathecal AZT trial in adults with AIDS dementia (described below), we are studying the distribution and elimination of AZT from the CSF during a continuous intrathecal infusion in a new nonhuman primate model. Preliminary data have demonstrated that intrathecal AZT is well tolerated by this route, but that it rapidly diffuses out of the CSF, such that drug levels in the lumbar CSF are considerably lower than ventricular levels at steady state. We will be evaluating mechanisms to enhance drug distribution in the CSF in this model.

3. Integrase Assay

We are initiating an effort in collaboration with a group in NIDDK who have cloned the gene for HIV integrase to develop a rapid fluorescent assay for this important enzyme in the HIV life cycle. By incorporating fluorescent probes (new fluorescent pteridine nucleoside analogs) into oligonucleotides we hope to be able to monitor the cleavage and integration reactions catalyzed by integrase by monitoring change in fluorescence (in real time). A series of these pteridine nucleoside analogs have been obtained from Dr. Pfleiterer in Germany and the fluorescent spectra are being defined. We have also successfully incorporated one of these compounds into an oligonucleotide. Although this assay would be useful for mechanistic studies with the enzyme, the ultimate goal is to develop an assay that can be used to screen for potential integrase inhibitors.

4. Pharmacokinetics of AZT in Children

We previously characterized the pharmacokinetics of AZT in 37 children with symptomatic HIV infection treated on one of two Pediatric Branch phase I/II trials of the drug. 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%. We demonstrated the pharmacokinetic advantage for delivering AZT by continuous infusion and defined a relationship between steady state plasma AZT concentration and neutropenia, the dose-limiting toxicity on the contiunuous infusion schedule.

Current pharmacokinetic studies of AZT are focused on patients being treated on an ongoing trial evaluating the utility of continuous infusion AZT in children with AIDS dementia. We hope to confirm pharmacokinetic-pharmacodynamic relationships noted in the phase I/II trial of continuous infusion AZT. As part of this trial we are also evaluating the neurochemistry of AIDS dementia by measuring of CSF neuropeptides, cholinesterases,

and monoamine neurotransmitters in an attempt to identify easily measured markers of the HIV CNS infection. Assays for this diverse group of compounds are being setup and validated. If a marker chemical can be defined, a similar approach may be instituted with CNS leukemia.

We are also evaluating potential pharmacokinetic drug interactions on a trial of combination AZT/ddI. In over 40 patients the plasma drug profile for both agents is unchanged when the drugs are administered together. 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. To date, two patients with renal failure have been 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.

5. Intrathecal AZT

A trial of continuous intrathecal infusion of AZT in adults with severe dementia who are intolerant of oral AZT has been developed in the NINDS with consultation and advice from our group. We will also collaborate in this trial by measuring CSF drug concentrations in the patients on the study. The rationale for using continuous infusion comes from our experience in the nonhuman primate model and an ongoing clinical trial of continuous intrathecal infusion of anticancer agents in patients with meningeal malignancies.

6. 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 initially 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 over 90 patients who have continued on the ddI phase I/II 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.

7. 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 dose schedule for phase I trials of this compound.

8. All-trans-retinoic acid pharmacokinetics in adults with Kaposi's sarcoma

All-trans-retinoic acid is a differentiating agent that has produced dramatic results in patients with APL. It is concentrated in the skin and will therefore be tested in Kaposi's sarcoma which is primarily a cutaneous malignancy. In collaboration with Drs. Pluda and Yarchoan who have a clinical protocol to study the combination of all-trans-retinoic acid in patients with KS, we plan to evaluate the pharmacokinetics of retinoic acid in adults on this trial.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: ZO1 CM 06890-13 PB

PERIOD COVERED: October 1, 1991, to September 30, 1992

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 T. Magrath | Head, Lymphoma Biology Section | PB, NCI |
| Melissa Adde | Nurse Specialist (Research) | PB, NCI |
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COOPERATING UNITS *(if any)*: NONE

LAB/BRANCH: Pediatric Branch

SECTION: Lymphoma Biology Section

INSTITUTE AND LOCATION: National Cancer Institute, Bethesda, Maryland

| TOTAL STAFF 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 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. Stemming from these studies, we are also investigating the possibility that the molecular abnormalities relevant to tumor pathogenesis (including viral genes) can be used as a target for tumor-specific treatment approaches. We are also undertaking molecular epidemiological studies pertinent to the scl and bcl-2 genes.

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 expand our molecular epidemiological data so as to develop a more detailed understanding of the molecular pathogenesis of the SNCL, and, ultimately, to develop novel therapeutic strategies based on these 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 values 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 of 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.) Explorations 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 examinations of the possibility that EBV may collaborate with the translocations and 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 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. *Explorations of the possible role of genes which inhibit apoptosis in SNCL*

9. *Defining the target cell in SNCL, by exploring the timing of the translocation*

10. *Exploring the molecular features of SNCL in HIV individuals*

SIGNIFICANCE

Therapeutic Studies

Protocol 89-C-41, should result in an improved outcome with a shorter duration of treatment 4(4 cycles for high risk patients, 3 cycles for low risk patients). We hope that the prognosis 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 had benefitted not only ourselves, but many other investigators.

Our studies are comprehensive, in that they encompass molecular epidemiology, molecular pathogenesis, clinical correlated 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 serious contemplated at present.

We have recently demonstrated the frequent involvement of p53 mutations in SNCL. In characterizing these mutation, we have been able to make novel observations with respect to the mechanisms whereby this gen - abnormalities of which constitute the most frequent genetic abnormality in cancer - contributes to neoplasia, not on 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 translocation, 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 under certain conditions both p53 and myc which appear to be involved in 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 remains 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 translocations, 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 American 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 juxtapositions with c-myc. 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 necessary to drive transcription from P1 and P2. We have also commenced a more detailed localization 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.

*Exploration of Mutations within the Translocated Myc Locus in SNCL*

Mutations in regulatory region of the myc gene (immediately 5', within the 1st exon and the MIF regions in the intron) have been

previously described. It has often been thought that these mutations arise as a consequence of the translocation to the immunoglobulin locus. These mutations have been confined to the noncoding regions of c-myc. To better understand the correlation between various factors in SNCL (1P, EBV, P53 and breakpoint locations) it would be advantageous to determine the speculations of mutations within the translocater c-myc allele. We have begun to study this using an SSCP screening promotor.

*The Target Cell in SNCL is a Pro-B Cell*

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 joining), such that, frequently the cell has not initiated rearrangement of its immunoglobulin genes. The translocation presumably prevents rearrangement of the translocated genes. The translocation presumably prevents rearrangement of the translocated allele, while of 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 (luficerase 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 promotors (P1 and P2) but contain the c-myc intronic promotor 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 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, eh 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 locations 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 by 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 (demonstrated 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 increased the expression of myc, and that this effect appears to be mediated via the juxtaposed immunoglobulin enhancer. We have also shown, in collaboration with L. Frappier, that EBNA-1 does not directly bind to myc sequences. These observations provide a potential mechanistic explanation of the pathogenetic significance of EBV in Burkitt's lymphoma. It is relevant to note that ENBA-1 appears to be invariable expressed in Burkitt's lymphoma, whereas other EBNAs are usually down regulated. We plan to explore the effect of these ENBAs 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. Examination of the c-myc locus in HIV related lymphomas suggests that in contrast to the published data, c-myc is infrequently rearranged even in tumor histologically classified as

SNCL.

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 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 feasibility 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 ration 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 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 difference 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 observations 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. Examination of a panel or primary relapsed tumors suggest that p53 mutations are associated specifically with relapse.

5. 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.

To further understand the molecular features of T cell ALL in developing countries we have recently explored the pathogenetic contribution of tal rearrangements in these neoplasms. The frequency of tal and SIL rearrangements from India and Egypt appear to be similar to the frequency rearrangements reports from the US and France. However, a specific type of tal rearrangement (B type) appears to be significantly lower in India.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06891-04 PB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

| | | | |
|---|---|---|---|
| PI: | Marc E. Horowitz | Senior Investigator | PB, NCI |
| Others: | Leonard H. Wexler | Clinical Associate | PB, NCI |
| | Linda L. McClure | Nurse Specialist (Res) | PB, NCI |

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI; 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

None

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 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.)

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. These studies are nearing completion. Subsequent studies will assess the activity of new agents in the pediatric solid tumors in a phase II "window" design.

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 80 protocol entries with the following diagnoses: Ewing's sarcoma, PN, primitive sarcoma of bone, rhabdomyosarcoma, and other soft tissue sarcomas. 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). Event-free survival at 3 years for patients with localized tumors is 65%. For those with metastatic disease at diagnosis, only 15% are event-free. Therefore, the addition of ifosfamide and etoposide have not improved the treatment of patients with metastatic sarcoma. The toxicity of this protocol has been significant.

Ninety-three percent 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.

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 $\mu$g/kg based on a recommendation by CTEP. Thirty-five 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. Thirty-five 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. Five patients have been entered on study.

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 23 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 $\pm$ 2 days. The study has determined that this regimen is active in PNET but not malignant glioma.

90-C-113 - A Phase I Study of R-verapamil with EPOCH Chemotherapy and Analysis of Drug Resistance Mechanisms in Relapsed Lymphomas and Pediatric Sarcomas

Preliminary evidence suggests that drug resistance of pediatric sarcomas may be mediated through the MDR gene product p170 glycoprotein. R-verapamil has been demonstrated to reverse MDR *in vitro*. In collaboration with the Medicine Branch we have initiated a trial in pediatric sarcoma patients with recurrent disease to determine the MTD of R-verapamil given with EPOCH (etoposide, prednisone, vincristine, cyclophosphamide and adriamycin). EPOCH is given without the reversing agent for the first two cycles. If there is no evidence of response the subsequent cycles of EPOCH are administered with R-verapamil. This will allow us to determine the dose of R-verapamil to use in future studies and to assess the response rate to EPOCH + R-verapamil in patients resistant to EPOCH alone. Five patients have been entered on study.

Publications:

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Kellie SJ, Kovnar EH, Kun LE, Horowitz ME, Heideman RL, Douglass EC, Langston JW, Sanford RA, Jenkins III JJ, Fairclough DL, Ogle L. Neuraxis dissemination in pediatric brain tumors: Response to preirradiation chemotherapy. Cancer 1992;69:1061-6.

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Friedman JM, Vitale M, Maimon J, Israel MA, Horowitz ME, Schneider BS Expression of the cholecystokinin gene in pediatric tumors. Proc Natl Acad Sci USA, 1992;89, in press.

Horowitz ME, Tsokos MG, DeLaney TF. Ewing's sarcoma. Ca-A Can J Clinicians, in press.

Horowitz ME, DeLaney TF, Malawer MM, Tsokos MG. Ewing's sarcoma family of tumors: Ewing's sarcoma of bone and soft tissue and the peripheral primitive neuroectodermal tumors. In: Pizzo PA, Poplack DG, eds. Principles and practice of pediatric oncology, 2nd edition. Philadelphia: JB Lippincott Co., in press.

Smith MA, Adamson PC, Balis FM, Feusner J, Aronson L, Murphy RF, Horowitz ME, Reaman G, Hammond GD, Fenton RM, Connaghan GD, Hittelman WN, Poplack DG. Phase I and pharmacokinetic evaluation of all-trans retinoic acid in pediatric patients. J Clin Oncol, in press.

Whang-Peng J, Knutsen T, Theil K, Horowitz ME, Triche T. Cytogenetic studies in subgroups of rhabdomyosarcoma. Genes, Chromosomes & Cancer, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 CM06892-03 PB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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,

| | | | |
|---|---|---|---|
| PI: | Lee Helman | Senior Investigator | PB, NCI |
| Others: | Caterina Minniti | Clinical Associate | PB, NCI |
| | Thea Kalebic | Visiting Scientist | PB, NCI |
| | Cindy Kappel | Biologist | PB, NCI |
| | Steven Hirschfeld | Clinical Associate | PB, NCI |
| | Maria Velez-Yanguas | Clinical Associate | PB, NCI |

COOPERATING UNITS (if any)
Stanford University Medical Center, Pediatrics (Dr. Rosenfeld).
National Cancer Institute, CPB (Drs. Sarter, Cooper, and Myers).

LAB/BRANCH
Pediatric Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, MD 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 3 | 0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☒ (b) Human ☐ (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), the most common soft tissue sarcoma of childhood, and osteogenic sarcoma (OS), the most common bone tumor of childhood. Several common growth factors are known to play a role in normal growth and maturation of skeletal muscle and bone such as TGF-beta and insulin-like growth factors (IGFs). In addition, the tumor suppressor gene p53 has been implicated in the progression of both these tumor types. We have focused our attention on the role of IGFs in these tumors.

We have identified IGF II as an autocrine growth factor in RMS and have begun a Phase II study using suramin, an agent that we have demonstrated is capable of interfering with this autocrine growth loop in vitro. We have also reported the largest series of RMS tumors analyzed for p53 mutations and found that a substantial number of these tumors have such mutations. In an attempt to determine the precise role of overexpression of IGF II in RMS tumors, we have transfected a human IGF II cDNA expression vector into mouse myoblast cells and are analyzing the effect on differentiation and proliferation potential, as well as on tumorigenic potential. To begin to sort out the molecular events that play a role in the metastatic potential of these cells, we have developed a series of subclones from a human embryonal RMS cell line with differing tumorigenic and metastatic potentials when injected into nude mice. Current experiments are ongoing to describe molecular differences between the various subclones and subtraction cDNA cloning is planned to isolate genes that may confer metastatic behavior to these cells.

Other investigators have demonstrated the presence of type I IGF receptors on the surface of OS cells and that IGF I is a mitogen to these cells. However, it is unclear whether some, all, or no OS produce autocrine IGF I. We are therefore currently characterizing a panel of 5 OS cell lines for IGF expression. In addition, since growth hormone is known to stimulate IGF I secretion and there are data that suggest growth hormone secretion is important in the development of OS, we are also currently involved in developing a clinical study using a somatostatin (SST) analog to inhibit GH secretion in patients with metastatic OS. This study will allow us to determine the effect of the drug on stimulated GH as well as circulating IGF I levels in patients with OS. In addition, we plan to determine whether OS cells express SST receptors that may mediate a more direct effect of SST analogues on the growth of these tumors.

Accomplishments and Results:

1. The role of IGF II in RMS: We have demonstrated that IGF II functions as an autocrine growth factor in RMS and its mitogenic signal is transduced through the type I IGF receptor. We have also demonstrated that IGF II functions as an autocrine motility factor in RMS cell lines and this activity is signaled through the type II IGF or cation-independent mannose-6-phosphate receptor. Thus therapy aimed at interfering with the action of IGF II in these tumors may have the ability to inhibit both growth and metastatic potential of these tumors.

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 IGFs 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 a Phase II study to test the activity of suramin in relapsed RMS. This protocol will be the first study of suramin in a pediatric population. After approval of this study, other investigators demonstrated that suramin inhibited the growth of osteosarcomas in a mouse xenograft model and we are therefore also entering patients with relapsed osteosarcomas onto this protocol.

We have also studied the ability of αIR-3, a blocking monoclonal antibody to the type I IGF receptor to inhibit the growth of RMS xenografts in nude mice. We have shown that co-injection of αIR-3 with tumor cells and continued treatment is able to completely abolish the formation of tumors while control animals all developed large tumors at the primary site. Subsequent experiments evaluated the ability of αIR-3 to block the growth of established tumors. In this protocol, animals were treated with antibodies after the development of primary tumors, usually 10 days post-injection of tumor cells. Under these conditions, animals treated with αIR-3 had arrest of tumor growth for approximately 30 days before breakthrough regrowth occurred. There were no tumor regressions however.

To determine what role deregulation of IGF II expression plays in "normal" myoblasts, we have transfected a human IGF II expression vector into a mouse myoblast cell line that normally differentiates under appropriate cell culture conditions. We have isolated multiple clones that we have documented to overexpress human IGF II protein. Preliminary data suggest that overexpression of IGF II in these cells leads to a block in differentiation, increased anchorage independent growth, and a higher saturation density in anchorage dependent growth assays.

2. Studies on the Metastatic Potential of RMS: In order to begin to study the mechanisms that play a role in the highly metastatic potential of RMS, we have subcloned a human embryonal RMS cell line and evaluated the growth characteristics of these lines in nude mice. We have currently established 10 such subclones with a wide range of growth rates and metastatic potential in nude mice. At least one subclone appears to be highly metastatic while other subclones never metastasize in this model. We are currently comparing the patterns of gene expression among the various phenotypes observed and have seen no correlation between expression of laminin receptor or nm-23 (proteins previously described to play a role in the metastatic phenotype) in these cell lines.

3. Analysis of p53 Mutations in RMS: We have completed our analysis of p53 structure in 6 tumor specimens and 5 cell lines. Mutations were found in 3/5 cell lines including a 4 bp deletion at codons 219-220, a point mutation at codon 280 changing an arginine to a serine, and a point mutation in codon 248 (a codon previously shown to be involved in germline mutations in the Li-Fraumeni syndrome) changing an arginine to a tryptophan. Two of six tumor specimens were also demonstrated to harbor mutations in the p53 coding sequence. One tumor was found to have deleted both alleles of the gene, and another tumor was shown to have a point mutation in codon 213 changing an arginine to a proline. These data suggest a relatively high frequency of involvement of p53 mutations in RMS and suggests a role for such mutations in the pathobiology of this tumor.

4. Studies on HIV-related encephalopathy: Based on previous study, we investigated whether human fetal cells may activate a latent HIV. By using glial cell lines SVG and POJ derived from human fetal brain, we demonstrated the release of soluble factors that 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 caused 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. Pretreatment of U1 cells with GSH or NAC did not suppress HIV expression in the cells stimulated with conditioned media derived from fetal glial cells SVG and POJ. However, the HIV-inducing capacity of TNFα was inhibited by its preincubation with glutathione (GSH), but not with N-acetyl cysteine (NAC). Our results in vitro suggest that fetal glial cells in vivo may contribute to the progression of encephalopathy in children with neonatally acquired HIV infection. Moreover, GSH and NAC may have different effects on various HIV-inducing cytokines, therefore a future in vitro study is needed to evaluate their potential use in anti-HIV therapy.

5. Studies on the effects of the reducing agents glutathione (GSH) and WR 12527 on activation of HIV expression in chronically infected cells: We described previously a suppressive effect on HIV activation of GSH and NAC mediated by TNFα and IL-6. GSH, which is decreased in AIDS patients, is also required for normal function of mitochondria.

Mitochondrial damage, which may lead to cellular death, is induced by AZT, the most commonly used anti-HIV drug. We are currently investigating whether glutathione may protect mitochondria from AZT-induced lesions. We have found that a new reducing agent, WR12157, previously used in anti-cancer therapy also has HIV suppressive effects. In the absence of cytotoxicity, WR 12157, at concentrations of 5 mM, suppresses induction of HIV expression in chronically infected cells stimulated with both TNFα and GMCSF. The suppressive effect of WR 12157 was demonstrated by monitoring production of total HIV proteins and HIV reverse transcriptase activity. Moreover, a preliminary study suggests that WR 12527 has a suppressive effect on the transcriptional activity of HIV-LTR. Regulatory mechanisms involved in WR 12527-mediated suppression of HIV expression will be investigated. In addition, the effect of WR 12527 on acute HIV infection will be studied. It is possible that WR 12527 may suppress induction of HIV, in vivo, and therefore it may be considered for anti-HIV adjuvant therapy.

6. Isolation and characterization of monocytic cell lines chronically infected with HIV: differential TNFα-mediated viral activation: In order to study the mechanisms which control TNFα-mediated activation of HIV expression, we isolated, by in vitro cloning, different cellular variants of U1 cells that respond differentially to TNFα stimulation. We have shown that in U3 cell line TNFα induces a higher level of HIV expression than in the parent U1 cell line. In contrast, the expression of HIV is hardly detectable in the U48 cell line stimulated with TNFα. In both cell lines, however, the activation of HIV expression by PMA is similar, suggesting that a selective loss of TNFα-mediated upregulation of HIV occurred in U48 cells. Lack of TNFα-mediated HIV activation in U48 cells and high level of HIV expression in TNFα-stimulated U3 cells was demonstrated by measuring HIV mRNA levels, total HIV proteins, and reverse transcriptive activity. The level of transactivation of wild type HIV-LTR linked to a CAT reporter gene in both cellular variants was found comparable, showing that both cellular environments are equally permissive for HIV-LTR expression. Sequence analysis of the regulatory region of HIV provirus from U3 and U48 is currently ongoing to determine whether a loss of response to TNFα is due to structural changes. This new cellular system may be a model to elucidate TNFα-triggering mechanisms of HIV activation.

PUBLICATIONS:

Crouch, GD, Helman, LJ. All-trans-retinoic acid inhibits the growth of human rhabdomyosarcoma cell lines. Cancer Res 1991;51:4882-7.

Minniti, CP, Maggi, M, Helman, LJ. Suramin inhibition of human rhabdomyosarcoma cell growth may occur through a block of the IGF-I receptor. Cancer Res 1992;52:1830-5.

Felix, CA, Kappel, CC, Mitsudomi, T, Nau, MM, Tsokos, M, Crouch, GD, Nisen, PD, Winick, NJ, Helman, LJ. Frequency and diversity of p53 mutations in childhood rhabdomyosarcoma. Cancer Res 1992;52:2243-7.

Minniti, CP, Kohn, EC, Grubb, JH, Sly, WS, Oh, Y, Rosenfeld, RG, Helman, LJ. The IGF-II/Mannose 6-phosphate receptor mediates insulin like growth factor-II induced motility in human rhabdomyosarcoma cells. J Biol Chem 1992;267:9000-4.

Park, J-G, Choe, GY, Helman, LJ, Gazdar, AF, Yang, H-K, Kim, J-P, Park, SH, Kim, YI. Chromogranin-A expression in gastric and colon cancer tissues. Int J Cancer 1992;51:189-94.

Helman, LJ, Horowitz, ME. Rhabdomyosarcoma. In: Tonini G-P, Sansone R, Thiele CJ, eds. Molecular genetics of pediatric solid tumors. Basic concepts and recent advances. Chur, Switzerland: Harwood Academic Publishers, 1992;337-58.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 00650-37 RO

PERIOD COVERED
October 1, 1991 to September 30, 1992

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: | F. Sullivan | Acting Clincal Chief | ROB, NCI |
| Others: | E. Glatstein | Chief | ROB, NCI |
| | T. Goffman | Head, Clin. Ther. Sect. | 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 10 | 4 | 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.)*

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:

| | | |
|---|---|---|
| R. Smith | Supervisory Clinical Nurse | CNS, CC |
| J. Smith | Clinical Nurse | CNS, CC |
| L. Dachowski | Clinical Nurse | CNS, CC |
| E. Feutsch | Clinical Nurse | CNS, CC |

Methods Employed

Formal and informal consultation with referring physicians and application of radiotherapy where appropriate with-rays and electrons in accordance with standard radiotherapy practice, as well as modified programs when necessitated by con adjuvant therapies.

Major Findings

Just under 550 patients were seen in formal consultation this years 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 in the Radiation Oncology Branch, or on collaborated 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06310-13 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Surgery Versus 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: | Joan Jacobson | Senior Investigator | ROB, NCI |
| Other: | 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 STAFF 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 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, MO 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 06320-13 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Response to 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 | Acting Branch Chief | 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

INSTITUTE AND LOCATION

Experimental Phototherapy Section

| TOTAL STAFF 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, necarcinostatin, novel metal derivatives, VP-16, and radiosensitisers 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 anti-neoplastic drugs and cellular response to these species, as well as sulfhydryl containing compounds as they relate to metabolism, activation, and detoxification of antineoplastic 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, cistplatin, VP-16, alkylators, and radiosensitizers. Alternately, 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 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 studies to determine other factors important in drug resistance. We have been studying the mechanisms of action of Taxol as it relates to modulation by cellular thiol concentration, cell cycle arrest, resistance and drug radiation interaction.

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, as well as the more fundamental mechanisms of drug action.

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, flow cytometry, EPR, 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. Additionally, we have found that intracellular thiol concentrations function to alter the cytotoxicity of taxol.

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.

Publications

1. Mitchell JB, Glatstein E. Radiation Oncology. Past achievements and on-going controversies. Cancer Res 1991;51:5065-73.

2. Krishna MC, DeGraff W, Tamura S, Gonzalez FJ, Samuni A, Russo A, Mitchell JB. Mechanisms of hypoxic and aerobic cytotoxicity of Mitomycin-C in Chinese hamster V79 cells. Cancer Res 1991;51:6622-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-13 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
| PI: | J.B. Mitchell | Acting Branch Chief | 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 STAFF 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.)*

The major objectives of this project are to identify approaches that will result in radiation enhancement of cell killing both i aerobic and hypoxic conditions and to better understand mechanisms of radiosensitization. Much of our previous work identified intracellular glutathione (GSH) as important in governing the cytotoxicity of certain chemotherapy drugs and hypoxic cell radiation sensitizers. We have recently shown that hypoxic radiation sensitization by two new nitroimidazoles is markedly enhanced with GSH depletion. In collaboration with the Surgery Branch, we have measured GSH levels from a number of lung tumor biopsies and compared than to normal lung GSH. Our findings show that 1) precise GSH measurement of tumor cells are complicated by infiltration of leukocytes (infiltration in some tumors exceeded 40% of the total tumor 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 3-5 fold higher GSH levels than found in normal lung, Such Studies support the idea of using an inhibitor of GSH synthesis to possibly enhance both radiation and chemotherapy cytotoxicity and indeed clinical trials have been initiated at other institutions to test this possibility. Presently we are working with a series of GSH esters that might be used to elevate GSH in cells and tissues with the idea of restoring cells/tissues to normal GSH levels after depletion and drug treatment. Additionally, we have demonstrated that non-lethal hyperthermia administered concurrently with continuous low dose irritation results in dramatic radiosensitization. This will be further explored including GSH depletion. Lastly, we have demonstrated that human B-lineage lymphoid precursor cells are radiosensitive.

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

GSH levels of cells taken from various types of human tumors 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. Low intracellular GSH levels markedly enhanced nitroimidazole mediated radiosensitization. Non lethal hyperthermia markedly enhanced continuous low dose rate irradiation.

Significance to Biomedical Research and the Program of the Institute

There is a need to improve cancer treatment. Learning of new approaches to enhance radiation sensitization of tumor cells and more importantly the biochemical/molecular mechanisms involved are vital to the stated goal of improving cancer treatment.

Proposed Course

More studies will be conducted at the cellular level on different radiation sensitizers and GSH modulation. Specifically, the role of taxol in radiation sensitization will be explored.

Publications

1. 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 Res 1991;51:4287-94.

2. Garg PK, Garg S, DeGraff WG, Zalutsky MR, Mitchell JB. 4-fluorobenzylamine and phenylalanine methyl ester conjugates of 2-nitroimidazole: synthesis and evaluation as hypoxic cell radiosensitizers. Int J Radiat Oncol Biol Physics 1991;22: 593-6.

3. Spiro IJ, McPherson S, Cook JA, Ling CC, DeGraff W, Mitchell JB. Enhanced radiosensitization at low dose rate using non-lethal hyperthermia. Radiat Res 1991;127:111-4.

4. Kaufman D, Mitchell JB, Russo A. Glutathione, a determinant of response to cancer treatment. Medical Radiology Continuous Infusion Chemotherapy & Radiation 1991;85-9.

5. Uckun FM, Mitchell JB, Obuz V, Park CH, Waddick K, Friedman N, Oubaha L, Min WS, Song CW. Radiation sensitivity of human B-lineage lymphoid precursor cells. Int J Radiat Oncol Biol Phys 1991;21(6):1553-60.

6. Mitchell JB, Cook JA, Krishna MC, Hahn S, Goffman T, Glatstein E. Prospect for modifying the radiosensitivity of oxic tumor cells. In: Dewey WC, Edington M, Fry RJM, Hall EJ, Whitmore GF, eds. Radiation Research: A Twentieth-Century Perspective, vol 2. New York: Academic Press, 1992; 745-50.

7. Biaglow JE, Mitchell JB, Held K. The importance of peroxide and superoxide in the x-ray response. Int J Radiat Oncol Biol Phys 1992;22:665-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-12 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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. Yeakle-Orr | Dosimetrist | ROB, NCI |
| | M. Thompson | Dosimetrist | 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, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2 | 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 Section continues to provide expert physical and technological support for radiation treatment, consisting of routine calibration and quality assurance of all radiation equipment. It includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to developing clinical needs.

1. The improvement of the quality assurance (QA) program for the three Varian accelerators (Clinacs 4, 18 and 20) is a continuing effort. Regular checking of dosimetric and technical set-up aspects of radiation treatment will continue. The M-22 Microtron has been removed.

2. The in-house developed MacIntosh II based treatment planning system is being phased-in for clinical use.

3. Adaptation of the radiation equipment and special supporting equipment and methodology for patient and its implementation is a continuing effort, tailored to the need of ongoing and new clinical research programs.

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 simulator, and the CT scanner. The mostly in-house developed mechanical patient 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.

A low dose rate 6 MV photon beam capability has been developed for the Clinac-20. This involved research for, and design, construction and implementation of a new flattening filter.

The rotating chair project has resulted in a versatile and stil open-ended treatment device which will be clinically evaluated in particular for low dose rate treatment, allowing selected patients to be treated more comfortably, with more normal tissue sparing. Extensive measurements have been made to improve dosimetry of customized small electron boost fields. **The rotating chair as a development project has been terminated.**

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 treatment chair is found to be an attractive device for patients who have difficulty lying down for prolongued periods of time, and for those were the nature of the target volume allows more normal tissue sparing in sitting position. Futher clinical evaluation is planned.

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.

Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning, delivery, monitoring and documentation 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. Continued transition to the new MacIntosh II treatment planning system.

2. Replacement of the Clinac-18 with a dual photon, multiple electron energy machine.

3. Preparation for the replacement of the Clinac 4.

4. Acquisition and implementation of Remote Afterloading Low Dose Rate Brachytherapy equipment.

5. Clinical evaluation of the here developed rotating treatment chair[1].

Publications

1. Miller RW, Orr K, Goffman TE, Harrington FS, van de Geijn J, Glatstein E. A Simple CT Aperture Emulator for Use With a Radiotherapy Simulator. Int J Radiat Oncol Biol Phys 1992;22:195-9.

2. Miller RW, Raubitschek AA, Harrington FS, van de Geijn J, Ovadia J, Glatstein E. An Isocentric Chair for the Simulation and Treatment of Radiation Therapy Patients. Int J Radiat Oncol Biol Phys 1991;21:469-74.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06330-12 RO

PERIOD COVERED

October 1, 1992 to September 30, 1992

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 |

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 STAFF 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.)*

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 vast improvement of the medical imaging and localization technology over the recent past has brought a challenge to physics and computer technology to extend the computerized treatment simulation (planning and optimization) capabilities. Our photon and electron beam radiation field models have been described before. One virtue of these models is that they require relatively few experimental data and thus can be implemented very easily.

The implementing of our beam treatment planning program on a MacIntosh II system is continuing. Currently, the system is phased-in for clinical use. 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.

3-dimensional dose calculation in particular is very time consuming. An exploratory project has been initiated with DCRT to explore the potential of connecting our MacIntosh II system to a massive parallel processing system to alleviate this aspect. In addition to computational challenges, the project faces problems with the real-time 2-way transmission of very large amounts of data.

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 of arbitrary shape, 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.

3. To develop ways to overcome processing and data transfer problems with large amounts of data.

Methods Employed

Modeling of computed dosimetry of photon beams modified with shielding blocks, and supporting experimental work have been carried out. 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. Both diode measurement and film densitometry techniques are used.

Novel methods based on mathematical morphology and imaging software techniques have been incorporated in our Mac II treatment planning software.

As to clinical computer treatment planning, current emphasis is on clinical implementation of the present version of the MacIntosh II system. In the mean time optimization of our own photon and electron beam models is continuing. Quality assurance will require a great deal of data acquisition and processing activity.

Interaction with DCRT is in progress to determine the potential of networking to a there available massive parallel processing system.

Major Findings

The Mac II system is being clinically tested in parallel with the existing VAX-750 based system. Continuous interacting with the users (dosimetrists, physicists) have lead to the assembling of a manual with several levels of user guidance. Initial experience shows great potential for clinical support as well as teaching of residents as well as technologists, as the system can run on any 8 MB Mac II connected to our internal network.

For extensive 3-D treatment planning, processing and data transfer time are bottlenecks. Efforts are underway to study the potential of massive parallel processing.

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.)

3. Intensive exploration of the potential of massive parallel processing.

Publications

A paper has been submitted to the Medical Physics journal, on a novel method for fast generation of 2-D beam photon energy fluence distributions, regardless of field shape.

A paper is being finalized on dosimetry of photon beams modified with arbitrarily shaped shielding blocks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06351-10 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
| PI: | J.B. Mitchell | Acting Branch Chief | ROB, NCI |
| | J.A. Cook | Staff Fellow | 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 STAFF 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. IdUrd tumor DNA replacement data has been obtained from a number of patients (head/neck, sarcoma tumors) receiving IdUrd and has thus far revealed replacement values ranging from 7.3 to 14.2%, much higher than was seen for gliomas. Ultimately, we wish to determine if a correlation exists between IdUrd replacement and treatment outcome. We have initiated a new clinical protocol involving IdUrd combined with high and low dose rate irradiation in the treatment of cervix cancer. Biopsies will be taken prior to, during and after irradiation to determine IdUrd incorporation, labelling index, and the potential doubling time of the tumor. These parameters will be compared/contrasted with the clinical response. Laboratory studies suggest that the higher the IdUrd replacement, the greater the extent of radiosensitization. We will provide data to directly test this hypothesis in humans.

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 IdUrd 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 performed using a fluorescent microscope linked to laser excitation and computer image analysis systems.

Major Findings

IdUrd tumor DNA replacement data has been obtained from a number of patients (head/neck, sarcoma tumors) receiving IdUrd and has thusfar revealed replacement values ranging from 7.3 to 14.2%, much higher than was seen for gliomas. Tumors with higher IdUrd replacements have generally responded better to treatment.

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

We will continue work on cellular and tumor quantitations of IdUrd and provide support to the clinical studies. Also we will 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. Mitchell JB, Coleman CN. Keynote address: Biochemical modification of therapeutic response. Int J Radiat Oncol Biol Phys 1992;22:483-4.

2. Cook JA, Glass J, Lebovics R, Bobo H, Pass H, DeLaney TF, Oldfield EH, Mitchell JB, Glatstein E, 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. Cancer Res 1992;52:719-25.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06353-10 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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. Garmenstani | Visiting Scientist | ROB, NCI |

COOPERATING UNITS *(if any)*

Lab. of Cellular and Molecular Bio., NCI; Metabolism Branch, NCI; Johns Hopkins Med. School, Balt., MD (M. Strand); Argonne National Lab., Argonne, IL (R. W. Atcher); U. 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 STAFF 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.

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 radionuclidic properties available. Thus, Copper-67 is a weak, short range beta emitter. Yttrium-90 is long range, high energy beta emitter and Bismuth-212 a short range alpha emitter.

Based on these studies, a successful Phase I trial of treatment of Adult T-cell Leukemia has been completed. A phase II trial is now underway.

The compound cyclohexyl DTPA was recently tested and used for radioimmunotherapy of the Rauscher leukemia in mice. Efficacy was exceptional in that complete remission of disease was induced with no histological evidence of toxicity.

Studies like these are providing for human medicine a basis for design of rational therapies of malignancies by selectively targeting cytocidal agents to tumors, as well as metastases and will in addition 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 Pb-212, Bi-212 and Y-90 labeled antibodies in tumor therapy.

Findings With The $\alpha$-Particle Emitters Lead-212, Bismuth-212

1. Use of the CHX-A-DTPA isomer for labeling the 103A antibody against cells invaded with the Rauscher leukemia virus was investigated. Complete remission of tumor was achieved at a dose of 150 $\mu$Ci with no histological toxicity.

2. Lead-203 studies showed that lead-DOTA complexes were stable in vivo and useful for tumor imaging. Lead-212 complexes are similarly stable.

Major Findings With The β-Particle Emitter Yttrium-90

1. Use of Y-90-anti-Tac radioimmunoconjugates in conjunction with granulocyte colony stimulating factor prolonged graft survival in primate allograft transplantation. Radiotoxicity was less than that observed in the absence of GCSF in a similar xenograft transplant study.

2. In a Phase I clinical trial, Y-90-anti-Tac immunoconjugate proved effective in the treatment of adult T-cell leukemia.

3. Newer ligands for the use of Y-90 were developed. The CHX-A-DTPA ligand reduces release of Yttrium for the ligand in serum by at least 50%.

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.

Protocols for production of chelator-linked antibodies are in place. We recently linked the 1B4M-DTPA ligand to a humanized and genetically engineered anti-Tac antibody for a new Phase I clinical trial. We will continue to improve efficacy of Y-90 therapy by developing and using more stable chelating agents.

Publications

1. Brechbiel MW, Pippin CG, McMurry TJ, Milenic D, Roselli M, Colcher D, Gansow OA. An Effective Chelating Agent for Labeling of Monoclonal Antibody with $^{212}Bi$ for α-Particle Mediated Radioimmunotherapy. J Chem Soc, Chem Commun 1991;1169-1170.

2. Gansow OA, Brechbiel MW, Pippin CG, McMurry TJ, Lambrecht R, Colcher D, Schlom J, Roselli M, Strand M, Huneke RB, Ruegg CL. Lead and Bismuth Complexes of Functionalized DTPA Ligands and of the Polyazacycloalkane-N-Acetic Acid DOTA. Antibody, Immunocon and Radiopharm 1991;4:413.

3. McMurry TJ, Brechbiel MW, Kumar K, Gansow OA. Convenient Synthesis of Bifunctional Tetraaza Macrocycles. Bioconjugate Chem 1992;3:108-117.

4. Brechbiel MW, Gansow OA. Synthesis of C-Functionalized Derivatives of Trans-Cyclohexyldiethylenetriaminepenta-Acetic Acid for Labeling of Monoclonal Antibodies with the $^{212}Bi$ Alpha-Particle Emitter. J Chem Soc, Perkin Trans 1992;1:1173-1178.

5. Parenteau GL, Dirbas FM, Garmestani K, Brechbiel MW, Bukowski MA, Goldman CK, Souza LM, Clark R, Gansow OA, Waldmann TA. Yttrium-90 Labeled Anti-Tac in Conjunction with Granulocyte Colony Stimulating Factor Prolongs Graft Survival in Primate Allograft Transplantation. Transplantation 1992, In Press.

6. Hay RV, Casalino DD, Kordylewski L, Atcher RW, Brechbiel MW, Gansow OA, Sharokhizadeh U, Fleming RM, Lathrop KA, Stark VJ, Harper PV. Accumulation of Indium-111-Labeled Human Low Density Lipoprotein in the Rabbit Aorta: Implications for Nuclear Imaging of Vascular Lesions. Cardiovasc Path 1992, In Press.

7. Gansow OA, Brechbiel MW. US Patent 5,124,471: A Bifunctional DTPA-Type Ligand, 1992.

8. Gansow OA, Brechbiel MW. US Patent 5,099,069: Backbone Polysubstituted Chelates-Protein Conjugate, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM06357-09 RO

PERIOD COVERED
October 1, 1991 to September 30, 1992

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: | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| Others: | E. Glatstein | Chief | ROB, NCI |
| | 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 10 | 100 | |

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:

| | | |
|---|---|---|
| Frank Sullivan | Senior Investigator | ROB, NCI |
| W. Sindelar | Senior Investigator | SB, NCI |
| H. Pass | Senior Investigator | CNS, CC |

Objectives:

There 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

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 and Phase II studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06358-09 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

| | | | |
|---|---|---|---|
| P.I.: | P. Riesz | Research Chemist | ROB, NCI |
| Others: | Heasook Kim | Visiting Fellow | ROB, NCI |
| | L.J. Kirschenbaum | I.P.A. | ROB, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 2.5 | |

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.)*

Phthalocyanines and their sulfonated derivatives show considerable promise to replace hematoporphyrin derivatives for photodynamic therapy (PDT) of human tumors. The ability of tissue-omnipresent ascorbic acid (Vitamin C) to deactivate photoexcited phthalocyanine with consequent formation of the electron spin resonance detectable ascorbate radical, brings new perspective to the understanding of the chemical mechanisms of photodynamic reactions. Our studies with chloraluminum phthalocyanine tetrasulfonate and riboflavin (Vitamin B2) as photosensitizers demonstrate free radical formation by direct reaction between ascorbate ion and the excited triplet state of the photosensitizer. This process is in competition with the reaction of ascorbate ion with singlet oxygen, a reaction which does not lead to the formation of ascorbate radicals. The chemical effects of ultrasound were investigated in relation to sonodynamic cancer therapy, a new approach in which the synergistic effects of combined ultrasound and anticancer drugs (e.g. Adriamycin) have been observed. The 50 kHz sonolysis of argon-saturated dimethyl sulfoxide-water mixtures was studied by electron spin resonance and spin trapping with 3,5-dibromo-4-nitrosobenzenesulfonate. While for previous sonolysis studies of aqueous mixtures of solvents more volatile than water, spin-adducts yields decrease at high mole fractions of the organic solvent because of the decreasing effective ratio of the specific heat at constant pressure to the specific heat at constant volume in the imploding argon cavitation bubbles, the pyrolysis product of dimethylsulfoxide increases with increasing dimethylsulfoxide concentrations.

Project Description

Objectives: The effects of ionizing and ultraviolet radiation and of ultrasound on biological macromolecules and 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 a 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 (an xanthine oxidase inhibitor) protect ischemic tissue 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 Cobalt 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 a 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

1. Photosensitized Formation of Ascorbate Radicals by Chloroaluminum Phthalocyanine Tetrasulfonate: An Electron Spin Resonance Study {with Heasook Kim, Louis J. Kirschenbaum, and Ionel Rosenthal (The Volcani Center, BetDagan, Israel)}

   The chloroaluminum phthalocyanine tetrasulfonate sensitized photooxidation of ascorbic acid to ascorbate radical ($A^{\bullet -}$) was followed by ESR spectroscopy. In air-saturated aqueous media, steady-state amounts of $A^{\bullet -}$ are rapidly established upon irradiation. The ESR signal disappears within a few seconds after the light is extinguished - more slowly under constant irradiation as oxygen is depleted. No photooxidation was observed in deaerated media. The effect of added superoxide dismutase, catalase, desferrioxamine, and singlet oxygen scavengers ($NaN_3$, and tryptophan) was studied as was replacement of water by $D_2O$ and saturation with $O_2$. The results are indicative of free

radical production by direct reaction between ascorbate ion and sensitized phthalocyanine, a Type I mechanism in competition with the Type II reaction of $HA^-$ with singlet oxygen, a reaction which does not produce ascorbate radical intermediates.

2. Photosensitized Formations of Ascorbate Radicals by Riboflavin: An ESR Study {with Heasook Kim, Louis J. Kirschenbaum, and Ionel Rosenthal (The Volcani Center, BetDagan, Israel)}

The riboflavin sensitized photooxidation of ascorbate ion ($HA^-$) to ascorbate radical ($A^{\bullet -}$) was followed by ESR spectroscopy in conjunction with oxygen depletion measurements. In air-saturated aqueous media, steady-state amounts of $A^{\bullet -}$ are rapidly established upon irradiation. The ESR signal disappears with a few seconds after the light is extinguished - more slowly under constant irradiation as oxygen is depleted. No photooxidation was observed in deaerated media. Similar results were obtained with other flavins and when ascorbyl palmitate was substituted for $HA^-$. The effect of added superoxide dismutase, catalase, desferrioxamine, and singlet oxygen scavengers ($NaN_3$ and tryptophan) was studied as was replacement of water by $D_2O$ and saturation with $O_2$. The results are indicative of ascorbate-free radical production via direct reaction between ascorbate ion and triplet riboflavin in the presence of $O_2$. While the presence of superoxide ion tends to reduce the steady-state concentration of $A^{\bullet -}$, a competition from the reaction of $HA^-$ with singlet oxygen is less apparent in this system (at $HA^- > 1$ mM) than in the previously studied aluminum phthalocyanine tetrasulfonate photosensitized reaction.

3. Sonolysis of Dimethylsulfoxide-Water Mixtures: A Spin Trapping Study {with Takashi Kondo (Fukui Medical School, Fukui, Japan), Louis J. Kirschenbaum, and Heasook Kim}

The 50 kHz sonolysis of argon-saturated dimethylsulfoxide (DMSO) water mixtures was investigated by ESR and spin trapping with 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS). Two distinct product regions are observed. At low DMSO concentrations, methyl radicals are formed due to the reaction of hydroxyl radicals, generated by the thermal

decomposition of water vapor in collapsing cavitation bubbles, which escape into the bulk of the solution and react with DMSO. The maximum $CH_3$-DBNBS yield is observed near 0.1% DMSO (v/v). An apparent maximum in the yield of the sulfur trioxide anion radical spin adduct of DBNBS ($SO_3$-DBNBS) was found at 75% DMSO (v/v). Sulfur dioxide has previously been identified as a major product of the pyrolysis of DMSO vapor. In the presence of water, the $SO_2$ produced in the collapsing cavitation bubbles is oxidized by DBNBS in the bulk of the solution to $SO_3^{\bullet -}$, which subsequently forms stable $SO_3$-DBNBS adducts. Whereas for previous sonolysis studies with aqueous mixtures of solvents more volatile than water, spin-adduct yields decrease at high mole fractions, because of decreasing effective g = Cp/Cv in the imploding argon cavitation bubbles, the thermolysis product of DMSO formed by sonolysis increases with increasing DMSO concentration.

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. 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;24-61.

2. Riesz P, Christman CL. Sonochemical exposure methods. In: Mason TJ, ed. Practical Sonochemistry-A Short Laboratory Text 1992, in press.

3. Kondo T, Riesz P. Effect of ultrasound and ionizing radiation on a sterically hindered cyclic secondary amine. An ESR study. Radiat Res 1991;127:11-8.

4. Kondo T, Krishna CM, Riesz P. Pyrolysis radicals formed by ultrasound in aqueous solutions. In: Niki E, ed. Magnetic Resonance in Medicine, vol 2. Tokyo: Nihonigakukan, 1991;137-42.

5. Krishna CM, Uppuluri S, Riesz P, Zigler JS, Balasubramanian D. A study of the photodynamic efficiencies of some eye lens constituents. Photochem Photobiol 1991;54:51-8.

6. Riesz P. The role of type II and type I mechanisms in photosensitization: Switching pathways with electron donors. Photomed Photobiol 1991;13:35-8.

7. Kim H, Rosenthal I, Kirschenbaum LJ, Riesz P. Photosensitized formation of ascorbate radicals by chloroaluminum phthalocyanine tetrasulfonate. An ESR study. Free Radical Biol Med 1992, in press.

8. Riesz P, Kondo T. Free radical formation induced by ultrasound and its biological implications. Free Radical Biol Med 1992, in press.

9. Riesz P, Kondo T, Carmichael AJ. Sonochemistry of acetone and acetonitrile in aqueous solutions. A spin trapping study. Free Radical Res Comm 1992, in press.

10. Kondo T, Riesz P. Effect of ionizing radiation and ultrasound on a sterically hindered cyclic secondary amine, 2,2,6,6-tetramethyl-4-piperidone. In: Mori S, Yoshikawa T, eds. Magnetic Resonance in Medicine, vol 4. 1993, in press.

11. Kondo T, Kirschenbaum LJ, Kim H, Riesz P. Sonolysis of dimethylsulfoxide-water mixtures: A spin trapping study. J Phys Chem 1993, in Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06361-08 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 | Acting Branch Chief | ROB, NCI |
| | H. Pass | Senior Investigator | ROB, NCI |
| | P. Smith | Senior Investigator | ROB, NCI |
| | W. Friauf | 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 STAFF 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 with 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/cm2 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 structures such as the esophagus, parietal and visceral pleura, heart can tolerate 35 J/cm2 red light. We have now finished the Phase I trial using DHE/630 nm light to treat unresectable intrathoracic mesothelioma and intraperitoneal ovarian/sarcoma/colon carcinoma. Currently, 32.5 J/cm has been used the clinical treatment of intrathoracic mesothelioma. We have begun a Phase III study for ovarian carcinoma and have submitted a protocol for retroperitoneal sarcoma. 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. Hematoporphyrin was covalently bound to the specific monoclonal antibody directed against the xenograft. The results show that tumor can be eradicated and that dermatophototoxicity 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.

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 toxicity 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. 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 Surg Medicine 1991, in press.

2. Progrebniak HW, Matthew W, Black C, Russo A, Smith P, Roth JA, Pass HI. Targetted Phototherapy with sensitizer-monoclonal antibody and light. Surgical Forum 1991;42:447-449.

3. Friauf WS, Smith PE, Russo A, Delaney TF, Pass HI, Cole HW, Gibson, CC, Sindelar WF, Thomas G. In Nagele TH, Tompkins WJ, Eds. Case Studies in Medical Instrument Design. IEEE. New York, 1992:127-38.

4. Sperduto PW, DeLaney TF, Thomas G, Smith P, Dachowski LJ, Russo A, Bonner R, Glatstein E. Phototherapy for chest wall recurrence in breast cancer. Int J Radiat Oncol Biol Phys 1991;21:441-6.

5. Bernstein EF, Glass JM, DeGraff WG, Schlegel R, Black C, Fisher JM, Cook SN, Glatstein E, Russo A, Mitchell JB. In vitro photodynamic treatment of normal and human papilloma virus-transfected keratinocytes with photofrin II and red light. Arch Dermatol 1991;127:683-7.

6. Bernstein EF, Friauf WS, Smith PD, Cole JW, Solomon RE, Fessler FF, Russo A. Transcutaneous Determination of Tissue Dihematoporphyrin Ether Content: A device to Optimize Photodynamic therapy. Dermatology, in press.

7. Pass HI, DeLaney T, Russo A, Mitchell J, Smith P, Friauf W, Thomas G. Feasibility of intrapleural photodynamic therapy: The first eight patients. SPIE 1992;1645:1-9.

8. DeLaney TF, Smith PD, Thomas GF, Tochner ZA, Sindelar WF, Pass HI, Harrington FS, Bonner RF, Mitchell JB. A light-diffusing device for intraoperative photodynamic therapy in the peritoneal or pleural cavity. J Clin Laser Med Surg 1991;Oct:361-6.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06378-07 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

QA of Treatment Delivery by means of Overlaid 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 |
| | H. Xie | Computer Specialist | ROB, NCI |
| | K. Yeakle-Orr | Dosimetrist | ROB, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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 employs graticules projecting onto all simulator films and all corresponding portfilms. A project has been started to overlay 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.

Project Description

Objectives:

1) To improve the quality of documentation on the proper implementation of beam treatment set-ups.

2) To integrate 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, centering 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 system MacTPS is being phased-in for routine clinical use. Lack of proper video camera, clinical satisfaction with the graticule facilitated visual film verification method, have lowered the priorities. The project should still be pursued ASAP.

Significance to Biomedical Research and the Program of the Institute

Short of real-time imaging the present proposed system should be attractive:

1. Quality assurance and verification would become much more efficient, self-contained and attractive to use.

2. Documentation of treatment delivery could be compacted for follow-up purposes and easier to use.

3. Quality assurance of joint studies could be made easier and more objective.

Proposed Course

To incorporate of this technique into the MacIntosh II TPS and start a pilot project as soon as means and personnel are available.

Publications

None yet.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06379-06 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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: | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| Others: | T.F. DeLaney | Senior Investigator | 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.25 | 0.25 |

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. This is followed by the delivery of light to the effective area using optical fibers coupled to an argon/pumped dye laser. Hematoporphryrin 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.





PHS 6040 (Rev. 5/92)

Professional Personnel Engaged on Project

| | | |
|---|---|---|
| H. Pass | Senior Investigator | SB, NCI |
| W. Sindelar | Senior Investigator | SB, NCI |
| W. Friauf | Engineer | 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 successfully 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.

Among 25 patients who have been treated on this protocol for tumors obstructing the trachea bronchus, eighteen had metastatic lesions and seven had tumors of the bronchus or the trachea. Sixteen (64%) had the lobe expanded or airway opened. One (4%) had partial reopening after 1 treatment with complete reexpansion with a second treatment. Two patients (8%) had transient airway opening. Two patients had a mixed response with 1 of 2 treated bronchi reopening and 4 patients (16%) had no response. Three complications were seen: 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 these findings, a formal phase I study of photodynamic therapy for intraperitoneal malignancies was initiated and completed (Project # Z01 CM 06393-02 R0) study of photodynamic therapy for intraperitoneal malignancies, which defines the maximum tolerated dose of photodynamic therapy which can be delivered at the time of surgery to the entire peritoneal surface.

Seven patients with tumors involving the pleural surface 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 these, formal study of Surgery and Photodynamic Therapy for Pleural Malignancies has been initiated in collaboration with Dr. Harvey Pass, Surgery Branch, NCI.

One patient with carcinoma of the nasopharnyx who had a recurrence in the primary site was treated with photodynamic therapy which induced a transient 5 month remission before he again recurred.

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. It offers potential organ preservation in some anatomic sites and is able to irradicate some tumors resistant to radiation or chemotherapy.

Proposed Course

Phase II study of photodynamic therapy for multi-focal peritoneal sarcomas and recurrent retroperitoneal sarcomas has recently opened. Phase I studies of photodynamic therapy for tumors in the pleural cavity and superficial urinary bladder carcinomas are nearing completion. A Phase II study of photodynamic therapy for patients with a refractory of ovarian cancers planned. 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.

2. DeLaney TF, Smith PD, Thomas G, Tochner ZA, Sindelar WF, Pass HI, Harrington FS, Bonner RF, Mitchell JB. A light diffusing device for intraoperative photodynamic therapy in the pleural cavity, J Clin Laser Med Surg 1991;October:361-366.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06381-06 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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: | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| | J. Mitchell | Acting Branch Chief | ROB, NCI |
| | R. Miller | Radiation Physicist | ROB, NCI |
| | J. Chen | Radiation Physicist | ROB, NCI |
| | E. Glatstein | Branch Chief | 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 STAFF 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.)*

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,and as well-targeted as possible. The limiting factor in tumor resistant is normal tissue reaction: Normal tissue reaction 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 empiricle, however. The present project continues the development and exploration of a theoretical description of time-dose response of tumors as well as normal tissues. Our work has concentrated on extension of the Linear-Quadratic model. A paper [1] was devoted to establishing the relationship between the $\alpha,\beta$ model, including repair of sublethal damage for conventional and (accelerated) hyperfractionation and the $\alpha,\beta$ model for continuous low dose rate. The study of implications of high dose rate versus low dose rate brachytherapy therapy is ongoing. An interactive computer program and graphical tools to help clinical guidance in the search for high dose rates compatible with constant tumor control as well as no more severe toxicity has reached a useful stage. Changed priorities related to changes in the medical staff have temporarily slowed-down the developments.

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

The present a,b model includes allowance for repair of sublethal damage, normal tissue repopulation and exponential tumor cell proliferation;

It assumes normal tissue cell loss and replacement are under homeostatic control;
clonogenic tumor cells are assumed to proliferate exponentially over time;
stem-cell proliferation is triggered only after some distress signal related to functionality cell levels drop below a certain threshold;
normal tissue tolerance represents the lower limit of normal tissue functionality.

Major Findings

1. The current extension of the "Linear Quadratic" (LQ) model of single dose response [1] covers both high dose rate fractionated (external beam) therapy and high dose rate as well as low dose rate brachytherapy.

2. Interactive computer programs developed by our group enable automatic search for acceptable parameters, based on reasonable estimated ranges of certain key parameters, a and b, cell doubling times, etc.

3. It is now possible under certain conditions, to extract TDR parameters for tumors from clinical data obtained with different fractionation schemes.

4. The current status of our work make it should be possible to select "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. These theoretical findings need to be tested against clinical findings. Thus, the future of this approach depends on the viability of clinical participation in developing clinical data.

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. Development and study of clinical data.

Publications

1. 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

Z01 CM 06387-05 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 |
| | J.B. Mitchell | Acting Branch Chief | ROB, NCI |
| | M.C. Krishna | Visiting Scientist | ROB, NCI |
| Others: | W. DeGraff | Biologist | ROB, NCI |
| | S. Hahn | Associate | ROB, NCI |
| | J. Liebmann | Associate | ROB, NCI |
| | F. Sullivan | Acting Clinical Chief | ROB, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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.)*

Our laboratory has shown that nitroxides, which have been used as EPR spin labels exhibit superoxide dismutase (SOD) activity and are quite effective agents in protecting cells against a wide variety of oxidative stresses. 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. We have demonstrated that Tempol protects both cells *in vitro* and mice against ionizing radiation. Thus, the nitroxides represent a *new class* of radiation protectors that may have widespread use in protecting humans against radiation. Preliminary studies using a rodent tumor model have shown that Tempol does not protect tumor tissue. Further chemical characterization of the SOD mimic activity of nitroxides has revealed the presence of an oxoammonium cation intermediate. This information will be used to rationally screen a series of nitroxides for maximal protective capacity based on their oxidation potential and charge. 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. Topically applied, Tempol has been shown to protect against radiation-induced alopecia in guinea pigs. Since these agents readily penetrate cell membranes, they may be of use in other areas of medical research such as ischemia/reperfusion injury studies.

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 in biological systems. 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 and against mutation induction in mammalian cells exposed to superoxide, hydrogen peroxide, and x-rays. These results establishes the nitroxides as a completely new class of radiation protectors with possible widespread use. Radiation-induced alopecia could be greatly reduced by topical application of Tempol. New chemical characterization of the SOD mimic reaction of nitroxides has revealed the oxoammonium cation intermediate. This finding will be used to rationally screen a series of nitroxides to identify compounds with maximal radioprotective properties.

Significance to Biomedical Research and the Program of the Institute

The study of nitroxides 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 damage. The nitroxides 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 refining differential radiation protection between normal versus tumor tissues for nitroxides. Since nitroxides protect against selected chemotherapy drug cytotoxicity they will be evaluated *in vivo*.

Publications

1. Mitchell JB, DeGraff W, Kaufman D, Krishna MC, Samuni A, Finkelstein E, Hahn SM, Gamson J, Russo A. Inhibition of oxygen dependent radiation-induced damage by the nitroxide superoxide dismutase mimic TEMPOL. Arch Biochem Biophys 1991;289:62-70.

2. Goffman T, Cuscela D, Glass J, Hahn S, Krishna MC, Lupton G, Mitchell JB. Topical application of nitroxide protects radiation-induced alopecia in guinea pigs. Int J Radiat Oncol Biol Phys 1991;22:803-6.

3. Hahn SM, Krishna CM, Samuni A, Mitchell JB, Russo A. Mn(III)-Desferrioxamine Superoxide Dismutase-Mimic: Alternative Modes of Action. Arch Biochem Biophys 1991;288(1):215-9.

4. DeGraff WG, Krishna MC, Russo A, Mitchell JB. Antimutagenicity of a low molecular weight superoxide dismutase mimic against oxidative mutagens. Environ Mol Mutagen 1992;19:21-6.

5. Krishna CM, Liebmann JE, Kaufman D, DeGraff W, Hahn SM, McMurry T, Mitchell JB, Russo A. The cathecholic metal sequestering agent 1,2-dihydroxybenzene-3,5-disulfonate confers protection against oxidative cell damage. Arch Biochem Biophys 1992;294(1):98-106.

6. Hahn SM, Tochner Z, Krishna CM, Glass J, Wilson L, Samuni A, Sprague M, Venzon D, Glatstein E, Mitchell JB, Russo A. Tempol, a stable free radical, is a novel murine radiation protector. Cancer Res 1992;52:1750-3.

7. Krishna MC, Grahame DA, Samuni A, Mitchell JB, Russo A. Oxoammonium cation intermediate in the nitroxide-catalyzed dismutation of superoxide. PNAS 1992;89:5537-41.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06388-05 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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: | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| Others: | T.F. DeLaney | Senior Investigator | ROB, NCI |
| | 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.25 | 0.25 |

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 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. Patients with high-grade in-situ in association with papillary tumor have a high frequency 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 a high risk for the development of invasive disease and may require removal of the urinary bladder (cystectomy). Recent work with hematoporphyrin derivative (HpD) 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 irradiation of tumor.

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 ($^{H}p^{D}$) and laser light, and to judge tumor response.

Methods Employed

Eligible patients receive hematoporphyrin by intravenous injection. They will subsequently undergo a cystoscopy at which time light is delivered to the bladder. Regular follow-up is performed to assess response to treatment.

Major Findings

Sixteen patients have been entered on the protocol since 7/89. Fifteen treatments have been given. Which resulted in 8 complete responses, 6 none responses, and 1 "atypia" at the time of restaging cystoscopy 3 months after treatment. Five patients remain free of disease 3 to 33 months after completion of photodynamic therapy. The other patients have recurred 3 to 12 months after photodynamic therapy. Patients have experienced transient acute bladder irritation secondary to photodynamic therapy which has been managed symptomatically. Usually patients were treated with a photosensitizer dose of 2 mg/kg given 48 hours prior to treatment with light doses between 2420 and 5032 joules to the entire bladder. Of 7 treatments given in this manner, 2 patients developed vesico-ureteral reflux which was accompany by severe chronic bladder irritability hydro-ureteral nephrosis in bladder volume loss in 1 patient. We subsequently lowered the drug dose to 1.5 mg/kg and treated patients at high doses of 2500 to 3500 joules. Of this regimen acute cystitis was milder. The 2 patients treated at 3500 joules developed vesico-ureteral reflux at the time of follow-up cystoscopy; no significant complications were treated at lower light doses.

We have developed a light monitoring device utilizing very thin optical fibers which can be passed through the cystoscope and permits measurement of light dose received at the bladder wall during photodynamic therapy. This is a substantial improvement in dosimetry and should have wide applicability. A patent application is pending.

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 significant advance in the treatment of superficial carcinoma of the bladder.

Proposed Course

We proposed to study a total of 18 patients on the protocol. The additional patients will allow us to establish a safe treatment regimen, which currently looks to be 1.5 mg/kg in association with 3000 joules to the entire bladder. No significant complications have been received at this dose. Two of three patients treated at this dose have had a complete response to treatment and they are free of disease at 12 months after treatment. Once a tolerable dose regimen has been established, a decision will be made about a Phase II study of patients with superficial bladder cancer at this dose level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06390-04 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Bifunctional Chelating Agents 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 |
| Others: | C.G. Pippin | Senior Staff Fellow | ROB, NCI |
| | M. Brechbiel | Chemist | ROB, NCI |
| | C. Wu | Visiting Fellow | ROB, NCI |
| | O. Gansow | Senior Investigator | ROB, NCI |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.4 | 1.4 | |

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 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 chemical literature elucidating the coordinating chemistry of Ga(III), we targeted two compounds as promising candidates for attaching Ga(III) isotopes to monoclonal antibody. 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 synthesis of p-NCS-Bz-NOTA has recently been completed in both C-14 labeled and in high purity C-12 form suitable for clinical experiments should the need arise. With both target compounds now in hand, we can begin to assess the *in vitro* and *in vivo* stability of the Ga(III) complexes and the imunoconjugates. Preliminary experiments show that both compounds are efficiently labeled with In-111, and serum stability studies are 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

Cyclization techniques used to efficiently prepare 12-and 14-membered ring macrocyclic tetraamines failed to produce the 9-membered triamine NOTA precursor due to an intramolecular reaction. Thus, an alternative strategy employing a Richman-Atkins tosylamide cyclization was developed. The cyclization conditions were found to be incompatible with the nitro-moiety commonly used as a latent isothiocyanate in the preparation of bifunctional chelating agents, but work efficiently using a benzamide protected aniline instead. The conjugates of p-NCS-Bz-NOTA with mAb B72.3, B3, and IgG were found to label efficiently with In-111.

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

1. McMurry TJ, Brechbiel M, Kumar K, Gansow O. Convenient Synthesis of Macrocyclic Bifunctional Chelating Agents. Bioconjugate Chemistry 1992;3:108-117.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06391-03 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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: | A. Epstein | Senior Investigator | ROB, NCI |
| Others: | T. Goffman | Head, Clinical therapy Sect. | ROB, NCI |
| | J. Mitchell | Deputy Chief | ROB, NCI |
| | A. Russo | Head, Exp. Phototherapy Sect. | ROB, NCI |
| | J. Cook | Senior Staff Fellow | ROB, NCI |
| | R. Smith | Supervisory Nurse Specialist | ROB, NCI |
| | S. Steinberg | Head | BDMS, NCI |

COOPERATING UNITS *(if any)*

Cancer Nursing Service, Clinical Center, 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 STAFF 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 17 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. We are trying to enlist participation by other centers that have a large experience with sarcomas.

Project Description

Professional Personnel Engaged on the Project:

| | | |
|---|---|---|
| J. Mitchell, Ph.D. | Acting Branch 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. | Supervisory Nurse Specialist | CNS, CC |
| S. Rosenberg, M.D. | Chief | SB, NCI |
| S. Steinberg, Ph.D. | Head | BDMS, NCI |

Objectives

The main objective is to assess the results of patients treated with IUdR as a radio sensitizer 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. Also, 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06392-03 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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: | A. Epstein | Senior Investigator | ROB, NCI |
| Others: | T. Goffman | Head, Clinical Theapy Sect. | ROB, NCI |
| | J. Mitchell | Deputy Chief | ROB, NCI |
| | A. Russo | Head, Exp. Phototherapy Sec. | ROB, NCI |
| | J. Cook | Senior Staff Fellow | ROB, NCI |
| | R. Smith | Supervisory Nurse Spec. | CNS, CC |
| | 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 STAFF 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: (current total 99) the focus this year continues to be obtaining biopsies as delineated in the original protocol for thymidine replacement. The results have been variable, but no toxicity was encountered in biopsy if 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 in Cancer. A long-term follow-up of the glioblastoma results has been published this year 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, and most recently have extended the project to include locally advanced cervix cancer.

Project Description

Professional Personnel Engaged on the Project:

| | | |
|---|---|---|
| J. Mitchell, Ph.D. | Acting Branch 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. | Supervisory Nursing Specialist | CNS, CC |
| H. Pass, M.D. | Senior Investigator | SB, NCI |
| S. Steinberg, Ph.D. | Head | BDMS, NCI |
| R. Lebovics | Senior Investigator | DCD, IR |

Objectives

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.

In selected patietns 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.

Method 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 historical controls.

Major Findings

Too soon to show.

Proposed Course

Continuation of current studies.

Publications

1. 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 phaseI/II study of glioblastoma multiforme treated with IdUdr and hyperfractionated irradiation, J Clin Oncol, 1992;10, 264-268.

2. Cook JA, Glass J, Lebovics R, Bobo H, Pass H, DeLaney TF, Oldfield EM, Mitchell JB, Glatstein E, 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. Cancer research, 1992; 52:719-725.

3. Epstein AH, Cook JA, Goffman T, Glatstein E. Tumor radiosensitization with the halogenated pyrimidines 5'Bromo (urIodo)deoxyuridine. Submitted to British Journal of Radiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06394-03 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Dev. insight into Radiolabelled Antibody Dosimetry by comp. sim. & exp. 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 |
| | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| | J. Carrasquillo | Nuclear Medicine Physician | NM, CC |

COOPERATING UNITS *(if any)*

Nuclear Medicine, Clinical Center

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 10 | 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 of 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 response in air is essentially independent of distance. The volume from which response is contributing is essentially a cylinder with a diameter about equal to that of the area of interest.

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. So far, 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. Current staffing and operational priorities have temporarily put this project on hold.

Publications

None; a provisional internal report is available.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06395-03 RO

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 STAFF 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 chemistry of metal ions utilized in radioimmunotherapy, especially lead and bismuth. To accomplish this objective, we need to learn the coordination chemistry of the metal ions, in particular, their kinetic and thermodynamic behaviors in solution. This information can then be used to develop radiolabeled antibody systems for use in therapy.

Substantive progress was made on two projects. First, Lead (II) complexes of the DOTA ligand were investigated in aqueous solution to model the behavior of the 212Pb $\alpha$-particle emitter we wish to use for radioimmunotherapy of cancer. The kinetics of formation of the complex were measured and a possible mechanism for complex formation established. This information was employed to create an efficient method for labeling antibodies with 212 Pb.

Secondly, the chemistry of bismuth (III) complexes with polyaminocarboxylate ligand DTPA and EDTA was investigated by physical methods including IR, NMR and X-ray diffraction. Both the CyclohexylDTPA complex which is stable in vivo and the parent DTPA complex show one carboxyl ligand that is less tightly bound than the others as evidenced in NMR spectra. The stability constant of the cyclohexyl derivative is, however, greater than that of the parent.

Objectives

The prime objective of this project is to develop an understanding of the fundamental chemistry of metal ions utilized in radioimmunotherapy, especially lead and bismuth. To accomplish this objective, we need to learn the coordination chemistry of the metal ions, in particular, their kinetic and thermodynamic behaviors in solution. This information can then be used to develop radiolabeled antibody systems for use in therapy.

Professional Personnel Engaged On The Project

| | | |
|---|---|---|
| Jim Silverton | Senior Investigator | NHLBI |

Methods Employed

Physical methods in chemistry were principally employed in this study. X-ray diffraction methods were used to determine crystal structures of bismuth with DTPA and EDTA ligands. Stopped-flow kinetics techniques were employed for measurement of kinetics of formation and dissociation of lead and bismuth complexes with these ligands. NMR spectra were obtained to investigate structures of the complexes in aqueous medium.

Major Findings

We have learned that the complex of lead with the DOTA ligand is quite stable, with a thermodynamic stability constant of $10^{23.8}$. As a result to kinetics studies, a mechanism for formation of lead-DOTA was devised which involves the rapid formation of a protonated intermediate which subsequently loses a proton during the rate limiting step. By comparison, lead-DOTA dissociates in acid by more than one pathway, forming the aquo Pb(II) cation and DOTA. These results were employed to develop a technique for labeling antibodies with $^{212}$Pb-DOTA.

Similarly, studies of this type with Bismuth (III) -DTPA complexes have lead to a substantive increase in yield of 212Bi labeled antibodies now being evaluated for use in radioimmunotherapy of cancer.

A separate study of the analytical chemistry of chelators linked to antibody led to the development of a method to quantitate the number of chelator ligands linked to a sample of immunoprotein.

Publication

Pippin CG, Parker TA, McMurry TJ, Brechbiel MW. Specroscopic Method for Determination of a Bifunctional DTPA Ligand in DTPA-Monoclonal Antibody Conjugates. Bioconjugate Chemistry, In Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06397-01-RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Study of Surgery and Photodynamic Therapy for Intraperitoneal Sarcomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | F. Sullivan | Acting Clinical Chief | ROB, NCI |
| Others: | 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 STAFF 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.)*

Visceral sarcomas frequently develop multi-focal involvement of the peritoneal cavity. Similarly patients with retroperitoneal sarcomas can also develop multi-focal sarcoma of the peritoneal cavity. There's no effective treatment strategy for either of these problems. In a prior study (Z01 CM 0693-02 RO, Study of Surgery and Photodynamic Therapy for Intraperitoneal Malignancies), we develop the technique and defined the maximum tolerated dose of photodynamic therapy that could be delivered to the peritoneal surface. As a natural extension of that prior study, we are moving forward with this Phase II Study of Resection and Intraoperative Photodynamic Therapy for Intraperitoneal Sarcomatosis or Recurrent Retroperitoneal Sarcomas. Patients receive the dihematoporphyrin ethers photosensitizer 2.5 mg/kg 48 hours prior to a surgical procedure which involves surgical removal of tumor and intraoperative light delivery for photodynamic effect to the entire peritoneal surface.

Professional Personnel Engaged on Project

| | | |
|---|---|---|
| W. Sindelar | Senior Investigator | SB, NCI |
| W. Friauf | Engineer | BEIP, NCRR |
| P. Smith | Laser Physicist | BEIP, NCRR |
| J. Taubenberger | Senior Investigator | LP, NCI |
| P. Choyke | Senior Investigator | DRD, CC |
| D. Venzon | Senior Investigator | BDMS, NCI |

Objective

Phase II study to evaluate response of intraperitoneal sarcomas to surgical resection of photodynamic therapy.

Major Findings

Five patients have been accrued for this study. Four patients were able to undergo surgical debulking and photodynamic therapy. No major treatment complications have been seen. Two of the four patients remain free of disease six to nine months after treatment.

Significance to Biomedical Research and the Program of the Institute

If substantial activity of this treatment regime is seen against intra-abdominal sarcomas, we believe it can be incorporated in the initial management of retroperitoneal sarcomas and visceral sarcomas.

Proposed Course

Studies approved for 24 patients. Estimated duration of study is 2 years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06398-01 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Phenolic Substituted Ligands for Diagnosis and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.W. Brechbiel | Chemist | ROB, NCI |
| Others: | T.J. McMurry | Senior Staff Fellow | ROB, NCI |
| | C.G. Pippin | Staff Fellow | ROB, NCI |
| | O.A. Gansow | Senior Investigator | ROB, NCI |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | | |

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 principal objective of this project is to obtain an understanding of the fundamental coordination chemistry of polyaminocarboxylate ligands which have had one or more carboxylates replaced with a phenolate metal binding site. Thus, synthesis of a series of novel acyclic ligands of various substitution patterns is required. Information acquired from evaluation of the solution chemistry of these compounds, their kinetic and thermodynamic properties, and their serum stability may be employed to gauge the potential usefulness of the complexes in vivo.

These studies will thus provide a basis for rational design of bifunctional phenolic azacarboxylate ligands for complexing metals useful as cytocidal or diagnostic agents.

Further synthetic efforts to incorporate a reactive protein linking substitutent into the synthesis of the useful chelating agents would be initiated and ultimately be applied to preparing useful monoclonal antibody conjugates.

Objectives

The initial target compound for study was HBDTTA, which was diethylenetriamine pentaacetic acid (DTPA) modified with two orthohydroxybenzyl groups symmetrically replacing carboxylate groups at the one and seven positions. This ligand was designed to take advantage of the extensive chemical literature of the EDTA analogue, HBED, which has demonstrated considerable stability for its metal complexes at physiological pH. Synthesis of the homolog, HBDTTA, then retains the symmetry of HBED while simultaneously expanding the number of binding sites to maintain the octadentate environment for complexing 7-9 coordinate metals ions such as Bi, Pb, Y, Tb, Eu, and Gd.

Further potential synthetic efforts within the DTPA analog would include the two possible mono-phenolate substituted and the asymmetric di-substituted compounds. Examination of the solution chemistry of these ligands, in comparison with HBDTTA, will be used to indicate which pattern and number of phenolate donors might be useful as antibody conjugates.

Methods Employed

Standard synthetic organic chemistry methodology is required for the preparation of both compounds. Their characterization is being performed by routine analytical techniques.

Major Findings

The target ligand, HBDTTA, and the 2 - (para-nitrobenzyl) substituted analogue have been synthesized, fully characterized, and routine purification methods are established for each compound.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06399-01 RO

PERIOD COVERED

October 1, 1991 to September 1, 1992

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

Thiol Containing Ligands for Pb(II) and Bi(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 |
| Others: | C.G. Pippin | Senior Staff Fellow | ROB, NCI |
| | M. Brechbiel | Chemist | ROB, NCI |
| | C. Wu | Visiting Fellow | ROB, NCI |
| | O. Gansow | Senior Investigator | ROB, NCI |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.4 | |

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 goal of the project is to synthesize bifunctional ligands which have a high affinity for Pb(II) and Bi(III) by virtue of the presence of sulfur donor atoms. Simple thiolate ligands such as dimercaptosuccinic acid have been shown to form complexes of high stability with both ions (Kf for the 1:2 Bi:DMSA complex is log 43.87), yet appropriate multidentate chelating agents suitable for linkage to monoclonal antibody are not yet available. We intend to systematically explore this chemistry by incorporating thiol donor atoms into both acyclic and cyclic chelating agents. Appropriate precursor molecules have been synthesized which will allow us to test methodology for the incorporation of thiols into new chelating agents.

Objectives

We plan to develop feasible strategies to incorporate thiolate donors into bifunctional acyclic and macrocyclic chelating agents. Because of the tendency of sulfur to alkylate and oxidize, our initial efforts will focus on finding an appropriate thiol protecting group which will allow us to introduce the desired thiol late in the synthesis, after which it can be unmasked efficiently.

The thermodynamic stability and physical properties of the Pb(II) and Bi(III) complexes will be determined by classical techniques, including potentiometry, spectrophotometry, electrochemistry, and NMR. Conditions for conjugation to protein and labeling will be investigated.

Compounds which exhibit a high affinity for Pb(II) and Bi(III) will be synthesized in a bifunctional version to permit conjugation to monoclonal antibody. Experiments to determine the *in vivo* stability and utility as carriers for Pb-212 and Bi-212 will then be performed.

The ability to prepare chelating agents with thiol donors will facilitate the systemic evaluation of the effect of ligand structure on the chemical fate of Bi-212 following the β-decay of Pb-212. Previous work has shown that following this event, a significant fraction of Bi212 is ejected from the coordination sphere of DOTA, currently our best ligand for the Pb/Bi-212 pair. We will investigate the radiochemistry of the Pb-212/thiolate complex to establish whether or not incorporation of the large polarizable thiolate anion in the chelating agent can affect the fraction of Bi-212 which is lost during β decay.

The development of new chelators for Pb-212 ($t_{1/2}$ 10.6 hrs) would enhance our ability to treat AIDS related lymphoma and other blood borne malignancies using radioimmunotherapy.

Methods Employed

Standard organic and inorganic synthetic techniques are required for the preparation of the new ligands.

Major Findings

Substituted benzyl groups (p-$OCH_3$, p-$NO_2$) moiety were found to provide efficient protection of the sulfur during alkylation of secondary amines. Efficient deprotection of the thiol has not yet been achieved, suggesting an alternative protecting group may be needed (eg. t-Bu).

Significance to Biomedical Research and the Program of the Institute

When combined with monoclonal antibody, Pb-212 (t1/2 10.6 hours) and its highly cytotoxic daughter Bi-212 ($t_{1/2}$ 1 hour) provide a potential *in vivo*

generator system for the treatment of malignant disease. While significant advances have been made in the area of sequestering Bi-212, current use of Pb-212 is restricted to systems in which the antibody is rapidly internalized into the malignant cell. This work directly addresses the goal of providing Pb-212 as a longer-lived alpha emitter for use in radioimmunotherapy by developing chelating agents with a high affinity for both Pb(II) and Bi(III).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07224-01 RO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Pulse wave EPR Spectroscopy & Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Russo | Senior Investigator | ROB, NCI |
| | J.B. Mitchell | Acting Branch Chief | ROB, NCI |
| Others: | Murali Krishna | Visiting Scientist | ROB, NCI |
| | John Bourg | Engineer/Comp | ROB, NCI |
| | Walter Friauf | Section Chief | EEES, BEIP |
| | Paul Smith | Physicist | EEES, BEIP |
| | Thomas Pohida | Engineer | EEES, BEIP |
| | Rolf Tschudin | Engineer | LCP, NIDDK |
| | B. Subramanian | Visiting Scientist | ROB, NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 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.)*

Electron Paramagnetic Resonance (EPR) provides a sensitive means of detecting and quantitating free radical species. Conventional constant wave (CW) EPR has provided insight into the basic chemistry of free radical reactions and has most recently been increasingly used to probe the intricacies of biological intermediates. At this time, although EPR is vastly more sensitive than nuclear magnetic resonance, its use as a biological tool as been limited by both the frequency used--routinely greater 1 MHz--and the paucity of detectable signal. The Radiation Oncology Branch is involved in the development of both a pulse wave (PW) EPR spectrometer and PW-EPR imaging. Because the electron decay are in the order of microseconds as compared to the vastly longer nuclear relaxation times, the use of nanosecond PW-EPR with extremely rapid signal averaging and processing offers the possibly of surmounting the previous limitations imposed by limitations imposed signal. Likewise, the previous limitations imposed by CW-EPR MHz frequencies need not remain an impasse to EPR based imaging when pulse field and tenths of MHz frequencies and gradient profiling is employed. The project is multi-disciplinary requiring state of the art electronic, computation, gradient design, etc. The project is designed to create a prototypical PW-EPR instrument for *in vitro* biologic studies and to establish the foundational framework to demonstrate the application of PW-EPR to *in vivo* imaging. Hopefully, it will ultimately yield insight into cellular events that occur at the microsecond time scales and should because of the inherent physics of rapid free induction decay of excited electrons yield practically real time imaging with voxel resolution not attainable by conventional nuclear magnetic resonance based magnetic resonance imaging.

Project Description

Objective: To develop pulse wave EPR spectroscopy and imaging for study of both the chemical mechanistic aspects of drug and/or radiation as it relates to treatment of cancer and to define and paramatize those aspects of imaging with either natural biologic paramagnetic ions or with developing contrast agents.

Methods Employed

Development of techniques for broad spectrum excitation, and design and fabrication of high gradient coils that are needed for Pulse wave EPR imaging, and construction of high sampler/averager.

Major Findings

At this stage only the preliminary aspects of the problems have been explored. We have been able to detect free radical species at nuclear magnetic excitation frequencies indicating that broad ban excitation is possible. That whole body real time pharmacology of stable free radicals such as nitroxides which are either small molecules or coupled to a larger protein can be studied with EPR.

Significance to Biomedical Research and the Program of the Institute

The development of the instrument will allow a better understand of the early events cell/drug interaction. The ultimate possibility may be to more fully appreciate the cellular pharmacodynamics of ionizing radiation and chemotherapy drugs. It is anticipated that this project will integrate with other projects that are ongoing within the ROB that are related to oxidative stress and development of malignant neoplasia, as well as design and study of drugs that have the potential to prevent development of cancer and/or to combat cancer. Further the development of the imaging compatibilities of EPR is virtually unlimited and will in ways surpass nuclear magnetic imaging and in other ways complement the use of imaging for not only the defining the anatomy of a malignancy but also to probe the physiology of malignant tumors so that staging and treatment may be more effective.

Proposed Course

The instrumentation aspects of the problem are predominant at this time and the first goal is to configure an instrument with a set of Hemholtz coils, pulse programmer, probe, radiofrequency generator, and data acquisition and processing unit run by PC. This goal will provide a means of processing approximately 200,000 FID's/second which should lead to the commencement of experiments on the reactions of quinoid-based antineoplastics such as anthracyclines, mitomycin, streptonigrin, etc. It is anticipated that the prototype will be functional in late 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03800-22 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 STAFF 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. Doherty GM, Doppman JL, Shawker JL, Miller DL, Eastman RC, Gorden P, Norton JA. Results of a prospective strategy to diagnose, localize, and resect insulinomas. Surgery 1991;110:989-97.

2. Norton JA, Doppman JL, Jensen RT. Curative resection in Zollinger-Ellison Syndrome: results of a 10-year prospective study. Ann Surg 1992;215:8-18.

3. Norton JA, Shawker TH, Doppman JL, Miller DL, Fraker DL, Cromack DT, Gorden P, Jensen RT. [Letter to the Editor]. Ann Surg 1992;215:877-88.

4. Doherty GM, Doppman JL, Miller DL, Gee MS, Marx SJ, Spiegel AM, Aurbach GD, Pass HI, Brennan MF, Norton JA. Results of a multidisciplinary strategy for management of mediastinal parathyroid adenoma as a cause of persistent primary hyperparathyroidism. Ann Surg 1992;215:101-6.

5. Lange JR, Norton JA. Surgery for persistent or recurrent primary hyperparathyroidism. Current Practice in Surgery 1992;4:56-62.

6. Danforth DN. Hormone receptors in malignancy. Clinical Reviews in Oncology/Hematology 1992;12:91-149.

7. Norton JA. Controversies and advances in primary hyperparathyroidism. Ann Surg 1992;215:297-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03801-22 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland 20892

| TOTAL STAFF 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. Rosenberg SA. Immunotherapy and gene therapy of cancer, Origins of Human Cancer: A Comprehensive Review. Cold Spring Harbor Laboratory Press, 1991;865-83.

2. Spencer WF, Linehan WM, Walther MM, Haas GP, Lotze MT, Topalian SL, Yang JC, Merino MJ, Lange JR, Pockaj BA, Rosenberg SA. Immunotherapy with interleukin-2 and a-interferon in patients with metastatic renal cell cancer with in situ primary cancers: a pilot study. J Urol 1992;147:24-30.

3. Rosenberg SA. The immunotherapy and gene therapy of cancer. J Clin Oncol 1992;10:180-99.

4. Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM. Molecular and cellular characterization of human renal cell carcinoma cell lines. Cancer Res 1992;52:348-56.

5. Sherry RM, Pass HI, Rosenberg SA, Yang JC. Surgical resection of metastatic renal cell carcinoma and melanoma after response to interleukin-2 based immunotherapy. Cancer 1992;69:1850-5.

6. Linehan WM. Molecular genetics of tumor suppressor genes in prostate carcinoma: the challenge and the promise ahead. Editorial, J Urol 1992;147:808-9.

7. Pass HI, Pogrebniak HW, Steinberg SM, Mulshine J, Minna J. Randomized trial of neoadjuvant therapy for lung cancer: interim analysis. Ann Thorac Surg 1992;53:992-8.

8. Linehan WM, Walther MM, Rosenberg SA. Metastatic renal cell carcinoma. In: Kursh R, ed. Current Therapy in Genitourinary Surgery. Hanover: Mosby, 1992;31-4.

SURGICAL SERVICES DEPARTMENT ANNUAL STATISTICS

APRIL 1991 - MARCH 1992

| TOTAL PROCEDURES | HOURS | INSTITUTES/OTHERS |
|---|---|---|
| 418.5 | 1227.50 | Ward (NCI) |
| 817.0 | 1674.00 | Consult (NCI) |
| 65.0 | 100.50 | Med. Br. (NCI) |
| 1300.5 | 3002.00 | TOTAL (NCI) |
| | | |
| 1300.5 | 3002.00 | NCI |
| 22.0 | 24.50 | NHLBI |
| 140.5 | 728.75 | NINDS |
| 153.0 | 208.25 | Med. Neuro |
| 55.5 | 143.00 | NEI |
| 117.0 | 185.00 | ENT |
| 59.0 | 152.00 | NIDR |
| 2.5 | 10.25 | Orthopedics |
| 1.0 | 1.25 | NICHD |
| 32.0 | 65.50 | Other (outside consults) |

| TOTAL | PROCEDURES |
|---|---|
| 53 | Emergencies |
| 111 | Add-Ons |
| 325 | Cancellations |
| 435 | OPD's |
| | 2WSCR |
| | ICU-2J |
| 2 | MICU-10D |
| 44 | Radiation |
| | |
| | |
| | |
| 1883 | Total Cases |
| 4520.5 | Total Hours |

MONTHLY SUMMARY

| | TOTAL CASES | TOTAL HOURS |
|---|---|---|
| April | 172.00 | 424.00 |
| May | 155.00 | 325.75 |
| June | 125.00 | 340.75 |
| July | 153.00 | 465.75 |
| August | 157.00 | 358.75 |
| September | 135.00 | 292.50 |
| October | 166.00 | 392.00 |
| November | 174.00 | 424.50 |
| December | 146.00 | 293.25 |
| January | 187.00 | 422.50 |
| February | 166.00 | 410.75 |
| March | 147.00 | 370.00 |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03811-18 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

P.I.: S.A. Rosenberg, Chief of Surgery, SURG, NCI

Others: P. Hwu, Clinical Associate, MB, NCI; O. El-Badry, 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; M. Ogasawara, Visiting Fellow, SURG, NCI; Z. Eshhar, Visiting Scientist, SURG, NCI; J. Treisman, Senior Staff 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 STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 14 | 8 | 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.)*

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. 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.

2. Rosenberg SA. Biologics in diagnosis and treatment of human diseases: portents of things to come. In: Harrison DC, Osterweis M, Rubin ER, eds. Preparing for science in the 21st century. Washington, DC: Assoc Academic Health Centers, 1991;34-9.

3. Morecki S, Karson E, Cornetta K, Kasid A, 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.

4. Mulé JJ, Jicha DL, Aebersold PM, Travis WD, Rosenberg SA. Disseminated human malignant melanoma in congenitally immune deficient (Bg/Nu/Xid) mice. J Natl Cancer Inst 1991;83:350-5.

5. Barth RJ, Mulé 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.

6. 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;88:3255-9.

7. 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.

8. 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.

9. Rosenberg SA. Development of adoptive cellular immunotherapies for the treatment of cancer. In: Broder S, ed. Baltimore: Williams & Wilkins, 1991;153-93.

10. Bock SN, Cameron RB, Kragel P, Mulé JJ, Rosenberg SA. Biological and antitumor effects of recombinant human macrophage colony-stimulating factor in mice. Cancer Res 1991;51:2649-54.

11. Asher AL, Mulé JJ, Kasid A, Restifo NP, Salo JC, Jaffe E, Rosenberg SA. Murine tumor cells transduced with the gene for TNF-alpha: evidence for paracrine immune effects of TNF against tumors. J Immunother 1991;146:3227-34.

12. Hom SS, Topalian SL, Simoni ST, Mancini MJ, Rosenberg SA. Common expression of melanoma tumor-associated antigens recognized by human tumor infiltrating lymphocytes: analysis by HLA restriction. J Immunother 1991;10:153-64.

13. Schwartzentruber DJ, Topalian SL, Mancini MJ, Rosenberg SA. Specific release of granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-alpha, and IFN-gamma by human tumor-infiltrating lymphocytes after autologous tumor stimulation. J Immunol 1991;146:3674-81.

14. 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, 1991;1-6.

15. Mulé JJ, Rosenberg SA. Interleukin-2: preclinical trials. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic Therapy of Cancer. Philadelphia: JB Lippincott, 1991;142-58.

16. Lotze MT, Rosenberg SA. Interleukin-2: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic Therapy of Cancer. Philadelphia: JB Lippincott, 1991;178-96.

17. Yang JC, Rosenberg SA. Adoptive cellular therapy: preclinical studies. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic Therapy of Cancer. Philadelphia: JB Lippincott, 1991;214-36.

18. Rosenberg SA. Adoptive cellular therapy: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic Therapy of Cancer. Philadelphia: JB Lippincott 1991;197-213.

19. Aebersold P, Hyatt C, Johnson S, Hines K, Korcak L, Sanda 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 1991;83:932-7.

20. Barth RJ, 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;140:269-79.

21. Rosenberg SA. Immunotherapy and gene therapy of cancer. Cancer Res 1991;51(suppl 1):5074-9.

22. 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 antitumor immune response. J Immunol 1991;147:1453-9.

23. Sherry RM, Rosenberg SA, Yang JC. Relapse after response to IL-2 based immunotherapy: patterns of progression and response to retreatment. J Immunother 1991;10:371-5.

24. Schwartzentruber DJ, Topalian SL, Rosenberg SA. Characterization of lymphocytes infiltrating human breast carcinomas: evidence of specific immune reactivity. Surg Forum 1991;42:480-2.

25. Schwartzentruber DJ, White DE, Zweig MH, Weintraub BD, Rosenberg SA. Thyroid dysfunction associated with immunotherapy of cancer patients. Cancer 1991;68:2384-90.

26. Alexander RB, Rosenberg SA. Adoptively transferred tumor infiltrating lymphocytes can cure established metastatic tumor in mice and persistent ong term in vivo as functional memory T lymphocytes. J Immunother 1991;10:389-97.

27. Pockaj BA, Rosenberg SA. Lack of antihypertensive effect of interleukin-2 administration in humans. J Immunother 1991;10:456-9.

28. Rosenberg SA. The immunotherapy and gene therapy of cancer. Origins of human cancer: a comprehensive review. Cold Spring Harbor Laboratory Press, 1991;865-83.

29. Jicha DL, Mulé JJ, Rosenberg SA. Interleukin-7 generates antitumor CTL against murine sarcomas with efficacy in cellular adoptive immunotherapy. J Exp Med 1991;174:1511-5.

30. Kawakami Y, Zakut R, Topalian SL, Stotter H, Rosenberg SA. Shared human melanoma antigens: recognition by tumor infiltrating lymphocytes in HLA-A2.1 transfected melanomas. J Immunol 1991;148:638-43.

31. Lotze MT, Rosenberg SA. Interleukin-2: clinical applications. In: DeVita VT Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1991;159-77

32. Weber JS, Yang JC, Topalian SL, White DE, Rosenberg SA. The use of interleukin-2 and LAK cells for the treatment of patients with non-Hodgkin's lymphoma. J Clin Oncol 1992;10:33-40.

33. 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 1992;147:24-30.

34. Rosenberg SA. The immunotherapy and gene therapy of cancer. J Clin Oncol 1992;10:180-99.

35. Linehan WM, Walther MM, Rosenberg SA. Metastatic renal cell carcinoma. In: Resnick MI, Kursh R, eds. Current therapy in genitourinary surgery. Hanover: BC Decker, 1992;31-4.

36. Rosenberg SA. Gene therapy of cancer. In: DeVita VT, Hellman S, Rosenberg SA, eds. Important advances in oncology. Philadelphia: JB Lippincott, 1992.

37. Alexander RB, Rosenberg SA. Tumor necrosis factor: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic Therapy of Cancer. Philadelphia: JB Lippincott, 1992;378-92.

38. Mulé JJ, Rosenberg. Combination cytokine therapy: experimental and clinical trials. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1992;393-416.

39. Mulé JJ, Rosenberg SA. Interleukin-2: preclinical trials. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1992;142-58.

40. Topalian SL, Rosenberg SA. Adoptive cellular therapy: basic principles. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1992;178-96.

41. Yang JC, Rosenberg SA. Adoptive cellular therapy: preclinical studies. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1992;197-213.

42. Rosenberg SA. Adoptive cellular therapy: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer. Philadelphia: JB Lippincott, 1992;214-36.

43. Sherry RM, Pass HI, Rosenberg SA, Yang JC. Surgical resection of metastatic renal cell carcinoma and melanoma after response to interleukin-2 based immunotherapy. Cancer 1992;69:1850-5.

44. Choyke PL, Miller DL, Lotze MT, Whiteis J, Ebbit B, Rosenberg SA. Delayed reactions to contrast media after interleukin-2 immunotherapy. Radiology 1992;183:111-4.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06654-15 SURG

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

P.I.: W.F. Sindelar Senior Investigator SURG, NCI

COOPERATING UNITS *(if any)*

Radiation Oncology Branch

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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 reactivity 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 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 performed including feasibility and development 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.

1. DeLaney TF, Smith PD, Thomas GF, Tochner ZA, Sindelar WF, Pass HI, Harrington FS, Smith PD, Bonner RF, Mitchell JB. A light-diffusing device for intraoperative photodynamic therapy in the peritoneal or pleural cavity. J Clin Laser Med Surg 1991;1:361-6.

2. Jensen JC, Pass HI, Sindelar WF, Norton JA. Recurrent or metastatic disease in select patients with adrenocortical carcinoma: aggressive resection vs. chemotherapy. Arch Surg 1991;126:457-61.

3. Kinsella TJ, DeLuca AM, Barnes M, Anderson W, Terrill R, Sindelar WF. Threshold dose for peripheral neuropathy following intraoperative radiotherapy (IORT) in a large animal model. Int J Radiat Oncol Biol Phy 1991;20:697-701.

4. 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.

5. Friauf WS, Smith PE, Russo A, DeLaney TF, Pass HI, Cole JW, Gibson CC, Sindelar WF, Thomas G. Light monitoring in photodynamic therapy. In: Nagle HT, Tompkins WJ, eds. Case studies in medical instrument design. New York: The Institute of Electrical and Electronics Engineers, 1992;127-138.

6. Tochner ZA, Pass HI, Sindelar WF, DeLuca AM, Grisell DL, Bacher JD, Kinsella TJ. Long term tolerance of thoracic organs to intraoperative radiotherapy. Int J Radiat Oncol Biol Phys 1992;22:65-9.

7. DeLaney TF, Sindelar WF, Thomas GF, DeLuca AM, Taubenberger JK. Tolerance of small bowel anastomoses in rabbits to photodynamic therapy with dihematoporphyrin ethers and 630 nm red light. Ann Surg, 1992; in press.

8. DeLaney TF, Sindelar WF, Tochner Z, Smith PD, Friauf WS, Thomas G, Dachowski L, Cole JW, Steinberg SM, Glatstein E. Phase I study of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumors. Int J Radiat Oncol Biol Phys, 1992; in press.

9. Sindelar WF, Kinsella TJ, Chen PW, DeLaney TF, Tepper JE, Rosenberg SA, Glatstein E. Intraoperative radiotherapy in retroperitoneal sarcomas: final results of a prospectively randomized clinical trial. Arch Surg, 1992; in press.

10. Sindelar WF, Kinsella TJ, Tepper JE, DeLaney TF, Maher MM, Smith R, Rosenberg SA, Glatstein E. Randomized trial of intraoperative radiotherapy in carcinoma of the stomach. Am J Surg, 1992, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06657-10 SURG

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Metabolic Studies with Cytokines

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: | D.L. Fraker | Senior Investigator | SURG, NCI |
| | H.R. Alexander | Expert | SURG, NCI |
| | M. Berg | Biologist | 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 |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Surgical Metabolism Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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.)*

This laboratory focuses on the role of cytokine mediators in clinical conditions of importance in caring for the cancer surgical patient.

Using a specific polyclonal rabbit antibody to murine tumor necrosis factor-α (TNF), we had previously demonstrated that TNF was partially responsible for the severe toxicity associated with interleukin-2 (IL-2) immunotherapy in mice. In a recent series of survival experiments with mice bearing pulmonary metastases, we show that TNF is also partially responsible for the anti-tumor efficacy of IL-2 therapy. The ubiquitous important nature of host-produced TNF was further demonstrated in a series of experiments with oxygen toxicity. Alveolar macrophages produced TNF in response to toxic amounts of inhaled oxygen. Antibodies to TNF protected against the toxic effect of oxygen.

A specific polyclonal antibody to murine interferon-γ (IFN) protected mice against the lethal effect of endotoxin (LPS) and also blocked the lethality and anti-tumor effects of high-dose TNF. Differentiation factor (D-factor) also called leukemia inhibitory factor, is a glycoprotein produced by macrophages in response to LPS. As we had previously demonstrated for TNF and IL-1, pretreatment with D-factor protected mice against the lethal effects of LPS. Unlike what has been seen with TNF and IL-1, the protective effects of D-factor were synergistic with small doses of TNF and IL-1. Work in progress demonstrates that a specific antibody to D-factor is also protective against the lethality of LPS.

1. Fraker DL, Langstein HN, Norton JA. Passive immunization against tumor necrosis factor partially abrogates interleukin 2 toxicity. J Exp Med 1989;170:1015-20.

2. Alexander HR, Sheppard BC, Jensen JC, et al. Treatment with recombinant human tumor necrosis factor-alpha protects rats against the lethality, hypotension, and hypothermia of gram-negative sepsis. J Clin Invest 1991;88:34-9.

3. Alexander HR, Doherty GM, Fraker DL, Block MI, Swedenborg JA, Norton JA. Human recombinant interleukin-1a protection against the lethality of endotoxin and experimental sepsis in mice. J Surg Res 1991;50:421-4.

4. Fraker DL, Thom AK, Doherty GM, Langstein HN, Buresh CM, Norton JA. Tumour necrosis factor mediates the survival benefit of interleukin-2 in a murine pulmonary metastases model. Surg Oncol. 1992;1:1-9.

5. Jensen JC, Pogrebniak HW, Pass HI, Buresh C, Merino MJ, Kauffman D, Venzon D, Langstein HN, Norton JA. Role of tumor necrosis factor in oxygen toxicity. J Appl Physiol 1992;72(5):1902-7.

6. Alexander HR, Wong GGH, Doherty GM, Venzon DJ, Fraker DL, Norton JA. Differentiation factor/leukemia inhibitory factor protection against lethal endotoxemia in mice: Synergistic effect with interleukin-1 and tumor necrosis factor. J Exp Med 1992;175:1139-42.

7. Doherty GM, Langstein HN, Alexander HR, Lange JR, Buresh CM, Norton JA. Evidence for interferon-gamma as a mediator of the lethality of endotoxin and tumor necrosis factor-α. J Immunol 1992; (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06659-10 SURG

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 |
| | J.R. Gnarra | Senior Staff Fellow | SURG, NCI |
| | J.P. Long | Clinical Associate (CO) | SURG, NCI |
| | R.E. Reiter | 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 STAFF 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.)*


Familial renal cell carcinoma In studies performed to localize the tumor suppressor genes associated with initiation and progression of familial as well as sporadic renal cell carcinoma, patients either affected or at risk for von Hippel-Lindau disease have been evaluated. The results of genetic linkage analysis indicate that the VHL gene is located in a small region of chromosome 3. A prospective clinical trial analyzing the use of presymptomatic detection of VHL disease by DNA polymorphism analysis determined that presymptomatic DNA analysis could accurately predict which at risk patients carry the disease gene for VHL. In situ hybridization, genetic and physical mapping localizes the VHL disease gene to the 3p25.5 locus. Candidate genes, one of which codes for a previously identified member of a family of genes involved in signal transduction has been identified. Full length cDNA cloning and germ line mutational analysis are currently underway in order to identify the familial kidney cancer disease gene.
Sporadic renal cell carcinoma In order to perform analysis of the tumor suppressor genes in initiation and progression of sporadic renal cell carcinoma, over 40 human tumor cell lines have been derived. RFLP analysis, Northern and Western analysis and immunohistochemistry, and polymerase chain reaction-single strand conformation polymorphismabnormalities are being evaluated at tumor suppressor gene loci on chromosomes 17, 13 and 18. Studies are ongoing to identify the sporadic kidney cancer disease gene on chromosome 3 and to evaluate abnormalities at other tumor suppressor gene loci which may be involved in progression of this malignancy.
Prostate carcinoma We are evaluating tumor suppressor gene abnormalities which may be involved in initiation or progression of this malignancy as well as the antitumor effect of a number of antineoplastic agents in human prostate carcinoma. Animal models of metastasis have been developed using human prostate carcinoma cell lines implanted in the prostate of athymic nude mice for this purpose. The role of the metastasis gene nm23 in progression as well as its effect hormonal regulation of growth of human prostate carcinoma are being determined.

The significance of this project lies in the identification of the tumor suppressor genes associated with kidney 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. Linehan WM, Walther MT, Sargent ER, Gomella LG, Robertson CN, Anglard P, Wade TP, Weiss GH, Ewing MW, Liu S. Studies of the endocrine and paracrine effects of tumor produced factors in human genitourinary cancers. In: Karr JP, Coffey DS, Smith RG, Tindall DT. eds. Molecular and Cellular Biology of Prostate Cancer. New York: Plenum Press. 1991;75-9.

2. Myers CE, LaRocca RV, Cooper M, Danesi R, Jamis-Dow CA, Stein CA, Linehan WM. The role of suramin in cancer biology and treatment. In: Broder S. ed. Molecular Foundations of Oncology. Baltimore: Williams & Wilkins, 1991;419-31.

3. Dawson NA, Wilding G, Gelmann EP, McCloud DG, Linehan WM, Frank JA, Jacobs JL. A pilot trial of chemohormonal therapy for metastatic prostate carcinoma, Cancer 1992;69:218-31.

4. Anglard P, Trahan E, Liu S, Latif F, Merino M, Lerman M, Zbar B, Linehan WM. Molecular and cellular characterization of human renal cell carcinoma cell lines, Cancer Res 1992;52:348-56.

5. Glenn GM, Linehan WM, Hosoe S, Latif F, Yao M, Choyke P, Gorin MB, Chew E, Oldfield E, Manolatos C, Orcutt ML, McClellan MW, Weiss GH, Tory K, Jensson O, Lerman MI, Zbar B. Screening for von hippel-lindau disease by DNA-polymorphism analysis, JAMA 1992;267:1226-31.

6. Linehan WM, Walther MM, Rosenberg SA. Metastatic renal cell carcinoma. In: Resnick MI, Kursh ED. eds. Current Therapy in Genitourinary Surgery. St. Louis: B.C. Decker, 1992;31-4.

7. Linehan WM. Molecular genetics of tumor suppressor genes in prostate carcinoma: the challenge and the promise ahead [editorial], J Urol 1992;147:808-9.

8. Myers C, Stein C, Cooper M, LaRocca R, Walther M, Weiss G, Choyke P, Chrousos G, Dawson N, Steinberg S, Uhrich MM, Cassidy J, Kohler D, Linehan WM. Suramin: a novel growth factor antagonist with activity in hormone-refractory metastatic prostate cancer, J Clin Oncol 1992;10:881-9.

9. Perry RR, Keiser HJ, 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 1992;212:6216-28.

10. Spencer WF, Linehan WM, Walther MM, Haas GP, 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 1992;147:24-30.

11. Alexander RB, Walther MM, Linehan WM. Immunotherapy of Renal Cell Carcinoma. Contemp Urology, In Press: 1992.

12. 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: Staehler G, Pomer S. eds. Basic Research on Renal Cell Carcinoma. Springer Verlag. In Press: 1992.

13. Choyke PL, Glenn G, Walther MM, Zbar B, Weiss GH, Hayes WS, Thakore KN, Linehan WM. The natural history of renal lesions in von Hippel-Lindau Syndrome, Am J Roentgenol In Press: 1992.

14. Doppman JL, Gill, JR, Miller DL, Chang R, Gupta R, Friedman TC, Choyke PL, Feuerstein IM, Dwyer AJ, Jicha DL, Norton JA, Linehan WM. The reliability of computed tomography in distinguishing hyperaldosteronism due to bilateral hyperplasia from unilateral aldosteronoma. Radiology, In Press: 1992.

15. Ewing MW, Liu S, Walther MM, LaRocca RV, Myers CE, Linehan WM. Effect of suramin on the mitogenic response of the human prostate carcinoma cell line PC-3, Cancer In Press: 1992.

16. Glenn GM, Daniel LN, Choyke P, Linehan WM, Oldfield E, Gorin M, Hosoe S, Latif F, Weiss G, Walther M, Lerman MI, Zbar B. Von hippel-lindau disease: distinct phenotypes suggest more than one mutant allele at the vhl locus, Hum Genet In Press: 1992.

17. Gnarra JR, Anglard P, Latif F, Lerman MI, Zbar B, Linehan WM. Molecular studies of sporadic and familial renal cell carcinoma. In: Bukowski RM. ed. Immunotherapy of Renal Cell Carcinoma. New York: Marcel Dekker In Press: 1992.

18. Latif F, Hosoe S, Brauch H, Glenn GM, Daniel L, Orcutt ML, Delisio J, Hampsch K, Linehan WM, Zbar B, Lerman MI. A single recombinant event between the von hippel-lindau disease (VHL) gene and D3S18 (3p26) loci on human chromosome 3, Am J Hum Genet In Press: 1992.

19. Linehan WM, Long JP, Steeg PS, Gnarra JR. Metastatic models and molecular genetics of prostate cancer [editorial], JNCI In Press: 1992.

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25. Walther MM, Malech H, Choyke P, Berman A, Venzon DJ, Linehan WM, Gallin JI. The urologic manifestations of chronic granulomatous disease, J Urol In Press: 1992.

26. Walther MM, Kragel PJ, Trahan E, Venzon D, Schlesinger PH, Blair HC, Jaimas-Dow C, Ewing MW, Myers CM, Linehan WM. Suramin inhibits osteoclast activity and reduces osteoblast number in a neonatal mouse calvarial bone resorption assay, Endocrinology In Press: 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06660-09 SURG

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

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 |
| | B. Averbook | Clinical Associate (CO) | Surg, NCI |
| | J. Balkissoon | Clinical Associate (CO) | Surg, NCI |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 3.5 | 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.)*


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 described that interleukin-6 incorporated into slow-release collagen matrix can generate TIL with enhanced in vivo activity. We have extended this to use tumor transduced with the gene for IL-6 as the means of local IL-6 delivery.

In addition, we are studying the migration of TIL in response to various stimuli. We are proceeding with the purification of a novel tumor-produced protein with chemotactic activity for TIL and other activated CTL. We have achieved partial purification and have demonstrated that this factor differs from other described factors with chemotactic activity. In associated on-going clinical trials, we have shown that cyclophosphamide improves the localization of TIL to sites of tumor, and that this tumor homing is associated with clinical responses to TIL.

Another clinical trial is investigating the efficacy of high-dose intensity IL-2 versus lower dose and dose intensity in a randomized, prospective trial in renal cell carcinoma.

Other clinical trials have been initiated or are on-going in the treatment of adult soft tissue sarcomas. A randomized adjuvant chemotherapy trial using doxorubicin, ifosfamide and G-CSF has begun and another investigation of the role of radio-therapy for low-grade sarcomas is continuing.

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4. Spencer WF, Linehan WM, McClellan MW, Haas GP, Lotze MT, Topalian SL, Yang JC, Merino MJ, Lange JR, Pockaj BA, Rosenberg S.A. Immunotherapy with interleukin-2 and α-interferon in patients with metastatic renal cell cancer with in situ primary cancers: A Pilot Study. J Urol 1992;147:24-30.

5. Yang JC, Shlasko E, Ritchey JL, Landry JG, White DE, and Rosenberg SA. Combination chemoimmunotherapy for metastatic colorectal cancer using 5-FU, leucovorin and interleukin-2. Eur J Cancer (In Press).

6. Sherry RM, Pass HI, Rosenberg SA, Yang JC. Surgical resection of metastatic renal cell carcinoma and melanoma after response to interleukin-2 based immunotherapy. Cancer (In Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06662-06 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 STAFF 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 for the treatment of malignancy. We have shown that conjugation of monoclonal antibody to a weak sensitizer will improve the specificity as well as the long-term cures in an in vivo model of photodynamic therapy. We continue with investigations in human trials to establish the maximum tolerated dose of phototherapy for intrapleural delivery. As of June of 1992 42 patients have been entered on an intrapleural photodynamic therapy trial with light dose escalation in cohorts of three patients. We have published the ability to monitor the light through these treatments. We have shown that the maximum tolerated dose of intraoperative pleural photodynamic therapy is 30 Joules/cm-squared of light after a 24 hour dosing of 2 mg/kg of Photofrin II. We found the next highest dose of phototherapy light (32.5 Joules/cm-squared) resulted in esophageal complications. We have also investigated use of a number of novel compounds to prevent tumor necrosis factor toxicity. Potassium channel activator, Nicorandil, was found to decrease macrophage TNF production from an LPS challenge possibly through a reduction in respiratory burst. Moreover, the nitrone PBN was found to abrogate the lethality of LPS in vivo through down regulation of serum production of tumor necrosis factor. Investigations of other compounds which may be useful in protecting against cytokine toxicity is continuing in our section within in vitro and in vivo models.

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2. Pogrebniak HW, Matthews W, Prewitt TW, Pass HI. Tumor necrosis factor alpha alters response of lung cancer cells to oxidative stress. Journal Thoracic Cardiovasc Surg. 1991;102: 904-7.

3. Friauf SW, Smith PE, Russo A, DeLaney TF, Pass HI, Cole JW, Gibson CC, Sindelar WF, Thoras G. Light monitoring in photo-dynamic therapy. In Nagle HT and Tompkins WJ, eds. Case Studies in Medical Instrument Design. The Institute of Electrical and Electronics Engineers Inc., New York, 1992.

4. Pogreniak HW, Matthews W, Pass HI. Alterations in macrophage free radical and tumor necrosis factor production by a potassium channel activator. J Surg Res 1992;52:395-400.

5. Pogrebniak HW, Merino, MJ, Hahn SM, Mitchell JB, Pass HI. Spin trap salvage from endotoxemia: The role of cytokine down-regulation. Surgery 1992; In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06663-03 SURG

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 STAFF 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 TNF alone and in combination on cell growth and 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 acts additively with IL-6 to inhibit growth and antagonize $E_2$ stimulation of growth of ER+ cells MCF-7 and T47D. TNF blocks $E_2$ stimulated growth and down-regulates the ER. This also indicates an important interaction between the immune and endocrine systems. The effects of the cytokines on steroid receptors are at the posttranscriptional level. TNF induces sustained expression of TNF mRNA and secretion of TNF protein and arrests growth at $G_0G_1$. TNF, however, does not act additively with IL-1. TNF and IL-1 both stimulate secretion of TGFß by MCF-7 cells in a time and dose-dependent manner; stimulation is antagonized by estradiol. TNF increases secretion of both the biologically active and inactive forms of TGFß. This occurs without a change in the structure of the subunit or glycosylated forms as determined by immunoblotting. Studies are in progress to determine the particular isoform(s) of TGF which is stimulated by TNF and IL-1, and whether these cytokines are acting at the transcriptional or posttranscriptional level to increase secretion.

A second important study is the establishment of long-term human breast carcinoma cell lines from primary solid tumors. We have developed a method involving enzymatic digestion of fresh tumor, maintenance of cell monolyers in defined condition media and the cloning of subcultures. Cellular characterization includes cell morphology, keratin staining and FACS analysis with antibodies specific for epithelial and breast carcinoma. Growth curves are constructed in vitro and in vivo in nude mice. Karyotyping studies are being performed to identify chromosomal aberrations, and in situ hybridization to identify specific genetic changes, including the evaluation of multi drug resistance and oncogene expression. These cell lines will allow more detailed characterization of chromosomal, genetic and transcriptional abnormalities, and the role of cellular and human cytotoxicity in the treatment of this malignancy.

1. Sgagias M, Danforth DN Jr. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibit growth and induce TNF mRNA in MCF-7 human breast cancer cells. Molecular Endocrinology; 1991;5:1740-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06664-03 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Recognition of Tumor Antigens by Human T 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 |
| Other: | D.J. Schwartzentruber | Senior Investigator | SURG, NCI |
| | P. Shamamian | Clinical Associate (CO) | SURG, NCI |
| | M. Mancini | Biologist | SURG, NCI |
| | R. Zakut | Expert | SURG, NCI |
| | Y. Kawakami | 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 STAFF 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. Melanoma-specific Ag recognized by $CD8^+$ T cells.
We have found that some melanoma Ag are commonly expressed in the patient population. $CD8^+$ T cells which specifically recognize autologous melanoma Ag can also respond to allogeneic HLA-matched melanoma cells sharing the same Ag, by cytolysis and/or cytokine secretion. Transfection of the HLA-A2.1 gene into HLA-mismatched melanomas has shown that they can also share melanoma Ag recognized by HLA-A2 restricted T cells. These Ag appear to be present on certain other neural crest derived tumors as well.

2. Other tumors specifically recognized by human T cells.
The function of cytokine secretion (TNF-$\alpha$, IFN-$\gamma$, GM-CSF) has been used to monitor $CD4^+$ and $CD8^+$ TIL recognition of tumor Ag. TIL derived from 4 of 10 colon carcinomas, 2 of 12 lymphomas, and 3 of 11 breast carcinomas specifically secreted cytokines in response to autologous and/or HLA-matched allogeneic tumors. In some cases, $CD4^+$ T cells were solely responsible for the observed response.

3. Function of human B cells in tumor Ag presentation.
We have identified an EBV-transformed B cell line capable of processing and presenting melanoma Ag to autologous $CD4^+$ T cells. Studies are in progress to identify the tumor-derived peptide recognized in this system, using immunoaffinity chromatography to isolate MHC II-peptide complexes.

1. Schwartzentruber DJ, Topalian SL, Rosenberg SA. Characterization of lymphocytes infiltrating human breast carcinomas: Evidence of specific immune reactivity. Surg Form 1991;480-2.

2. Weber JS, Yang JC, Topalian SL, White DE, Rosenberg SA. The use of interleukin-2 and LAK cells for the treatment of patients with non-Hodgkin's lymphoma. J Clin Oncol 1992;33-40.

3. Kawakami Y, Zakut R, Topalian SL, Stotter H, Rosenberg SA. Shared human melanoma antigens: Recognition by tumor infiltrating lymphocytes in HLA-A2.1 transfected melanomas. J Immunol 1992;638-43.

4. Spencer WF, Linehan WM, Walther MM, Haas GP, Lotze MT, Topalian SL, Yang JC, Merino MJ, Lange JR, Pockaj BA, Rosenberg SA. Immunotherapy with interleukin-2 and α-interferon in patients with metastatic renal cell cancer with in situ primary cancers: A pilot study. J Urol 1992;24-30.

5. Alexander RB, Bolton ES, Koenig S, Jones GM, Topalian SL, June CH, Rosenberg SA. Detection of T lymphocytes with specific immune reactivity to cellular antigens by determination of intracellular calcium concentration using flow cytometry. J Immunol Methods 1992;131-41.

6. Schwartzentruber DJ, Solomon D, Rosenberg SA, Topalian SL. Characterization of lymphocytes infiltrating human breast cancer: Specific immune reactivity can be detected by measuring cytokine secretion. J Immunother 1992 (In press).

7. Topalian SL, Hom SS, Kawakami Y, Mancini M, Schwartzentruber DJ, Zakut R, Rosenberg SA. Recognition of shared melanoma antigens by human tumor infiltrating lymphocytes. J Immunother 1992 (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06665 03 SURG

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Studies of the Gene Therapy and Immunotherapy of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. J.J. Mulé Microbiologist SURG, NCI

Others: P.A. Cohen, Clinical Associate, SURG, NCI; P.Hwu, Clinical Associate, SURG, NCI; S. Karp, Visiting Associate, SURG, NCI; F. Marincola, Clinical Associate, SURG, NCI; S. Kishter, Clinical Associate, SURG, NCI; N.P. Restifo, Senior Staff Fellow, SURG, NCI; M. Custer, Microbiologist, SURG, NCI; R.J. Barth, Jr.; Biotechnology Fellow, SURG, NCI; D.L. Jicha, Clinical Associate, SURG, NCI

COOPERATING UNITS *(if any)*
W.D. Travis, M.D., Laboratory of Pathology, NCI
S.I. Katz, M.D., Dermatology Branch, NCI
P. J. Cohen, M.D., Dermatology Branch, NCI

LAB/BRANCH
Surgery Branch, NCI

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 9.0 | 9.0 | 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.)*

Experimental studies were undertaken to investigate the antitumor effects and mechanisms of action of recombinant cytokines and gene-modified tumor and effector cells in immunotherapy of cancer.

A. Tumor Infiltrating Lymphocytes (TIL). We have functionally and molecularly characterized human TIL retrovirally transduced with the TNF cDNA and have utilized soluble TNF receptor to accurately measure secretion of TNF in vitro.

B. Gene-Modified Tumor Cells. We have molecularly engineered murine tumor cells to secrete various cytokines. TNF and IL-2 cDNAs have been introduced into both weakly and non-immunogenic tumors. The host cellular immune response to these cytokine-secreting tumors have been characterized. Interferon-gamma gene-modification corrects an antigen presentation defect in a non-immunogenic sarcoma and elicits therapeutically effective CD8+ TIL reactive against the wild-type tumor, which had not previously produced effective TIL.

C. Interleukin 6 and 7. We have demonstrated a potent role for IL-6 in the treatment of established solid tumors in mice that have the capacity to elicit both CD4+ and CD8+ T cell responses. IL-7 has been shown to mediate the generation and expansion of murine antitumor CTL. Adoptively transferred, IL-7 activated CTL have potent antitumor effects in vivo.

D. Dendritic (Langerhans) Cells (DC). We have demonstrated that epidermal Langerhans cells and splenic dendritic cells process and present tumor-associated antigens to primed CD4+ T cells in mice. Tumor-pulsed DC are extremely potent stimulators of helper T cell proliferation in vitro.

PUBLICATIONS

1. Asher AL, Mulé JJ, Kasid A, Restifo NP, Salo JC, Jaffe G, Fendly B, Kriegler M, Rosenberg SA. Murine tumor cells transduced with the gene for TNF-alpha: evidence for paracrine immune effects of TNF against tumors. J Immunol 1991;146:3227-34.

2. Karp SE, Salo JC, Jaffe G, Mulé JJ, Rosenberg SA. Regression of a non-immunogenic murine tumor following retroviral mediated insertion of the IL-2 gene. Surg Forum 1991;42:467-9.

3. Jicha DL, Mulé JJ, Rosenberg SA. Interleukin-7 generates antitumor CTL against murine sarcomas with efficacy in cellular adoptive immunotherapy. J Exp Med 1991;174:1511-5.

4. Jicha DL, Schwarz S, Mulé JJ, Rosenberg SA. Interleukin-7 mediates the generation and expansion of murine allosensitized and antitumor CTL. Cell Immunol 1992;141:71-83.

5. Mulé JJ, Custer MC, Travis WD, Rosenberg SA. Cellular mechanisms of the antitumor activity of recombinant interleukin-6 in mice. J Immunol 1992;148:2622-9.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06667-02 SURG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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 |
| | A. Mixon | Biologist | SURG, NCI |

COOPERATING UNITS *(if any)*
Surgery Branch, NCI (PA Cohen)
Navy Medical Research Institute, Bethesda, Maryland (CH June)
Laboratory of Immunoregulation, NIAID (S. Koenig)

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

| TOTAL STAFF 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.)*

We have developed methodology to detect individual T cell reactivity to cellular antigens and demonstrated the specificity and utility of this system. This technology is being applied to the study of human T cells obtained from patients with metastatic renal cell carcinoma. We are also engaged in a cooperative research venture with Dr. Peter Cohen of the Surgery Branch, NCI to detect proliferation of T cells from patients with renal cell carcinoma following exposure to tumor pulsed antigen presenting cells. We are studying the proliferation of these cells by $^{3}H$ thymidine proliferation assay. We have also perfected a technique for the rapid purification of CD4 T cells from human lymphoid compartments for use in these experiments.

The laboratory is also engaged in the flow cytometric analysis of peripheral blood lymphocytes from patients with metastatic cancer undergoing experimental therapy with interleukin-6. We have identified some activation markers in peripheral blood T cell compartment caused by the IL-6 administration. This work is ongoing.

The significance of these projects is the detection of individual T cell reactivity to tumors. The production of T cell populations with specific antitumor reactivity would provide very useful information in understanding the nature of the immune response to cancer in patients with metastatic or localized disease. Such cells could also be used in therapy trials.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM

DIVISION OF CANCER TREATMENT

OCTOBER 1, 1991 - SEPTEMBER 30, 1992

## I. INTRODUCTION

The Radiation Research Program (RRP) was established in 1982 in the Division of Cancer Treatment (DCT), to develop and maintain a program for the support of cancer-related radiation research activities in the extramural research community. Research in which various types of radiations are used in the diagnosis, staging, treatment planning, treatment, and post-treatment evaluation of the patient with cancer is the principal focus of RRP activities. The RRP comprises two closely related branches, the Radiotherapy Development Branch (RDB) and the Diagnostic Imaging Research Branch (DIRB). This combination was established because a portion of the imaging activities of DIRB provide visualization, planning, monitoring, and image management activities that are indispensably required in the process of radiation treatment.

Diagnostic oncologic radiology research applications which do not readily apply to other specific Institutes of NIH are assigned to NCI, so that the Diagnostic Imaging Research Branch provides the general focus for such research programs at NIH.

For scientific and administrative guidance the Radiation Research Program 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
   G. Stephen Brown, M.D., Associate Director
   Sandra Zink, Ph.D., Cancer Expert
   Wendy R. Fredericks, Biologist
   Richard V. Stepney, Computer Specialist
   Laura F. Young, Secretary to the Associate Director

2. Administrative Office
Steven Hammond, Administrative Officer
Christina Rademakers, Administrative Technician

3. Radiotherapy Development Branch
Francis J. Mahoney, Ph.D., Acting Chief
Thomas A. Strike, Ph.D., Program Director
Helen B. Stone, Ph.D., Cancer Expert
Kathleen Kocher, Branch Secretary

4. Diagnostic Imaging Research Branch
Faina Shtern, M.D., Chief
Matti S. Al-Aish, Ph.D., Program Director
Roger S. Powell, Program Director
Patricia E. Schrock, Branch Secretary

B. Recruitments

Clerk-typist
Chief, Radiotherapy Development Branch

RRP BUDGET

Fiscal Year

| Grants | 1991 Number | 1991 Dollars in Thousands | 1992 Number | 1992 Dollars in Thousands |
|---|---|---|---|---|
| Traditional (R01) | 224 | 48,181 | 240 | 55,975 |
| Program Projects (P01) | 23 | 25,001 | 25 | 28,584 |
| Conference, New Investigator, and Small Grants | 3 | 21 | 10 | 299 |
| MERIT Awards | 14 | 4,384 | 15 | 5,270 |
| FIRST Awards | 32 | 2,938 | 28 | 2,623 |
| Cooperative Agreements | 7 | 1,612 | 5 | 1,030 |
| (RFAs) Request for Applications | 18 | 7,927 | 11 | 2,052 |
| Exploratory/Development | 0 | 0 | 0 | 0 |
| SBIR Awards | 43 | 5,596 | 60 | 5,881 |
| Shannon Grants | 5 | 250 | 0 | 0 |
| Total Grants | 369 | 95,910 | 394 | 101,644 |
| Contracts | | | | |
| Regular | 9 | 2,191 | 7 | 1,926 |
| SBIR | 2 | 479 | 0 | 0 |
| Total Contracts | 11 | 2,670 | 7 | 1,926 |
| TOTAL SUMMARY BUDGET | 380 | 98,580 | 401 | 103,570 |

RDB BUDGET

Fiscal Year

| Grants | 1991 Number | 1991 Dollars in Thousands | 1992 Number | 1992 Dollars in Thousands |
|---|---|---|---|---|
| Traditional (R01) | 127 | 25,107 | 135 | 28,791 |
| Program Projects (P01) | 14 | 16,895 | 17 | 21,381 |
| Conference, New Investigator, and Small Grants | 3 | 16 | 8 | 205 |
| MERIT Awards | 12 | 3,979 | 12 | 4,305 |
| FIRST Awards | 21 | 1,959 | 16 | 1,542 |
| Cooperative Agreements | 0 | 0 | 0 | 0 |
| (RFAs) Request for Applications | 8 | 5,842 | 4 | 444 |
| Exploratory/Development | 0 | 0 | 0 | 0 |
| SBIR Awards | 15 | 2,175 | 20 | 2,097 |
| Shannon Grants | 3 | 150 | 0 | 0 |
| Total Grants | 203 | 56,123 | 212 | 58,765 |
| Contracts | | | | |
| Regular | 9 | 2,191 | 7 | 1,926 |
| SBIR | 2 | 479 | 0 | 0 |
| Total Contracts | 11 | 2,670 | 7 | 1,926 |
| TOTAL RDB BUDGET | 214 | 58,793 | 219 | 60,691 |

DIRB BUDGET

Fiscal Year

| Grants | 1991 Number | 1991 Dollars in Thousands | 1992 Number | 1992 Dollars in Thousands |
|---|---|---|---|---|
| Traditional (R01) | 97 | 23,074 | 105 | 27,184 |
| Program Projects (P01) | 9 | 8,106 | 8 | 7,203 |
| Conference, New Investigator, and Small Grants | 0 | 5 | 2 | 24 |
| MERIT Awards | 2 | 405 | 3 | 965 |
| FIRST Awards | 11 | 979 | 12 | 1,081 |
| Cooperative Agreements | 7 | 1,612 | 5 | 1,030 |
| (RFAs) Request for Applications | 10 | 2,085 | 7 | 1,608 |
| Exploratory/Development | 0 | 0 | 0 | 0 |
| SBIR Awards | 28 | 3,421 | 40 | 3,784 |
| Shannon Grants | 2 | 100 | 0 | 0 |
| Total Grants | 166 | 39,787 | 182 | 42,879 |
| Contracts | | | | |
| Regular | 0 | 0 | 0 | 0 |
| SBIR | 0 | 0 | 0 | 0 |
| Total Contracts | 0 | 0 | 0 | 0 |
| TOTAL DIRB BUDGET | 166 | 39,787 | 182 | 42,879 |

III. MAJOR ACTIVITIES

The Radiation Research Program (RRP) develops, administers and evaluates basic science and clinical research areas in radiation therapy, nuclear medicine, diagnostic imaging, and their related subspecialty areas.

IV. SCIENTIFIC OVERVIEW

RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch (RDB) stimulates and supports scientific research in radiation therapy with conventional photons, fast neutrons, proton beams, hyperthermia, radiation sensitizers, radiation protectors, systemic radiation therapy (SRT), photodynamic therapy (PDT), 6boron neutron capture therapy (BNCT), radiobiology, radiation physics, and the use of knowledge-based infra-systems in the radiologic sciences.

A high priority research area of the Radiotherapy Development Branch has been the completion of the fast neutron therapy clinical trials project. After more than a decade of clinical research and development, Phase III trials in head-and-neck, prostate and lung cancer were closed. Over the 10-year period, the participating institutions included the University of Washington, Seattle, Washington; UCLA, Los Angeles, California; University of Texas Cancer Center, M. D. Anderson Hospital, Houston, Texas; Cleveland Clinic Foundation, Cleveland, Ohio; and Clatterbridge Hospital, Liverpool, England. While preliminary results show no clear overall advantage for either neutrons or best conventional therapy, longer follow-up is needed to assess the final results of the Phase III studies. The efficacy of neutron beam therapy for the treatment of malignant salivary gland tumors has been demonstrated and many world-wide patients are treated routinely at the Seattle-based neutron therapy facility. Early evaluation of data suggests an advantage for neutron therapy of cancer of the prostate.

Exciting areas of research supported by the Radiotherapy Development Branch include the following:

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 FY 1990, $5 million in FY 1991 and $4 million in FY 1992. Two planning grants were awarded in FY 1990 to the Massachusetts General Hospital and the Lawrence Berkeley Laboratory of the University of California. Two continuation awards were made in FY 1991 and two continuation awards will be made in FY 1992. Completion of the planning process should occur in 1994. After that each site will require approximately $30 million each to construct and equip a research and treatment proton facility.

Data from the separately funded 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.

International scientific activities in the area of proton therapy continue to be pursued and supported by the Radiation Research Program. Feasibility of an international dosimetry intercomparison between proton beam facilities in Russia and the United States was investigated this past year with preliminary measurements in the proton beam of the Institute of Theoretical and Experimental Physics (ITEP), Moscow, in cooperation with and through the help of the National Institutes of Standards and Technology, Gaithersburg. Using identical dosimetric materials as those used at ITEP, similar measurements will be carried out in the Harvard Cyclotron and the proton beam facility at Loma Linda, California. Comparison of the results will give scientific investigators the means to compare the doses delivered by their respective beams. Six Russian scientists were supported by NCI to give papers at the biannual meeting of the Proton Therapy Cooperative Oncology Group (PTCOG) meeting in Boston and one United States scientist spent a month with the ITEP project in Moscow. Continued interaction and exchange of ideas between the Russian and American scientists is anticipated to benefit both countries as new accelerator and proton beam gantry designs are considered for the new hospital-based facilities.

Phase II and III clinical trials of intraoperative radiation therapy are continuing for the treatment of locally advanced gynecological and rectal tumors, retroperitoneal sarcomas and gastric cancers. Trials are being conducted in the United States and Europe.

Radiation modifiers: The radiation sensitizer contract continues to identify and develop substances that enhance the

effectiveness of radiation. Encouraging results with SR-2508 (etanidazole) 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. Phase I trials of the bioreductive agent SR 4233 have been initiated.

Clinical investigations in the use of hyperthermia as an adjunct to radiotherapy and chemotherapy are continuing. Difficulties in delivering uniform heat throughout deep tumors and the precise measurement of temperatures in the tumor volume still represent problem areas that require resolution before hyperthermia is accepted as an effective treatment modality for cancer. Efforts are being directed to finding solutions to these problems.

The exciting area 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 second generation compounds are now entering clinical trials. 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. Research in radionuclide conjugate dosimetry is extremely important as this therapeutic approach is experiencing rapid growth.

The "Patterns of Care" study (PCS) in radiation oncology is a research effort being carried out by the American College of Radiology to establish "best management" for radiotherapy practices in the treatment of carcinoma of the prostate, cervix, Hodgkin's disease, and cancer of the breast and rectosigmoid. In addition to establishing consensus guidelines for the five cancer sites, the PCS study has a fully developed educational and informational program to communicate the guidelines as well as the results of long-term follow-up studies to the radiation oncology community. Ten and 15-year follow-up data on prostate, cervix and Hodgkin's disease patients are being obtained, as well as 5-year follow-up data on breast patients. The survey of all radiotherapy facilities in the United States shows an increase in

the number of free standing treatment facilities in the last five years when compared with those that are academically based or are part of a cancer center. Another related study is assessing the effect of different fractionation schemes on treatment outcome in tonsillar cancer tumors. The effect of large dose fractions on the complication rates for normal structures is of particular interest. The contract with the American College of Radiology was recently extended, scheduled to complete in October 1993.

The Radiotherapy Treatment Planning Tools Collaborative Working Group has completed 3 of the 5-year contracts to three institutions, charged with the development of portable software that takes three-dimensional treatment planning from the research environment into routine clinical use. Prototype software tools for a number of 3-D planning activities have been demonstrated on different operating systems and hardware platforms at the three institutions. Tools have been developed for (1) object definition of anatomical landmarks in a CT study; (2) creation of data sets for 3D display; (3) tumor localization; (4) target definition; (5) first-guess plan generation; (6) treatment plan evaluation; and (7) treatment verification through portal imaging and simulation image registration. Evaluation of the tools in each of the individual institutions' clinical environments is planned for the next year.

A new initiative launched by the Radiation Research Program with the National Library of Medicine (NLM) resulted in the National Cancer Institute (NCI) supporting four post-doctoral fellows in medical informatics as part of NLM's Medical Informatics Research Training Grant Program. The NCI funds support trainees whose projects specifically deal with the application of medical informatics approaches to the problems of cancer diagnosis and therapy. Four fellows have been identified among the highest-ranking institutions and their projects deal with (1) melanoma screening by computerized detection; (2) evaluation of medical images for radiotherapy treatments; (3) evaluation of imaging quality and computer interface design; and (4) modeling variability of diagnostic film readers and developing studies for analyzing prospective diagnostic accuracy. This new program, the first of its kind, is designed to develop trainees in the radiological sciences to have skills and expertise in medical informatics. The trainees will complete degree programs in Medical Informatics at their respective institutions.

Another training program initiative was developed in cooperation with the Cancer Training Branch (CTB) of the Division of Cancer Biology, Diagnosis and Centers. The CTB staff administers all postdoctoral training grants for the NCI. A Program Announcement was developed in cooperation with the CTB staff inviting applications for postdoctoral National Research Service Award fellowships in the radiological sciences for cancer-related projects. The Program Announcement generated considerable

interest in the radiological science community and the first applicants are in the process of evaluation. Applicants are evaluated through peer review and must compete on the basis of their relative ranking and availability of funds.

Boron Neutron Capture Therapy (BNCT) is a therapeutic method which has the potential for achieving tissue and cell specific radiation therapy. When a boron compound is deposited in a tumor and the boron excited by low energy neutrons, a subsequent nuclear disintegration of the boron atom results in the release of focal radiation. Early BNCT trials in the treatment of malignant gliomas were carried out in the United States in the 1950's and 1960's but were discontinued because of unacceptable normal tissue side effects. However, Dr. Hatanaka, a neurosurgeon in Japan, has continued using BNCT for the treatment of patients with malignant brain tumors. A Japanese dermatologist, Dr. Mishima, has used 10 borono-phenylalanine to study melanoma in an animal model (pig). He is performing clinical trials in the use of BNCT for patients who have peripheral melanoma. These encouraging results should be followed by further research in the use of this innovative therapeutic approach. On the topic of promising future BNCT compounds, the Board of Scientific Counselors, DCT/NCI, was given a thorough update at its February 1992 meeting by four grantees who were funded in 1991 as a result of an RFA issued in 1990.

RADIATION BIOLOGY

The NCI supports a major portion of the radiation biology research in the United States, primarily through the Radiotherapy Development Branch. This research is dedicated to improving radiation therapy as a treatment modality. Research activities encompass tumor and normal tissue radiobiology at the molecular, cellular and animal levels. The following examples illustrate the breadth and diversity of this program:

The radiation sensitivity of cell lines derived from cancer patients treated with radiotherapy corresponds in many cases to the curability of the tumors in those patients. At Georgetown University, certain proteins have been found to be consistently expressed in sensitive cells, and others in resistant cells. Studies are underway to identify these proteins and their role or roles in cellular radiosensitivity. Cells in which oncogene function was altered experimentally became more sensitive to radiation killing.

Further evidence that inherent radiosensitivity of cells is under genetic control has been shown by researchers at M.D. Anderson Hospital in experiments that involved fusing radiosensitive cells with normal cells. The radiosensitive cells were deficient in their ability to repair radiation damage. One cell line resulting

from the fusion of the repair-deficient and normal cells had radiosensitivity similar to that of the normal parent, and one was radiosensitivity resembling the repair-deficient parent. Although the amount of radiation damage produced in the two fused lines was similar, they differed in their ability to repair that damage. These findings imply that mutations in the genes that are responsible for repair processes cause radiosensitivity. When these genes are functioning normally, repair is normal and radiation resistance is imparted to the cell.

The radiation response of 16 different tumor lines in mice showed wide variations in the incidence of apoptosis or programmed cell death (PCD) following irradiation. In a lymphoma, 90% of the cells died by apoptosis within 3 hours after a dose of 10 Gy. In several mammary tumors, 30-40% of the cells died by apoptosis after 10 Gy. Fibrosarcomas, however, were resistant to this form of cell death, showing little or no response after doses as high as 25 Gy. This study was done at M.D. Anderson Hospital in Houston.

Cells isolated from normal tissues of different individuals differ in their intrinsic cellular radiosensitivity. Furthermore, lymphocytes and fibroblasts from the same individual may differ in their radioresponse, although there is more variability in radiosensitivity between patients than within each patient. Studies are in progress to determine whether either of these cell types predicts normal tissue sensitivity in individual patients treated with radiotherapy. Both early and late normal tissue responses are being examined. This study was done at M.D. Anderson Hospital.

Squamous cell carcinoma cells were inhibited by epidermal growth factor (EGF) when grown in monolayer cultures, but were stimulated by EGF when grown in 3-dimensional culture as multicellular spheroids. In spheroids, there was a 90% reduction in surface-expressed EGF-receptors, and an increase in tyrosine-phosphorylated proteins, but little change in protein tyrosine phosphatase. This illustrates the importance of culture conditions on experimental results. This study was performed by scientists at SRI International.

Preliminary results of studies at Colorado State University on intraoperative radiotherapy in dogs have indicated that the incidence of peripheral neuropathy and of ureteral constriction increases with the volume of tissue exposed in para-aortic and ureteral fields, respectively. In another study of dogs with naturally-occurring osteogenic sarcomas treated with limb sparing surgery, the animals that were treated at the site of the tumor with Cisplatin in a slow-release polymer carrier had a local recurrence rate of only 6%, compared with 25-40% not treated with the drug. Local toxicity was mild.

Inclusion of normal brain or spinal cord in radiotherapy fields can result in demyelination. The glial cells and their progenitors may be the primary targets for this type of injury, and therefore studies of their response has been undertaken at M.D. Anderson Hospital using primary cultures of these cells. It was found that terminally differentiated oligodendrocytes were especially radiosensitive, with significant increase in cells undergoing apoptosis (programmed cell death) within 1-6 hours after doses of 1-10 Gy. This suggests that radiation-induced apoptosis of oligodendrocytes may be involved in early demyelination.

A new assay, the "comet assay," has been developed at the British Columbia Cancer Foundation to determine the fraction of hypoxic cells in tumors. This assay measures DNA damage in the form of single strand breaks in individual cells isolated from irradiated tumors. The rationale for this assay is that about 3 times as many single strand breaks are produced by irradiation of aerobic cells as of hypoxic cells. The single strand breaks are detected by lysing the individual cells in an agarose gel using an alkaline solution, thereby separating the two strands of DNA. The cell lysates are subjected to electrophoresis, and the DNA is stained. The broken strands migrate more readily in the electric field than intact strands, and the amount and migration distance of the "tail" of the comet-shaped stained area is proportional to the amount of damaged DNA in that cell. Assays require only a few thousand cells isolated from fine needle aspirates, and are more rapid than others for measuring hypoxic fraction. The method currently requires exposures of more than 4 Gy, and is able to detect hypoxic fractions greater than 5%, but techniques are being refined to improve the sensitivity of the assay.

Another assay for the presence of hypoxic cells in tumors involves a derivative of the hypoxic cell radiosensitizer misonidazole. This compound, CCI-103F is reduced metabolically in hypoxic cells to a product that binds to macromolecules in viable hypoxic cells, such as may occur in solid tumors. Fluorescent-tagged antibodies to the bound CCI-103F metabolite allow investigators to identify tumor regions containing hypoxic cells in histological specimens.

An important question in tumor radiobiology has been answered by scientists working at the Massachusetts General Hospital: When a tumor is destroyed by radiation, does this occur as a result of radiation effects on the tumor cells directly, or indirectly as a result of radiation injury to vascular and stromal components? These researchers transplanted a tumor line both into nude mice, which have normal radiosensitivity, and into SCID mice, which are 2.5-fold more radiosensitive. The vascular and stromal tissues in a transplanted tumor are derived from the host. If tumor response to radiotherapy were determined by vascular and stromal

radiosensitivity, the TCD50s (radiation dose required for local tumor control in 50% of the mice) would be different in the two types of mice. However, they found that the TCD50 was the same in both mouse strains. The same results were found for both murine and human tumor lines. There is no support, therefore, for the hypothesis that damage to the vascular cells is an important factor in tumor destruction by radiation.

Measurements of intravascular concentrations of oxygen within tumors have shown, for the first time, that blood flowing in vessels within the center of tumors can be hypoxic. It is known that low oxygen tension can affect the rigidity of red blood cells, and this in turn can affect blood viscosity. Dr. Mark Dewhirst and his colleagues at Duke University have found that leukocyte-endothelial cell interactions are greatly depressed in tumors, and that this first step in the inflammatory process is unresponsive to attempts to induce it using chemical and biological mediators. Since tumor cells that metastasize have surface receptors that are similar to leukocyte receptors, this finding may provide one explanation for how tumor cells escape the primary tumor and enter into the blood stream. Therapeutic strategies involving treatments that could induce an inflammatory response, such as hyperthermia, photodynamic therapy, radiation therapy, and chemotherapy, might be unable to do so in tumors. These studies were performed at Duke University.

Pentoxyfilline, a drug commonly used to treat intermittent claudication in humans, increases blood circulation and oxygen concentration in diseased muscle. It has been found to increase the oxygenation and radiosensitivity of rodent tumors at doses lower than that used in humans. Clinical trials using this agent in conjunction with radiotherapy have been initiated.

The possibility that tumor cells may proliferate during the course of radiotherapy suggests that shorter overall treatment times would be preferable to longer times. Using various fractionation schedules in a mouse tumor, investigators at M.D. Anderson Hospital have found evidence that the doubling time of clonogenic cells is shorter in tumors in which severe cell depletion has occurred, such as after irradiation, than in untreated tumors.

## HYPERTHERMIA

The research community continues to express a high level in hyperthermia as 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 of adding hyperthermia to radiation or chemotherapy.

Investigators at the University of Maryland have studied the effects of hyperthermia on RIF-1 multicellular spheroids as models of tumors containing hypoxic cells. Hypoxic cells surviving in the center of the spheroids after hyperthermia treatment were more thermotolerant than surviving aerobic cells at the surface of spheroids. Following a dose 8 Gy of X-rays, a dose that killed most of the aerobic cells, thermotolerance could be induced in survivors by a milder heat treatment than required in unirradiated spheroids. However, the magnitude of thermotolerance was as great as that induced by severe heating (44°C., 30 min.). These results suggest that if heat doses are not high enough to kill hypoxic cells, the surviving hypoxic cells become very thermotolerant and may determine the outcome of fractionated hyperthermia in patients.

The life cycle of enveloped viruses is influenced by host cell membrane organization, which is disrupted by hyperthermia. Investigators at the Oregon Health Sciences University have shown that hyperthermia alters virus budding protein production and intracellular processing in cultured murine cells. Both 44°C/45', and 42.8°C/135' substantially decreased cell-free virus 8 to 48 hours post-heating. Virus from heated cells was as infectious as virus from control cells. Thirty to >90% of heated cells were killed after 42.8°C/135'; however, survivors became thermotolerant to both heat-killing and decreased virus budding. The ability of cells to be infected by viruses and to express infection was markedly reduced in cells heated 6-48 hours before exposure to the viruses.

Damage to tumors by hyperthermia is mediated in large part by vascular damage, accompanied by decreases in pH and oxygen concentration. Investigators of the University of Minnesota recently reported that tumor vessels become thermotolerant when tumors are heated sublethally and relatively high temperatures cannot lower intratumoral pH and oxygen concentration. These investigations on heat-induced changes in blood flow in various organs will provide critical information for treating deep-seated tumors with hyperthermia.

At the Mallinckrodt Institute of Radiology, researchers are investigating changes in the nuclear association of specific proteins in normal and thermotolerant cells. Heating time dependent increases in specific proteins was noted in thermotolerant cells and their relative rate of removal was faster in thermotolerant than in non-thermotolerant cells. The proteins appear to inhibit repair by DNA damage perhaps by masking damaged sites and making them less accessible to

repair enzymes.

Investigators at the University of Texas M.D. Anderson Cancer Center are attempting to modulate heat shock proteins. They found that treatment with bacterial endotoxin lipopolysaccharide (LPS) markedly modulated the rate of synthesis of proteins in the heat stress protein (HSP) 70 family in macrophages. The rate of synthesis of the HSP 70 family was slightly reduced if cells were incubated with LPS 4 hours prior to heating at 43°C for 1 hour, but was greatly reduced as the triggering time approached the initiation of heating and was nearly completely abrogated if LPS triggering immediately preceded or followed heating. Near-normal rates of HSP 70 synthesis occurred if triggering was delayed until 1 to 2 hours after heating ended. The LPS-triggered release of tumor necrosis factor (TNF) was also reduced as LPS addition time neared the heating time, but this depressed release preceded the efforts on HSP 70 synthesis and did not recover for up to 3 hours after heating. The effects of LPS on HSP 70 synthesis also occurred in a murine monocyte cell line, PU5-1.8, that releases TNF in response to LPS and in a murine fibroblast cell line, NIH/3T3, indicating a lack of restriction of these effects to cells of monocyte/macrophage lineage. The nature of the transcriptional or translational mechanisms controlling these responses is unknown.

Researchers at Texas Tech University have developed a non-invasive magnetic resonance imaging (MRI) technique to study the mechanism of hyperthermic damage of tissues at the molecular level. Using different isotopic hindered fractal diffusion models, molecular parameters i.e. diffusion coefficients, impermeable barrier sizes and fractal dimension of water diffusion in brain, kidney, muscle and other tissues was obtained in-vivo. All of the above water diffusion parameters were increased irreversibly after hyperthermia treatment, except in muscle. The results indicate that water inside the tissues becomes less hindered and more chaotic following hyperthermia.

Investigators at the Ellis Fischel State Cancer Center of the University of Missouri, have shown that hyperthermic perfusion of rat liver results in lipid peroxidation. A source of oxidative stress was shown to result from the conversion of xanthine oxidase to the oxidase form. This conversion resulted in loss of glutathione from the perfused liver, mainly as oxidized glutathione. Inhibition of xanthine oxidase by allopurinol and chelation of available iron by desferrioxamine substantially ameliorated the oxidative stress incurred during hyperthermic liver perfusion. It was noted also, that protection from oxidative stress and the associated heat induced hepatotoxicity can be accomplished without significant reduction of the tumoricidal effects of hyperthermia.

At the University of Kentucky thermochemotherapy studies are being conducted to determine the effect of chemotherapeutic agents and hyperthermia on the mouse fibrosarcoma FSa-II both in-vitro and in-vivo. Activation energies for cisplatinum, bleomycin, 5-fluorouracil, and BCNU were measured following in vitro treatments and thermal enhancement ratios (TER) for tumor response were determined by tumor growth assays in-vivo. A good relationship was found between the activation energy determined at temperatures of 37 and 41°C in vitro and TER in vivo. Drugs with large activation energy showed greater TERs. BCNU was most significantly enhanced by heat while 5-FU was least. Cisplatinum and bleomycin were intermediate.

A major problem in the clinical use of hyperthermia continues to be the difficulty of achieving adequate heating of the tumor. Analysis of data from patients treated at Duke University since 1984 clearly indicates that the tumors of a majority of patients were not heated adequately (therapeutic temperature of 43°C) with currently available technologies. The analysis showed, however, that significant improvement in response could be achieved if temperature distributions were elevated by approximately 1.5°C. Studies in tumor-bearing dogs have shown that this may be accomplished by combining whole-body hyperthermia with local hyperthermia or by using vasodilators such as nitroprusside which can shift tumor temperatures upward by 1.5-1.8°C. It is now possible to deliver adequate clinical hyperthermia.

Another approach to achieving adequate heating of the tumor is being explored at several institutions: Insertion of ferromagnetic "seeds" into the tumor. When placed in an oscillating magnetic field, the seeds heat until they reach their Curie temperature (the temperature at which they lose their magnetism, and do not heat further). The Curie temperature depends on the composition of the seeds. Ferromagnetic seeds have been used for hyperthermia in conjunction with seeds containing radioactive iodine for the treatment of intraocular melanoma in animals. In a study performed at the University of Wisconsin with radiation alone, using the iodine seeds, the radiation dose required to control half the tumors was 41.7 Gy, but when hyperthermia was given as well, only 9.5 Gy was required, a reduction in dose by a factor of 4.4. Hyperthermia alone gave a 20% tumor response rate, but the responses were only temporary and not curative.

The use of hyperthermia as an adjunct to chemotherapy is receiving increased attention by clinical investigators. At Duke University researchers have shown that the uptake of cisplatin and carboplatin by the prostate is increased 3-5 fold by the use of hyperthermia. This finding prompted development of a protocol exploring the feasibility of using regional hyperthermia with cisplatin and radiation in patients with locally advanced unresectable carcinoma of the prostrate.

At the University of Colorado several studies are being initiated evaluating the used of whole body hyperthermia and chemotherapy in various types of canine tumors. In separate studies, doxorubicin and mitoxantrone will be evaluated above as combined with whole body hyperthermia in dogs with lymphomas. Maximum tolerated drug dose and progression free interval will be used as endpoints in these studies.

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) continues to struggle for acceptance as a potential treatment modality for some cancers. PDT is based upon the principal that systemically administered photosensitizers 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 at or near the maximum for the photosensitizer, singlet 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.

New classes of photosensitizers continue to be synthesized, characterized spectrally, and evaluated biologically. These classes include merocyanine dyes, indoles, oxazines, thiazines, bacteriochlorophylls, napthalocyanines, benzoporphyrins, chlorins, purpurins and phthalocyanines. All of these classes appear to be potent photogenerators of singlet oxygen. A representative of each of the latter four classes is currently or shortly will be in clinical trial.

Researchers at the University of Rochester have investigated a class of chemically-defined compounds, the synthetic "picket fence porphyrins" (derivatives of tetraphenylporphine with acrylamide substituents at the ortho-position of the phenyl groups). This class of porphyrins exhibit several attractive features that make them promising candidates as biological photosensitizers for photodynamic therapy. It was shown that by altering the side chain length and atropisomer configuration of the parent compound (tetraphenylporphine) the effectiveness of these compounds in vitro can be altered. In vivo some of these compounds were as effective as Photofrin II in controlling tumor growth. Since these porphyrins can be modified with ease, synthesis of a chemically-defined photosensitizer superior to Photofrin II is anticipated.

Investigations at Children's Hospital of Los Angeles have shown that both photodynamic therapy (PDT) and hyperthermia (HT) induce the same family of heat shock proteins. However, cross resistant between HT and PDT was not observed. It was also shown that porphyrin mediated PDT induces transcriptional and translation

expression of heme oxygenase. One of the metabolic products of heme oxygenase (i.e. carbon monoxide) is now believed to play a major role in signal transduction. Cellular exposure to porphyrin and to photochemically generated singlet oxygen independently induce increased expression of this gene (which may initiate molecular modulation of oxidative damage in cells). Four cell lines resistant to porphyrin-mediated PDT have been isolated from the murine tumor line RIF-1. Comparing these lines to the parent line has shown that the resistance is not the result of differences in uptake of porphyrin, levels of antioxidant enzymes, or levels of constitutive stress proteins. However, cellular level of several other unique proteins appear to be over expressed and are higher in the resistant cell lines than in the parent lines. Glucose regulated proteins are induced when cellular protein glycosylation is inhibited during PDT, and are over expressed in cells tolerant to PDT.

RADIATION MODIFIERS: SENSITIZERS AND PROTECTORS

The field of chemical modifiers of radiation response is evolving as emphasis shifts from the traditional hypoxic cell sensitizers to other classes of modifiers. There is increasing interest in bioreductive agents and agents that exploit or modify tumor pathophysiology, as well as agents that modify oxidative damage produced by radiation. Both radiosensitizers and radioprotectors are being studied in combination with chemotherapeutic agents as well as with radiation.

Clinical trials of several chemical modifiers of radiation response are in progress. The hypoxic cell radiosensitizer etanidazole (SR-2508) is being tested in Phase III clinical trials for use in the treatment of head and neck cancer, and Phase II trials in advanced prostate cancer. SR-4233, a bioreductive agent and hypoxic cell cytotoxin, and buthionine sulfoximine, which depletes cells of the natural radioprotector glutathione, are undergoing initial toxicity tests in patients.

Glucose is an important modulator of the toxicity of thiols used as radioprotectors because it is the ultimate source of the reducing equivalents used in cellular detoxification of peroxidation products caused by oxidizing thiols. This has been confirmed in studies at Massachusetts General Hospital using cells deficient in the enzyme G6PD that are unable to show pentose cycle stimulation. These mutant cells have a much greater sensitivity than their parent cells to cell killing by thiols or by hydrogen peroxide. This information has wider application to fields such as arthritis, oxygen toxicity to the lung, heart disease, and ischemia-reperfusion injury where thiols may be used as therapeutic agents. Additional studies have shown that charge on a thiol compound can affect its ability to radioprotect, which is important in the design of more effective radioprotectors.

PARTICLE RADIOTHERAPY

Some tumors are located adjacent to critical normal tissues such as the spinal cord. Conventional photon and electron therapy beams cannot be focused adequately to irradiate these tumors and also spare the normal tissues. Proton beams, however, have a finite penetration distance into tissue that is a function of the energy of the incident beam. There is also less scattering than with conventional therapy beams. Therefore, protons can be used to achieve extremely precise radiation dose distributions, so that smaller volumes of normal tissues surrounding tumors are exposed to high doses, and higher doses can be delivered to tumors, increasing the probability of local control and improved survival.

A total of $4 million appropriated in FY 92 was awarded to Massachusetts General Hospital and Lawrence Berkeley Laboratory/University of California Davis for continuation of the planning process for Proton Research and Treatment Facilities that will be hospital-based. The planning process will require an additional $4 million for completion in 1994. If continued funds are forthcoming, two facilities could be constructed to begin treating patients in 1997, for a total cost of each facility of about $40 million.

The National Cancer Advisory Board of the NCI appointed a select committee to review and evaluate the scientific rationale and current status of proton beam therapy. The NCAB accepted the report of the committee at its May 1992 meeting. The report recommendationed that the NCI should "endorse the funding of two hospital-based proton therapy facilities in the United States, strategically located to treat patients for whom a prima facie case for the therapeutic superiority of proton therapy has been established, and to conduct peer reviewed research into the value of proton therapy for other tumor types".

Radiotherapy with either charged or uncharged particles continues to receive a significant portion of the RDB budget. Neutron therapy Phase III trials have been supported for the last several years to 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. Neutrons have a high linear energy transfer ratio which increases the relative biological effectiveness of neutron treatments when compared with conventional x-rays for the same radiation dose. Charged particle therapy with protons, on the other hand, shows an advantage for radiotherapy treatments through improved dose distributions. The highly focused beams of protons and charged particles result in greater sparing of normal adjacent tissue and a higher tumor dose, resulting in increased local control and

improved survival. Charged particles are now successfully used in 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.

A two-year study to carry out therapy-simulated Monte Carlo calculations for proton particles in the energy range of 60-250 MeV was initiated the past year through an Inter-Agency Agreement with the National Institute of Standards and Technology. Although protons are generally assumed to have a biological effectiveness very similar to conventional x-rays, there is clinical evidence that in the region of the Bragg peak the biological effect is somewhat greater. The data will provide a foundation for biological models to explain the difference. The project will result in Monte Carlo codes that will be in the public domain for use by scientific investigators.

A high priority research area of the Radiation Research Program for more than a decade has been the fast neutron therapy clinical trials project. The interest in neutrons stems from the fact that fast neutrons possess a higher energy transfer ratio than standard x-rays or photons for the same radiation dose. Thus, the relative biological effectiveness of the neutron beams is much greater than is possible with photon treatments, enabling treatments to take place in a much shorter time scale. The Radiation Research Program awarded contracts in 1979 for the design, development and construction of state-of-the-art hospital-based neutron therapy facilities and the conduct of Phase II and Phase III trials. After more than a decade of effort, Phase III trials were completed in early 1991 for the treatment of prostate cancer, non small-cell lung cancer and head-and-neck cancer. The efficacy of neutron beam therapy for the treatment of malignant salivary gland tumors was demonstrated early on and the Phase III study closed in 1986. The institutions participating in the Phase III studies were the University of Washington, Seattle; University of California, Los Angeles; the University of Texas Cancer Center, M. D. Anderson Hospital, Houston; Cleveland Clinic Foundation, Cleveland, Ohio; and Clatterbridge Hospital, Liverpool, England. Preliminary analyses of the three studies currently indicate no significant difference in survival between fast neutrons and conventional therapy, although some tumors show a complete response much sooner with neutron treatments, particularly in advanced head-and-neck disease. Final evaluation of the results of the recent Phase III studies must await long-term follow-up.

## RADIOIMMUNOTHERAPY AND OTHER NUCLIDE RADIOTHERAPY

Cancers that have estrogen receptors, such as breast, ovarian, and endometrial cancers, may be targeted with estrogens carrying radionuclides. Iodine-123-labeled estrogens show selective toxicity in vitro to cancer cells with estrogen receptors, using concentrations achievable *in vivo*.

Radioimmunotherapy utilizes differences between surface molecules on tumor cells and normal cells to direct therapy to the tumor cells themselves. The clinical feasibility of radioimmunotherapy depends on the presence of unique molecules on tumor cells, the number of such molecules/cell, and the ability of the therapeutic molecules to reach tumor cells in sufficient numbers to achieve a therapeutic effect, as well as many other factors. Radioimmunotherapy shows some promise for treatment of lymphomas and neuroblastomas, where cell killing appears to exceed that from equivalent doses of external-beam irradiation. Currently, uncertainty in dosimetric estimates is the limiting factor in selecting the dose (both in milligrams of antibody and in mCi of radioisotope) to be administered for a particular radioimmunotherapy patient.

Studies are being performed at the University of Washington using trace-labeled antibodies to predict the behavior of antibodies labeled with therapeutic amounts of radioactive isotopes. Serial images taken by a gamma camera have demonstrated that this method can detect patients with abnormal rates of clearance. Methods for quantitative autoradiography and immunoperoxidase staining are being developed to determine microscopic distribution of the antibody within the tumor. These will be used to calculate the microscopic distribution of radiation doses. Differences in the distribution of radioactivity between follicular lymphomas and non-involved perifollicular zones have been observed.

A comparison of two radiolabeled antibodies differing by a factor of 35 in their affinity for a common antigen has shown that more cell killing was produced by the antibody with lower affinity, which was distributed more homogeneously in spheroids.

Investigators at Harvard University have shown that it takes about 25 radioactively-labeled immunoconjugate molecules/cell to kill 90% of lymphoma cells, using the a-particle emitting isotope 212-bismuth. Calculations showed that about 4 a-particles traversed the cell's nucleus from this exposure. Electron micrographs of the treated cells indicated that they were dying in interphase through the process of apoptosis, rather than dying as they attempted mitosis.

## BORON NEUTRON CAPTURE THERAPY

Boron Neutron Capture Therapy (BNCT) is a potential treatment modality for cancerous tumors that has resurfaced because of a resurgence of interest by members of the medical community. The concept of BNCT is based on the nuclear reaction that occurs when a non-radioactive isotope of boron (10B) is irradiated and absorbs low energy (thermal) neutrons. The unstable boron (11B) that is formed undergoes instantaneous nuclear fission to yield a lithium (7Li) nucleus and a highly energetic alpha particle. Theoretically, a single alpha particle can kill a cancer cell if part of its energy is released in the cell nucleus. The rationale for using BNCT is to exploit the short range of the alpha particle. If boron (10B) atoms could be selectively concentrated in the tumor, then the subsequent irradiation of the tumor would minimize the radiation dose to the normal tissue. The early studies in experimental animals and the clinical trials in man used inorganic boron compounds which distributed ubiquitously throughout the body. The non-specificity of these boron compounds resulted in boron concentrations which were higher in the blood than in the tumor. As a result, the vascular damage which occurred caused extensive destruction of the normal tissue, as well as the tumor. These initial clinical studies showed no therapeutic advantage for the use of BNCT and interest in this treatment modality waned. It was clear, however, that better boron compounds needed to be developed to achieve the necessary high tumor-to-normal tissue ratios, and the high tumor-to-blood ratios. Some limited compound development followed the initial BNCT clinical studies. Two anionic sulfhydryl compounds showed promise and one of these (the sodium salt of B12H11SH2) was used subsequently by Hatanaka in Japan to treat brain tumor patients using BNCT. The reported results of his studies (the average survival of BNCT-treated glioblastoma patients exceeded 28 months vs. 5-17 months with conventional therapy) created worldwide renewed interest in BNCT. The renewed interest in BNCT has accentuated the need to develop second generation boron-containing compounds for use with BNCT.

In order to stimulate the development of boronated compounds for the clinical application of BNCT, the Radiation Research Program sponsored a Workshop (May 3-4, 1988) directed to this goal. As a result of the Workshop a request for grant applications was issued (January 5, 1990) to synthesize compounds for BNCT. Four awards were subsequently made and these grants are now beginning to show results.

At Emory University, investigators are synthesizing a series of carboanyl-pyrimidine nucleosides which will be evaluated for their ability to phosphorylate intracellularly into DNA. Although these compounds are expected to be used to treat brain tumors, they appear to have anti-viral activity as well.

Investigators at Ohio State University are synthesizing a variety of carboranyl nucleosides and masked nucleotides with the aim of

delivering large numbers of boron atoms selectively into tumor cells in a form that will be retained after incorporation into nucleic acid. Oligonucleotides with carborane groups on the sugars will also be investigated.

At the University of California, Los Angeles, research efforts are being directed toward using monoclonal tumor-targeting proteins as selective delivery systems for 10B. The goal being the precise chemical synthesis of compounds containing large arrays of boron atoms which can be attached to immunoreactive species in an orderly manner to produce conjugates capable of strong binding to tumor-associated cell surface antigens such as CEA. Substantial advances into the synthesis and characterization of boron-rich peptide and oligophosphate macromolecules have been made in the last year. Multigram quantities of an engineered chimeric CEA-recognizing antibody has also been made from which a large scale production of immunoreactive F (ab) 2 antibody fragments was possible. These investigators are now ready to attach the boron containing compound to the delivery system.

Workers at the University of California, San Francisco are attempting to synthesize a wide variety of water-soluble porphyrins and phthalocyanines containing large fractions of boron in the form of polyhedral carboranes and/or borane anion derivatives. One of the boronated photoporphyrins (BOPP) was shown to selectively localize in C6 intracerebral glioma xenografts implanted in mice at ratios as high as 400:1 relative to normal brain 48 hours after i.v. injection. At the same time point the tumor/blood ratio was 11:1. BOPP appears to localize in the mitochondria and in tumor concentrations that exceed the levels required to achieve tumor necrosis. BOPP has many characteristics that make it a strong candidate to test clinically.

The activation of intracellular boron requires a neutron source capable of providing a beam of specified flux and spectral characteristics. Presently there are only 6 reactors in this country capable of producing suitable a beam for BNCT. The paucity of neutron sources is a major problem that needs to be resolved before BNCT can achieve wide acceptance as a treatment modality for cancer.

A small start has been made toward resolving this problem. Investigators at the Science Research Laboratory, Inc. in Somerville, Massachusetts, a small business grantee, is developing new electrostatic accelerator (Tandem Cascade Accelerator) which will produce a beam comparable to the reactor-produced epithermal beams required for BNCT. Fabrication of a prototype accelerator is currently in progress.

RADIOTHERAPY TREATMENT PLANNING

The Radiation Research Program has had a major involvement 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 software to reduce the time-consuming and labor-intensive tasks of 3-dimensional radiation therapy treatment planning. The group has developed new software engineering and documentation standards which have yielded 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 advances will have important consequences for the future development of sophisticated software that can then be adapted to community-based health care centers.

Prototype demonstrations of the new portable software tools have been demonstrated at all three institutions and will be clinically evaluated in the next year. The tools consist of (1) object definition of anatomical landmarks in a CT study; (2) creation of data sets for 3D display; (3) tumor localization; (4) radiation target volume definition; (5) first-guess plan generation; (6) treatment plan evaluation; and (7) treatment verification through portal imaging and simulation image registration. The implementation of the software tools into the radiotherapy treatment planning systems of the three institutions is the next stage of the project for the purpose of clinical evaluation. Evaluation of the tools in each of the individual institutions' clinical environments is planned for the next year. 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 in the radiotherapy community, many of the barriers to the use of this advanced technology will be removed, enabling more widespread use of 3-D conformal radiation therapy.

The goal of conformal radiation therapy is to tailor the radiation treatment field to the shape of the tumor, thus maximizing the radiation dose to the tumor while minimizing the dose to the adjacent normal tissues. Conventional treatment planning methods are inadequate for planning complex innovative computer-controlled delivery techniques involving several beams, beam directions, and beam shapes. The treatment plans involve consideration of the probabilities of normal tissue

complications, which in turn depends on both dose and volume in the irradiated field. The development of the procedures involves collaboration of physicists and computer programmers in searching for the optimal beam shape, dose, and treatment volume.

Physicists, engineers, and computer programmers at the University of Texas Medical Branch at Galveston are developing and testing techniques and programs for conformal radiotherapy.

Electron beams are used in radiation therapy to treat superficial lesions, mainly in the head and neck, and occasionally in the chest wall and intraoperatively. Electrons have a finite range and can provide the best sparing of normal underlying tissue of any conventional treatment modality. There has been a great deal of uncertainty in the dose distributions near regions of heterogeneous tissue density, such as air cavities and bone. Conventional methods do not predict these effects accurately enough. Direct prediction of dose distributions from electron beams is being investigated at the University of Wisconsin using Monte Carlo simulation, and this method is being linked with a 3-D radiotherapy treatment planning system. Dose predictions from this method are being compared with those from other methods and with measured dose distributions.

The depth of penetration of electron beams can be modified during radiotherapy using "dynamic range shifters," which are being developed at the University of Utah. These show promise for achieving a uniform dose to the target volume in breast cancers while sparing the critical normal tissues underlying the breast, namely, lung and heart.

PATTERNS OF CARE

The Radiation Research Program is currently funding a contract with the American College of Radiology (ACR) 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 hyper-fractionation (two or more 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 fourth year of performance and has completed the updated facility master list survey, data collection for the long-term follow-up studies and consensus guidelines for five disease sites. Newsletters on the guidelines have been disseminated world-wide and several lectures and refresher courses have been delivered at professional meetings. All data collection for the patterns of fractionation project has been completed and analysis of local control and survival as a function of fractionation patterns is proceeding. The contract with ACR has recently been extended to complete in October 1993.

## SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

In FY 1992, the RDB funded 2 SBIR contracts (Phase II) and 17 SBIR grants (11 Phase I, and 6 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.

WORKSHOPS

Radiation Resistance - September 17-19, 1992

A panel of experts discussed the possible mechanisms for the cellular resistance exhibited by tumor cells exposed to ionizing radiation. It was the recommendation of the workshop participants that exploration of the molecular biology of gene regulation of the radioresistant cells would be most useful. As a result of the workshop, an RFA was issued in December, 1991, with an application receipt date of March 13, 1992. Twenty-two applications were received as a result of the solicitation. These grants were reviewed the last week of July, 1992. It is expected that four awards will be made.

Hyperthermia - September 24-25, 1992

Key investigators representing the various disciplines involved in clinical hyperthermia met to discuss the present state of the art, the problems, and potential solutions. Recommendations were made on how to advance the field so that clinical hyperthermia can become an accepted treatment modality.

Chemical Modifiers of Radiation Response - February 11, 1992

Experts in the field of radiosensitizers met to determine the future directions of the field of chemical modifiers of radiation response. It was agreed that emphasis on traditional hypoxic cell radiosensitizers should be reduced, and that new modifiers should be developed in the classes of bioreductive agents, agents that exploit tumor pathophysiology, modifiers of repair of radiation damage, and agents that modify free radical damage. An RFP was issued for synthesis of chemical modifiers of radiation damage in August, 1992, with a receipt date for applications of December, 1992.

INTERNATIONAL ACTIVITIES

US/RUSSIA

The Radiation Research Program initiated a scientific exchange program in 1990 between United States and Russian scientists in the field of charged particle proton therapy. A United States-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 Central Scientific Research Institute of Roentgenology and Radiology, Leningrad. Three Russian scientists were provided tours of the charged particle facilities in this country in the fall of 1990 and several scientists were supported to present papers at the biannual meeting of the Proton Therapy Cooperative Oncology Group (PTCOG) in Boston in the spring 1991. In spite of the break-up of the Soviet Union, communications and scientific exchange between the United States and Russian scientists continue. An American scientist spent a month at the Institute of Theoretical and Experimental Physics (ITEP), Moscow, to initiate international dosimetry intercomparisons between proton facilities in the United States and Russia and to translate papers into English for publication in the United States. A Russian scientist will be supported to spend a year at the Loma Linda University proton therapy facility in Loma Linda, California, to gain experience in 3-D treatment planning and to carry out dosimetry intercomparisons. Plans for the future include international workshops in the areas of accelerator and proton beam gantry and design of clinical protocols. Continued exchange of scientific ideas and delegations is anticipated to yield benefits to both countries as the respective groups complete plans for the hospital-based facilities in both the United States and Moscow. 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. Final evaluation of local control and survival of the patients when compared with conventional radiotherapy awaits appropriate long-term follow-up.

FUTURE DIRECTIONS (RDB)

A number of scientific concepts were approved in the past year by the Board of Scientific Counselors for development and funding. These include:

1. Synthesis of Chemical Modifiers of Radiation Response. One contract for four years was approved for recompetition. This contract supports the development, synthesis, and preliminary in vitro and in vivo testing of novel agents to be used in conjunction with radiotherapy.

2. Radiation Resistance. Up to four awards will be made for the study of the molecular biology of gene regulation of radioresistant cells.

3. 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.

4. 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 were solicited through a Request for Applications (RFA) issued in December, 1991. The 22 grant proposals submitted in response to this RFA are 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. Four grants are expected to be awarded.

The Radiotherapy Development Branch continues to stimulate, develop, and administer research in radiation biology, chemistry, and physics at the levels of both basic science and the clinic. In addition, it supports the development of advanced computer-based tools to improve treatment planning and the delivery of radiation therapy.

1. The use of hyperthermia as a treatment modality continues to interest the medical community. However, further research is needed to develop and improve methods for achieving reliable and uniform heating of tumors, especially deep-seated tumors. Improvements in thermometry are needed as well, especially methods for non-invasive thermometry. Hyperthermia as an effective adjunct to radiation and chemotherapy needs to be confirmed by standardized, randomized clinical trials for specific tumors and anatomic sites.

2. Photodynamic therapy (PDT) is being explored as a possible treatment modality. Better chemical compounds for light-stimulated radiation treatments are being sought, and will require clinical testing. Development of improved light-producing lasers and light delivery systems will be encouraged, as well as systems and methods for photodosimetry.

3. Interest in boron neutron capture therapy (BNCT) is increasing as research advances unfold in the chemistry and biology of boron containing compounds that concentrate in tumors. Irradiation of a boron compound with low energy neutrons causes emission of a short range alpha particle that deposits an intense

radiation dose within the cell. This is an example of possible cellular radiotherapy.

4. Radiolabeled immunoconjugates and cell specific receptors are being investigated for use in systemic radiation therapy (SRT), another form of cellular radiotherapy. This type of therapy seeks to take advantage of differences between tumor cells and normal tissue cells. Further support is needed for research on the dosimetry of these radionuclide tagged compounds. Radiolabeled immunoconjugates for therapy and diagnosis will continue to be a high priority research area of the Radiation Research Program.

5. Chemical modifiers of radiation damage continue to be investigated for use in conjunction with radiotherapy. The goal for their use is to increase the sensitivity of neoplastic tissue to radiation. Attempts to improve the efficacy of these agents and to decrease their toxicity are continuing. Many of these agents have radiosensitizing and chemosensitizing activity, while others act as prodrugs that are activated in tumors. 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.

6. 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 1990s 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.

7. 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.

8. 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.

V. SCIENTIFIC OVERVIEW

DIAGNOSTIC IMAGING RESEARCH BRANCH

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 are conducted by the Radiologic Diagnostic Oncology Group (RDOG). The goal of the RDOG is to use single or multiple new imaging technologies to diagnose, stage, and monitor cancer and to develop optimal algorithms for the appropriate sequential, cost effective selection of diagnostic procedures.

The Diagnostic Imaging Research Branch provides leadership for the NIH-wide Diagnostic Radiology Coordinating Committee (DRCC) mandated by Congress. The committee is charged with developing a five-year research plan for diagnostic radiology/imaging science at the NIH. Additional functions of the DRCC include improvements in the dissemination and management of information related to imaging science.

The DIRB 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 $42,879 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, computer-assisted radiology and collaborative clinical diagnostic imaging research.

## 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. 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.

## NON-IONIZING RADIATION SECTION

### MAGNETIC RESONANCE IMAGING (MRI) AND SPECTROSCOPY (MRS)

The frontiers of research in this area have been extended both by continued progress in existing projects and by the appearance of new developments. Many investigators have been continuing to develop and refine new equipments and techniques to provide clinical images of higher spatial and contrast resolution, in shorter scanning times or in real time, and for specialized application to the imaging of important specific organs, tissues, or systems. Most of the research and clinical use of MRI employs the imaging of hydrogen nuclei, or protons, since they are readily abundant throughout the body and provide the strongest intensities of magnetic signals, and thus the most desirable signal-to-noise ratios in terms of sensitivity. A few centers carry out physical science and clinical imaging research with

other magnetic nuclei, such as fluorine, phosphorus, and sodium.

There is a greatly increased interest in the development and use of new magnetic contrast agents for MRI image enhancement and detection of tumors as well as their use in studying dynamic functions, such as flow or metabolism. There is also a growing activity in the study of MRS to assess and monitor the effects of tumor therapy, using especially phosphorus and proton MR spectroscopy, and occasionally carbon. Finally, a few important studies are being carried out on potential bioeffects of high magnetic fields.

Instrument and Technique Development:

Work carried out on the SBIR-supported program at Advanced NMR Systems, Inc., ( now in Wilmington, Massachusetts) has provided the fastest available MRI system, which can depict the motion of any portion of the body in any plane in real time. Individual images are made in as little as 25 milliseconds and "assembled" sequentially to create a moving picture of the beating human heart or the action of the temporomandibular joint. The success of this "real time", fast imaging technique based on echo-planar imaging principles, has led to a great increase in the practical use of MRI systems in sports medicine, where the injured joints, members, or tissues can be examined quickly and in a visual manner which permits the member to be seen in action as it is flexed. The first phase of another SBIR grant with this company has been aimed at designing a fast, low cost, MRI system which is dedicated to the examination of the breast. If such a system can be developed for a few hundred thousand dollars or less capital cost, as is the goal, a significant new method for clinical examination of the breast for potential cancers may become practical. Conventional MRI is presently being studied and used clinically for breast examination in diagnostic workup or in equivocal cases, despite its very high cost per procedure, but not for screening. A significant breakthrough has been made elsewhere in the past two years in the use of a new magnetic contrast agent, based on gadolinium, which has a tendency to seek tumor receptor locations and to reside there long enough to enhance the MRI image for improved tumor detection sensitivity.

Similar fast MRI techniques and designs are being studied under an SBIR program at Intermagnetics General Corporation in Guilderland, New York and are being aimed at improved imaging and detection of prostate cancer.

New success at the Mayo Research Foundation is providing the unusual capability of being able to correct MRI images, pixel for pixel, after they have been produced in a blurred or distorted form because of patient motion, so that they can be

restored to a clearer, more accurate form for improved quality in diagnosis. This is particularly useful in clinical application to abdominal imaging in adults, where respiratory, cardiac, or organ motion, degrades the original image, or in pediatric imaging , where sedation might then be reduced. Other research at Mayo has continued to improve more rapid MR scanning so as to facilitate the use of MRI in a manner somewhat akin to that of x-ray fluoroscopy, where patient placement and precise desired angle of imaging can be more quickly and efficiently achieved at reduced cost in dollars and time.

Another new way of achieving a fast conventional MRI scan in a few seconds has been successfully developed at Stanford University, based on squared spiral scans rather than the conventional rectilinear scans. This technique provides new high speed imaging of tumors of the abdomen and thorax. By another improvement, known as correlation filtering, it is now possible to visualize lung parenchyma; this was not possible before. Other improvements in high speed dynamic MRI techniques are also being pursued at the University of California at Irvine.

An SBIR investigator at Huntsville, Alabama under Alabama Cryogenic Engineering, Inc., is developing an improved cryogenic cooling system, based on engineering knowledge derived from space technology, to be used with MRI medical systems.

A major program project continues at Columbia University in the long range study and evaluation of MRI clinical applications, quality control, and methodology of MRI systems operated at very high magnetic fields, at intensities of 2.0 and 3.0 Tesla in animal and human subjects. Particular clinical emphases in their research have been placed on studies of brain tumors, trauma, and stroke because of the high order of neuroradiological experience at this institution. Such high fields are necessary to permit research using sodium, with its low magnetic moment (i.e., poor sensitivity) in addition to phosphorus and protons, and it has been possible to quantitate fractions of sodium in intracellular and extracellular water. A unique 5.0 Tesla whole body imaging system has recently been designed and installed at Columbia, which will extend this research and increase our knowledge of possible bioeffects at such high magnetic field intensities. Advances at 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 patients during treatment.

A new approach to the imaging and detection of prostate cancers has been developed over the past few years, particularly at the University of Pennsylvania, in which special coils are inserted in the rectum to give high resolution images of prostate tissue while the patient is lying within a conventional high field MRI

system.

Outstanding physics and engineering contributions to the clinical application of MRI have been made at the Medical College of Wisconsin at Milwaukee in the design, construction, and clinical evaluation of specially designed NMR body and surface coils to fit around or next to different body parts. These coils are now routinely used greatly to increase the spatial detail and 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 is one of the most valuable new uses of MRI in conventional diagnostic medicine and in sports medicine Similar special coils have also been developed for use in research and clinical studies in MR spectroscopy.

Special coil and instrument 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 a desktop MR microscope capable of a spatial resolution of 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 tissue heating and separate reversible from irreversible tissue change. 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 in both diagnosis and in treatment planning and monitoring.

Special MR techniques have been perfected at the University of California at Irvine for measurement of the velocity of blood and other body fluid flow 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 surroundings are suppressed.

A new technique for imaging molecular 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 laboratory research in vivo in animals using magnetic resonance spectroscopy (MRS) to measure and 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 which can be introduced in contrast agents or in treatment pharmaceuticals. Important progress has been made at Wayne State University in determining which peaks of the MRS phosphorus-31 spectrum that are characteristic of metabolic products within tumors can be used as predictive markers of response to chemotherapy. At Memorial Hospital in New York a number of MRS studies have been carried out with phosphorus-31 to follow the metabolism of sarcomas under treatment and the radioresistance and radiosensitivity of tumors under radiation treatment. Fluorine-19 MRS measurements have assisted in the in vivo monitoring of changes in 5-fluorouracil metabolism induced by methotrexate.

With high magnetic fields it is also possible to make MR images of these same 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 distri-bution of oxygen, and sodium-23 MR spectra have 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 prostate tumors in mice. The work may be extended to all types of tumors.

Early pioneering research by a team at the Johns Hopkins University has continued to use MRS to measure tumor oxygenation and radiosensitivity and to study the effects of both radiation therapy and chemotherapy in vivo in mice using RIF-1 and EMT6 tumor models as well as human MCF-7 breast carcinomas implanted in mice. Multinuclear studies are involved. The team uses 31-phosphorus to measure bioenergetics, 13-carbon to measure the flux through glycolysis, the hexose monophosphate shunt and the TCA cycle, and protons (1-hydrogen) to monitor lactate.

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 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 Chicago 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.

Currently, the interesting and potentially exciting use of MRS to attempt to assess and monitor the effects of tumor therapy is limited principally by two physical constraints: (1) the sensitivity or signal-to-noise ratio that is possible with the available natural magnetic nuclei, especially phosphorus and protons, vis-a-vis (2) the ability to localize the volume of the magnetic field probe so that the sample is also homogeneous. These two factors militate against each other. However, another factor that is also of enormous importance is the need for thorough understanding of the complex and dynamic phenomena of tissue and tumor metabolism and pathology and the ways in which the variations in the MRS spectral changes can be correlated with these biochemical phenomena. Validation of these relationships will require very sophisticated knowledge of biochemistry and continued laborious experimentation; thus much research will continue to be needed to achieve this understanding.

Investigators at Harvard University have been studying magnetic field effects on iron oxide-loaded lung macrophages to study 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 motions 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 results are more irreversible.

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.

New superfine magnetic particles (2.86 nanometers in diameter) are now available as monocrystalline iron oxide nanocompound (MION) and are being used at Massachusetts General Hospital to study receptor imaging of tumors. Another superfine magnetic contrast agent has been made by OmniQuest,Inc., in Atkinson, NH on an SBIR grant; it tends to form microclusters, is nonmagnetic below 50 Angstroms and superparamagnetic above 500 Angstroms. Another SBIR team has developed a non-protein MR contrast agent for breast imaging; it has the ability to highlight selectively primary and metastatic breast carcinomas.

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 candidates. A program at the University of Arizona is concentrating on the development of new liposome-entrapped contrast agents for the detection of liver and splenic metastases. They look promising for their ability to deliver the desired magnetic contrast material to the desired sites in the body without prior degradation or dilution. Contrast agents are being used pharmacokinetically at the Pittsburgh NMR Institute and the Massachusetts General Hospital to study tissue perfusion in the spleens and placentas of rats and rabbits. Agents employed include Gd-DTPA, Gd-DTPA-albumen, 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 underlay the production of intrinsic contrast differences in MRI, is the subject of basic research at Yale University.

Bioeffects:

Research has been done on the thermophysiologic effects of magnetic resonance imaging at Cedars-Sinai Medical Center in Los Angeles and at UCLA. Theoretical and experimental studies have examined particularly 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. A group at the University of California at Berkeley has also undertaken a specific study of the effect of very high magnetic fields on lymphocyte calcium-2+ metabolism. 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 easy to control.

## NEW DEVELOPMENTS IN X-RAY TECHNOLOGY FOR BREAST IMAGING

Since digital methods of image computation underlie the entire dramatic recent history of radiology, starting with the CT and then the MRI scanners, it is not surprising that digital techniques should play a part in trying to improve breast imaging. The first major attempts began several years ago by groups, including the team at the University of Michigan, attempting to digitize mammographic films for subsequent computer-aided discrimination and analysis. The method consists of scanning the photographic films with a light-spot scanner for variations in optical density and then using the digital computer as an expert radiology assistant to assist in the detection of abnormalities.

More recently, other regular grant and SBIR grant investigators, have developed new CCD (charge-coupled-device) cameras to permit direct acquisition of the x-ray image information in digital form without the use of film. The cameras are made up of arrays of x-ray sensitive solid state materials whose electrical responses can be digitally quantized and fed directly into the computer for analysis and processing. Groups working to perfect the sensitivity and resolution of these systems and to carry out clinical testing with them are located at Lorad Medical Systems in East Hartford, CT and the University of Arizona at Tucson. It is anticipated that this approach, when perfected, will aid the human radiologist as an expert radiology assistant, providing her or him with greater sensitivity, accuracy, and security in the task of finding all the significant breast lesions at the earliest time.

A new SBIR program at Sunnyvale, CA is supported to develop a novel mammographic system based on a new material microstructure that is intended to improve the spectral quality of the x-ray beam and provide a mammogram of higher quality at lower dose and lower cost.

## OPTICAL IMAGING AND SPECTROSCOPY

One of the most interesting new fields that is developing as an optical spectroscopic tool in the near infrared and potentially as a new imaging modality is the technique of pulsed time-resolved spectroscopy, based on the introduction of picosecondpulses of light from a source and subsequent detection and analysis of the diffusely scattered light after its passage through tissues. Some investigators are also studying the more direct, "ballistic" pathways of light propagation. (A picosecond is a millionth of a microsecond. Light pulses as short as this can now be timed with almost incredible precision.) Several laboratories are investigating this new technological capability in America, Japan, Germany, and elsewhere, including the University of Illinois, the University of Utah, the City University of New York, and the University of Pennsylvania. In this latter institution, time-resolved spectroscopy has been demonstrated to provide quantitative assessment of 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 and near infrared spectral regions may one day permit the reconstruction of images of the breast.

## COMPUTER-ASSISTED RADIOLOGY RESEARCH

Computer-assisted radiology research has shown steady growth in the last few years, as shown by the accompanying graph. The major areas of computer-assisted radiology research include (1) improved methods of digitally creating, collecting and storing x-ray images; (2) techniques for providing computer-assisted diagnosis; (3) radiology workstation design; (4) implementation of Picture Archiving and Communication Systems (PACS) and teleradiology systems; and (5) three-dimensional (3-D) studies that include segmentation, surface and volume computation and 3-D display. Specific projects in these five areas are discussed below.

### 1. Digital Imaging Research

A novel approach to optimize single and dual-energy digital radiography systems dedicated to chest imaging is being explored at Thomas Jefferson University. The instrument design uses a filter-wheel equalization system to compensate for over- and under-penetrated areas in chest images. By analyzing numerous chest images, organ-specific compensation patterns are to be developed that will reduce or increase the entrance exposure appropriately so that the sensitivity and specificity to pulmonary nodules and other chest pathologies is optimized. Development of an advanced, large field-of-view digital chest imager is the subject of another research grant at the University of Tennessee. The imager is expected to yield high spatial and

contrast resolution with low patient dose. Another chest radiography unit is under development at the University of Alabama. Equipped with a scanning slit, copper filtration and advanced image processing optimization methods, the unit is expected to deliver digital chest images instantaneously with spatial and contrast resolution equivalent to film technology.

Image representation methods such as 3D display depend on optimization of basis functions for each particular imaging modality. Development of new basis functions of the image representations for emission tomography (PET and SPECT) is the subject of a research effort at the University of Pennsylvania. By optimizing the computer algorithms for PET and SPECT that incorporate realistic models of the data acquisition process, the full potential of these functional imaging devices can be realized. At the University of California, Los Angeles, research over the last several years has focused on the implementation and operation of electronically distributed images to several hospital locations as well as the radiology department. In one particular phase of the work, the development of new methods for digital acquisition of PET data are being explored. The research program calls for the development of efficient computer algorithms and application-specific implementations, such as methods of display of volumes and surfaces, using PET data from realistic phantoms. At the University of Pittsburgh, the primary diagnosis of chest images, using digitally acquired images, is being investigated. Using normal and abnormal chest images acquired with a high-resolution, high contrast sensitivity storage phosphor system investigators are developing the parameters for the acquisition and display properties of the digital system for optimal diagnosis of chest nodules, disease of the pneumothorax and interstitial disease. The successful detection of lung disease is an essential attribute of an electronically functioning radiology system.

A practical and cost-effective approach to dual-energy chest radiography using phosphor plate technology is being explored by a California-based small business through the development of an energy-selective cassette that is compatible with general purpose computed radiography systems. Phosphor storage screens are the subject of another research project by a small business grantee in Maryland, with the objective to develop and assemble a prototype digital read-out and display system suitable for diagnostic mammography. The resulting product is expected to yield a cost-effective system that is superior in performance to existing screen and film systems.

2. Computer-Assisted Diagnosis

Computer-aided diagnosis for the detection of lung nodules and quantitative analysis of lung textures in digital chest images, as well as detection of microcalcifications in digital mammograms

is the subject of one research effort at the University of Chicago. Another related study at the same institution is aimed at the detection and classification of pulmonary nodules in digital chest images (including conventional posterior-anterior images, energy-subtracted images and CT images), and masses and parenchymal distortions in digital mammograms. The computer-aided diagnostic schemes, which are designed to provide the radiologist with the location and/or quantitative measures of suspicious lesions, have the potential to improve diagnostic accuracy in the detection of cancer by reducing the "miss-rates" associated with unaided radiologists' readings.

A new approach for the computer-aided detection of skin melanoma is the subject of a research project at Oregon Health Sciences University. By comparing digitized images of large areas of a patient's skin taken at different times with a video camera, the computer will identify new or unrecognized lesions for physician examination. The methodology is readily adaptable for many other uses such as surveying large or patchy lesions, counting skin lesions, and objectively measuring non-melanoma skin diseases such psoriasis.

Development of a computer-based expert radiology assistant to aid in the diagnosis of breast cancer is the subject of a small business grant in Indianapolis. Using clinical findings, x-ray mammograms, and ultrasound images as input data, the prototype system produces interpretations comparable to expert radiologists. The system will be extensively tested over the course of the two-year project to evaluate the practically attainable diagnostic accuracy, overall clinical utility, and potential for commercialization.

3. Radiology Workstation Design

New image-accessing, image-visualizing, and image-measuring tools that are available quickly and smoothly to the radiologist and attending physician are essential elements of a new computer workstation design under development at the University of North Carolina. The advanced medical-image-display techniques hold the potential for improved clinician productivity and significantly improved medical-image interpretation. In addition to providing rapid access to images, methods for relating information in more than one image will be developed and evaluated. The most effective methods for comparing images (side-by-side or sequentially) will be evaluated and a 3D interactive tool for quickly and accurately measuring the volumes of anatomical objects, such as organs and tumors, will be developed and experimentally compared with manual measuring.

At the University of Arizona, investigators are refining the Arizona Viewing Console (AVC) so that speed of access is improved to medical images available through the Picture Archiving and

Communications System (PACS) and provide a new software environment that provides for greater and more flexible image control. The console performance will be evaluated for its acceptance by radiologists. A Maryland-based small business is developing a low cost, high performance ACR-NEMA interface technology that will result in the development of a single board ACR-NEMA/FDDI Network Interface Unit (NIU). The unit will then be evaluated in a prototype medical imaging network as the first step towards PAC systems that can provide peer to peer interconnectivity.

Two research efforts are involved in the development of compression algorithms for the efficient electronic transmission of digital images with no loss in diagnostic quality. At the University of California, Los Angeles, the first goal has been to establish that the quality of images reconstructed from compressed data is acceptable for diagnosis for all imaging modalities. The second goal is to demonstrate that the hardware compression module can be integrated into a radiologic image management system to be used in clinical environment. It is expected that the resulting research will show that irreversible image compression for a 2,048-pixel (picture element) image (in four seconds) with a compression ratio of 10:1 or higher is achievable and will not exhibit any degradation in diagnostic quality. At Stanford, the goal of the research effort is to extend existing techniques and develop new techniques for image coding and compression that are well matched to specific medical image applications. The specific aims of the research are: (1) to develop algorithms for compressing medical images to one bit per pixel (compression ratio of 8:1) and less with little or no loss in perceived image quality or diagnostic accuracy; and (2) to evaluate clinically the quality of the compressed images at various compression ratios. The primary medical imaging applications include computer tomography (CT) and magnetic resonance (MR) images (including CINE mode MR images consisting of sequences of MR heart images.)

4. Picture Archiving and Communication Systems (PACS)

The University of California, Los Angeles, has invested considerable effort over the last several years in the development and implementation of Picture Archiving and Communication System (PACS) in radiology in a large medical center. Initially, two PACS prototypes were implemented in pediatric radiology and in the coronary care unit. In the current research effort five additional PACS modules are to be implemented over a 5-year period: three in intensive care units, one in neuroradiology, and one in chest radiology. The clinical loads of these five specialties represent about 35% of the examination procedures in the UCLA department.

5. 3-D Studies

At the University of North Carolina, a number of investigations are in progress to evaluate methods for fast, interactive 3D visualization of medical image information, directly from the image intensity data. Object definition methods that operate in both 2D and 3D and which are based on multiresolution image descriptions will be developed, so as to support the 3D display investigations. Here evaluation will be based on the time to produce an accurate definition of an object of clinical interest. Contrast enhancement, workstation design for effective navigation through large image sets, and fundamental aspects of visual perception of contrast in grey-scale images, leading to effective display, are also targets of investigation using both 2-D and 3-D image datasets.

The goal of a University of Massachusetts project is the development and refinement of an accurate three-dimensional (3D) edge detection system in SPECT imaging. Such an edge detection system would serve as a key component when employing SPECT for volume and activity quantitation. The proposed edge detection system is based upon calculating the 3D gradient of the count density. Variations in object size, shape, closeness to other objects, inclusion of other objects, background level and texture, system transfer function, and Poisson noise level will be investigated as to their impact on the accuracy of the algorithms.

At the University of Pennsylvania, the overall goal of the research effort is to develop a transportable and easy-to-use software system for the three-dimensional (3D) display and analysis of tomographic medical image data. Currently, the application of 3D display and analysis techniques is actively pursued in a variety of medical areas including craniofacial surgical planning, neurosurgery, orthopedics, analysis of psychiatric disorders based on magnetic resonance imaging, cardio-pulmonary analysis, and radiation therapy planning. There is no software package currently available in the public domain that incorporates a variety of advanced 3D imaging tools developed at various centers, is transportable and is easy to use in both imaging research and medical environments. The proposed software is intended to fill this gap.

At the University of Chicago, research focuses on the development of software that can integrate a large number (e.g. 100) of cross-sectional images (e.g., CT, MRI or PET) of the brain which may have different geometrical orientations and which portray different aspects of brain anatomy and function. Specifically, spatial integration will be achieved by using computer graphics to create 3-D renditions of brain anatomy or function from each modality. Multimodality integration will be accomplished by fusing these separate 3-D views into a single, comprehensive

model of the brain. Interactive software will be developed for exploring and manipulating this 3-D model, so that anatomical and functional information can be examined at any point and from any viewing angle. This software is expected to be useful for medical diagnosis, surgical planning, radiation therapy planning, and medical education.

The development of computerized methods for the routine, accurate 3D volume estimation and display of soft-tissue lesions and organs is the objective of a research proposal at the University of Michigan. Current technology can accomplish these goals exclusively on high contrast boundaries. The research effort focuses on the application of new, robust, automatic surface finding algorithms which require minimal operator intervention to define the spatial extent of low contrast, soft tissue lesions and organs in x- ray computed tomography (CT) and magnetic resonance imaging (MRI). Algorithm development will include adaptation of a new 3D segmentation algorithm originally developed for computer vision under funding from the National Science Foundation for segmentation of 3D medical data sets. The major clinical beneficiaries of this research will be 3D radiation therapy treatment planning and routine and inexpensive quantitative assessment of tumor response to therapy.

At Pennsylvania State University, an interactive automated 3-D/4-D radiological image analysis system is under development for the (1) extraction of the endocardial and epicardial borders from 3-D/4-D X-ray computed tomography (CT) images; (2) analysis of true 3-D angiograms; (3) extraction and analysis of the upper airway in 3-D Magnetic Resonance Imaging (MRI) images; and (4) analysis of congenital heart defects in 3-D MRI images. Using a broad spectrum of iconic and symbolic problem cues through a flexible display interface the desired anatomical regions are extracted automatically. Evaluation of the resulting prototype will be done by quantitatively comparing automatically generated results to manually generated results.

## IONIZING RADIATION SECTION

The Ionizing Radiation Section (IRS), Diagnostic Imaging Research Branch (DIRB), supports and administers research leading to the advancement of basic, applied, and clinical research in diagnostic imaging with emphasis on cancer diagnosis. The ultimate goal is non-invasive specific anatomical, and functional diagnosis.

According to the NCI organization, this section is actually an artificial amalgamation of two officially recognized sections, (1) X-Ray Imaging Section and (2) Nuclear Medicine Section. The first section supports meritorious research leading to the advancement of imaging technology, technology transfer, and

clinical trials to evaluate and implement the newly developed technology in cancer diagnosis. The second section supports a wide range of nuclear medicine research such as the development of new radiolabeled compounds, their biodistribution, toxicity, use in various modalities, e.g. PET, and SPECT, and monoclonal antibodies.

There are three mechanisms of research support at this section; (1) grants, (2) collaborative agreements, and (3) contracts. Grants, especially traditional grants (ROI) constitute most of this section portfolio. Nine out of the twelve program projects grants (POI) supported by DIRB are in this section. Five program projects deal with nuclear medicine research and the remaining four POI's cover wide range of research in physics, engineering, computer sciences, and radiology. This includes digital radiography, PACS, 3-D imaging, instrument development, and technology transfer. There is one contract supported by this section, "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization." It deals with the development of TC-99m radiolabeled compounds for use in SPECT. Currently, this is the only contract at DIRB. This section was the first and only section at DIRB to establish collaborative research groups. There are three collaborative research groups, namely, Radiological Diagnostic Oncology Groups, "RDOG I", "RDOG II", and "RDOG III". There are 17 institutions involved in this clinical research at an estimated cost of $1,752,000 in FY-'91. Furthermore, this section established a network of three interactive institutions to conduct clinical research in the early diagnosis of prostate cancer using ultrasonography and other methods.

NUCLEAR MEDICINE

The National Institute of Health has been supporting research in nuclear medicine for over three decades. Since 1982 the number of funded grants in nuclear medicine at NCI has been increasing rapidly. Currently, nuclear medicine research comprises a major portion of the traditional research project grants (R01) in DIRB. Development of new radiolabeled compounds, their biodistribution, toxicity, use in various modalities, i.e., PET and SPECT, and chelate conjugated antibody research are just a few examples to illustrate the wide range of this program. Program project grants funded by our program deal with various aspects of nuclear medicine research. A program project deals with the development and evaluation of promising compounds which when radiolabeled, have the potential to scintigraphically diagnose and at higher doses treat cancer. A second program project continues to explore the improvement of image information by the administration of a novel contrast agent; improvement of the qualitative and quantitative aspects of imagery by SPECT and utilization of pharmacoangiography to improve information content and understanding of functions of imaged organs. An important

development by another program project is to design and develop a scintillation probe for intraoperative tumor detection. By using two detectors, the newly constructed probe effectively discriminates against background radiation that might otherwise be mistaken for tumor, 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 and used routinely in surgery. Unavailability of funds resulted in the loss of one program project and the inability to fund any new program projects, this fiscal year.

Polypetides and proteins in the form of enzymes and hormones are essential to life processes. Radiolabeled molecules are frequently needed to trace their action and fate in living cells. A novel approach to label proteins and polypeptides with tritium is being studied by our grantees at the University of California, San Francisco. Labeling large biomolecules with tritium requires some understanding of the dynamics of the hydrogen in the molecule and avoiding denaturation. They were able to achieve specific tritium labeling of large steroids, benzodiazepines, alkyl halides and other molecules with high radiopurity and without the use of precursors. Further they extended the use of this technique to produce radiolabeled leu-enkephalin, cyclosporin A, insulin, protease inhibitors and small peptides with high specific activity on alumina and in the absence of metal catalyst. Improving these techniques and extending the labeling for other physiologically significant large biomolecules is the future aim of this highly productive group.

Emission computed tomography is a fundamental technique for *in vivo* radionuclide quantitation. Quality quantitation requires an accurate model of objects and gamma ray cameras. Monte Carlo simulation has been widely sued to develop such models. Our grantees at Duke University are world leaders in this field. They proposed a solid geometry based approach to model objects with irregular shape and non-uniform material characteristics for the simulation. The solid geometry approach is based on a set of objects such as ellipsoids, elliptical cylinders, tapered elliptical cylinders, and rectangular solids, each with a uniform material characteristic. An object is composed by a combination of intersection, union, and difference. Due to simple parametric representation of the boundaries, this approach is useful in diagnostic imaging, especially, to model the thorax with a beating heart for myocardial tomography and blood pool perfusion quantitation. The models created by the Duke group are widely used in *in vivo* studies and in the clinical setting.

In addition to the planning, design, and synthesizing new imaging important radiocompounds, the researchers at the University of Michigan are engaged in research to devise various strategies for accomplishing the site-specific delivery of radiopharmaceuticals to specific organs and tumors. A specific aim has been to select

appropriate molecules to serve in the transport of radioiodine and other radionuclides to the desired target. The research is currently focused on two classes of agents, radioiodinated phospholipid ethers (PLE) and radioiodinated triglyceride analogs (TG). They have shown in previous studies that PLE have the unique property of accumulating in tumors as opposed to normal tissue. During this past year they have gained considerable evidence to support the view that PLE are retained in tumors because of their inability to be metabolized and clear readily. Moreover, studies indicated that clearance of PLE from normal cells is due to the action of phospholipase D and/or phospholipase C.

The triglyceride studies have focused on glycerol-2-palmitoyl-1,3-15(*p*-iodophenyl) pentadecnoate (DPPG) as a potential hepatic diagnostic agent and as a probe for studying alterations in liver lipid metabolism in normal and diseased states. Studies in rats showed DPPG to rapidly associate with circulating lipoproteins upon administration. The lipoprotein-associated DPPG is then taken up in the liver by mechanisms involving hepatic lipase. Once in the liver, DPPG is hydrolyzed to the free fatty acid, p-iodophenyl pentadecanoic acid (IPPA). Beta oxidation of IPPA and subsequent conjugation with glycine results in the production of iodohipuric acid. The iodohippuric acid is then excreted in the urine. Hepatic lipase and lysosomal lipase are known to be decreased by a number of factors including gender, alcoholic liver disease, diabetes or the presence of tumors. Decreased hepatic lipase activity resulted in a decreased uptake of radioactivity in the liver following administration of DPPG. Moreover, decreased lysosomal lipase activity resulted in a decrease in the clearance of radioactivity from the liver. Thus, DPPG has potential not only for liver imaging but also for evaluating aspects of lipid metabolism. This would be advantageous, because currently used liver imaging agents do not provide information about the biochemical functioning of the liver.

Another group of scientists at the same university completed the development of a miniature imaging device, Single Pholon Ring Tomograph (SPRINT II). It is fully functional and have been used with a special purpose aperture designed for imaging rats and mice to obtain SPECT images with 2.8mm resolution. Images of a miniature Jaszczak phantom show good definition of 2mm spots and improved sampling should allow them to resolve 1.5mm spots. This will have major impact on the development and testing of new radiopharmaceutical agents for detection and treatment of cancer since longitudinal studies can be performed on the same experimental animal. This will dramatically decrease variance and reduce the number of animals required. Further, simulations of a simple, inexpensive video measurement of patient motion shows 3 dimensional motion is easily quantified to within 1mm in nearly real time. The ultimate aim is to permit real-time motion

correction during acquisition of either SPECT or PET data. Patient motion may well be one of the major courses of image degradation in Nuclear Medicine imaging.

Last year, grantees at George Washington University demonstrated that indocyanine dyes with two linear aliphatic chains containing 12 to 16 carbon atoms would intercalate irreversibly into cell membranes. When labeled with radioiodine, these dyes prove useful for cell labeling of leukocytes to follow their organ distribution in vivo. They should prove particularly useful for trafficking the in vivo distribution of lymphocytes because excessive radiation toxicity from intracellular or intranuclear Auger electrons from other cell labeling agents are avoided. This year, they have synthesized a [p-didecylamino styryl] pyridinium dye with the addition of tributyl tin to facilitate radioiodination. Like the previously used indocyanine dyes, it possesses two long aliphatic chains for intercalation into cell membranes, but has a less complex chemical structure.

They have also evaluated the effect of growth factors, in dogs, particularly granulocyte colony stimulating factor (G-CSF) on the proliferation and distribution of granulocytes. They have shown, with relatively modest administered doses, that the bone marrow blood flow is increased quickly by a factor of three over baseline values. G-CSF also has a profound effect on the distribution of bone-seeking agents such as Tc-99m methylene diphosphonate commonly used for skeletal radionuclide imaging.

MONOCLONAL ANTIBODIES

For more than a decade this section has been encouraging research leading to the development and evaluation of new monoclonal antibodies (MoAb) for diagnostic imaging in both human and laboratory animals. The number of grants supported in this field is increasing rapidly since the issuance of an RFA several years ago as a result of a workshop. Noticeable progress has been made and important publications have resulted from this effort. The need for more and effective human tumor specific MoAb's, however, continue 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 McAb's to advance this promising field in both diagnostic imaging and tumor therapy.

Labeling of antibodies with radionuclides such as Cu-67 or Y-90 at the University of California, Davis results in agents with promising radioimmunoimaging and radioimmunotherapy. The structure of the bifunctional chelating agent along with the properties of the chelated metal ion play essential roles in achieving high stability of the metal complex under physiological conditions. Antibody conjugates of polyazamacrocyclic bifunctional chelating agents previously

developed in their laboratory, have shown remarkable kinetic inertness *in vivo* and *in vivo* human serum. They have extended the peptide-based synthetic route to other macrocyclic bifunctional chelating agents in order to improve understanding of the structial features responsible for stable chelates. They synthesize two new macrocyclic bifunctional chelating agents with serum stabilities of their Cu-67-antibody conjugates comparable or better than available compounds.

The primary aim of this project at the Center for Molecular Medicine and Immunology, Newark, NJ is to determine within a second antibody can be used to remove circulating radiolabeled antibody from the blood, and in so doing improve radioimmunotherapy. The model is being tested in nude mice bearing human colonic tumor xenografts. $^{131}$I-labeled NP-4 IgG, a mouse anti-CEA MoAb is the primary antibody used for therapy, and the second antibody is a mouse anti-NP-4 idiotype antibody. Despite the reduction in tumor uptake seen in animals given second antibody, biodistribution studies have shown that radiation doses to the tumor may be increased by 75 % over that of $^{131}$I-labeled NP-4 IgG alone. $^{111}$In-labeled NP-4 suggested a second antibody approach would produce unacceptable radiation doses to the liver in relationship to the tumor. They are also examining other antibody systems to determine if the loss in tumor uptake after the administration of the second antibody can be reduced if primary antibodies of higher affinity or with higher tumor uptake and retention than that seen with NP-4 are used. Therapy studies are underway to compare the efficacy of the MTD of $^{131}$I-NP-4 IgG alone to $^{131}$I-NP-4 IgG with second antibody given at 48 h (optimal time). These results are also compared to $^{131}$I-labeled NP-4 F(ab')$_2$.

Radiolabeled monoclonal antibodies can be highly useful for scintigraphic imaging or therapy of malignant lesions. At Thomas Jefferson University Hospital our grantees developed efficient, yet simple methods to label, IgM, IgG or (F(ab')$_2$ types of antibodies with Tc-99m for imaging and with Re-186 for therapy.

The methods utilize ascorbic acid that reduces one or two native disulfide groups on an antibody molecule to sulfhydryls and provide strong binding sites for direct labeling with the radionuclides. The yields are high, and analytical evaluations combined with animal studies indicate that the antibodies are neither fragmented nor are their biological specificities altered. Several papers have been published, describing the methodology and imaging tumors and inflammatory foci. The method has been extended to label peptides and one antibody labeled with Tc-99m to image patients with abscesses. Data of this clinical trials is being accumulated and analyzed.

## RADIOLOGIC DIAGNOSTIC ONCOLOGY GROUP

Radiologic Diagnostic Oncology Group (RDOG) was formed in September, 1987, in response to an RFA. The RDOG objective is a timely evaluation of current and emerging imaging modalities in the management of patients with cancer. The development of multi-institutional clinical trial groups allow 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 the 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 determiniing 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 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 America 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 the 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 arameters 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)

Sixteen applications were received in response to the RDOG IV RFA published in February, 1992. Eight to ten institutions are expected to be funded in FY 92.

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 15-10%). Thus, there is a great need to improve diagnostic imaging of ovarian cancer.

In Fy 90-91 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 Counselors 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 Counselors 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, RDOG V entitled "Non-palpable Breast Lesion Characterization: Image-Guided Stereotactic Breast Biopsy" was supported by the BSC-DCT in June, 1992 and is expected to be funded in FY 93. The goal of RDOG V is to examine image-guided biopsy as a minimally invasive, low morbidity alternative to surgical open biopsy for minimal breast cancer. (Estimated increase in budget: $1,500,000 per year for four years, and ten new institutions are expected to be funded.)

NIH PROSTATE ULTRASOUND WORKING GROUP

In addition to RDOG I, the 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 a Prostate Ultrasound Group which has been investigating the capability of ultrasound imaging 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 $4000,000.

PLANS

The multi-institutional working group entitled "Imaging-Guided Tissue Diagnosis of Prostate Cancer" is considered to be proposed 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.

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 Panes 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 lost of important new areas of diagnostic research. The next meeting is planned for late spring-summer, 1993, 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: (1) it will help various institutes to facilitate and coordinate support of the most important areas of radiologic science; and (2) it will educate and stimulate extramural radiologic scientific community.

FUTURE DIRECTIONS (DIRB)

As a result of several recent workshops and RFAs, new areas of research were planned to be established in the DIRB. Examples of such projects include:

1. RFA CA-92-02 entitled "Radiologic Diagnostic Oncology Group IV: Ovarian Cancer and Pediatric Solid Tumors" was issued in early 1992. Sixteen applications were received and recently reviewed. Funded grants will be added as RDOG IV to the on-going collaborative RDOG clinical research. The objective of this RFA is to support clinical research to diagnose, stage and monitor ovarian cancer and pediatric solid tumors.

2. In the area of digital imaging, DIRB will establish a National Digital Mammography Development Group which will combine four critical components (digital imaging technology, image processing, computer-aided diagnosis and teleradiology) in order to assure integrated technology development for breast cancer detection and characterization. This initiative was supported by the DCT BSC in October, 1991.

3. In December, 1992, NCI panel on PET clinical oncologic imaging is planned to take place at the major annual radiologic meeting. Radiologic Society of North America. This panel is expected to develop a research agenda for multi institutional clinical PET imaging of cancer. Based on the panel findings, program staff will consider to establish a new collaborative group patterned after RDOG and entitled "Positron Emission Tomography Diagnostic Oncology Group" (PET-DOG).

4. Another new initiative, "Radiologic Diagnostic Oncology Group V: Imaging-Guided Stereotactic Tissue Diagnoses for Breast Cancer" is expected to be published in early FY 93, and ten new institutions will be funded. The objective of this project is to

establish a multicenter collaborative group to evaluate imaging guided stereotactic breast lesion biopsy as the minimally invasive alternative to open surgical excision for minimal breast lesion characterization.

5. RFA entitled "Clinical Diagnostic Studies of AIDS-Affected Brain Using PET and Other Imaging Modalities" is in its final stages of NIH approval. The objective of this RFA is to support meritorious research using neuroimaging techniques to improve our understanding and knowledge of the disease processes associated with HIV encephalopathy and the onset of AIDS.

6. A new Program Announcement (PA) entitled "Novel Non-Ionizing Technologies for Breast Cancer Imaging" was approved by the DCT BSC in October, 1991 and published by the DIRB in March, 1992. At least thirty new grant applications are expected in response to this PA, which was formulated on the basis of the Breast Imaging workshop of September, 1991.

7. A new PA entitles "MRS and Cancer Treatment" was published by the DIRB in June, 1992. This research solicitation will stimulate multi-institutional clinical study of MRI-guided localized MRS as the means to predict and early detect tumor response to treatment. This announcement is based on the DIRB advisory group meeting of August, 1990 and workshop of December, 1991.

8. RFA entitled "Quantitation of Tumor Response to Treatment: A 3D Approach" was published in January, 1992, and twenty three applications were submitted in response to this program solicitation. Three to five new institutions are expected to be funded in early FY 93.

9. The inter-agency agreement was developed between the DIRB and NASA in order to establish a joint NCI/NASA technology transfer working group. The first meeting which took place in July, 1991, identified specific future collaborations in the area of computer science.

10. 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", was funded in FY 91. The workshop devoted to PACS and related issues took place in March, 1992. New initiatives in the area of PACS, medical informatics and teleradiology/telemedicine are currently under consideration.

11. The multidisciplinary DIRB workshop entitled "MRS an Tumor Cell Biology" was convened in December, 1991 in order to define a research agenda for bridging the currently existing gap between cancer cell biology and basic biochemistry of MRS. This conference 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 is planned to be developed in FY 93.

12. RFA in the area of acoustic microscopy is planned to be published in early FY 93. This proposal will stimulate the development of in vivo microscopic device.

The following workshops are planned for FY 92: (1) Tumor Receptor Imaging; (2) MR Contrast Agents for Quantitative Functional Tumor Imaging; and (3) New Directions in Imaging Science.

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