olser 1, 1989–September 31, 1990

annual report

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Cancer Treatment

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ANNUAL REPORT

October 1, 1989 through September 30, 1990

CONTENTS FOR VOLUME II

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

DIVISION OF CANCER TREATMENT

October 1, 1989 - September 30, 1990

GENERAL ORGANIZATION

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

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

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

The Regulatory Affairs Branch (RAB) monitors the conduct of clinical trials performed in the NCI-supported clinical trials network. It also assures that clinical investigators using experimental agents are in compliance with federal regulations regarding the use of such agents. At the start of the clinical testing of each investigational 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 review is administered within the OAD by a central Protocol and Information Office (PIO) which is also the receipt point at NCI for all protocols entered into the PDQ system. The Program Analysis and Management Office (PAMO) has responsibility for the technical management of CTEP's grants and contracts and carries out analyses, as needed, of certain fiscal and administrative issues of particular interest to the program. The OAD is responsible for overall program supervision and budgetary allocation.

ORGANIZATIONAL AND PROFESSIONAL STAFF CHANGES

David Parkinson, M.D., formerly Chief, Section of Biological Therapy of Solid Tumors, Department of Clinical Immunology and Biological Therapy, Division of Medicine, The University of Texas, M.D. Anderson Cancer Center has been named Head, Biologics Evaluation Section, Investigational Drug Branch.

John Brennan, M.D., was recruited as a Medical Officer in the Clinical Investigations Branch after serving as a Clinical Instructor, Uniformed Services University of the Health Sciences, Bethesda, Maryland.

Hoo Chun, M.D., a Cancer Expert in the Developmental Chemotherapy Section, Investigational Drug Branch is leaving to continue his career in academic medicine.

Paul Hiranaka, Senior Clinical/Research Pharmacist in the Drug Regulatory Affairs Section of the Regulatory Affairs Branch has retired.

Joseph High, a pharmacist in the Clinical Center, NIH has been recruited to the Drug Management and Authorization Section of the Investigational Drug Branch.

Langdon Miller, M.D., joined the Biologics Evaluation Section of the Investigational Drug Branch as a Senior Investigator. He was formerly an Associate Investigator at the Palo Alto V.A. Medical Center, Palo Alto, California.

Henry Stevenson, M.D., transferred to the Biologics Evaluation Section of the Investigational Drug Branch from the BRMP.

Malcolm Smith, M.D., of the Clinical Oncology Program will be spending the last year of his fellowship in the Clinical Investigations Branch where we will be a Senior Investigator in the Pediatric Section.

Estelle Russek-Cohen, Ph.D., completed a year in the Biometric Research Branch as an IPA from the University of Maryland.

Alison Martin, M.D., resigned as a Senior Investigator in the Biologics Evaluation Section, Investigational Drug Branch to join the Washington Clinic, Washington, D.C.

HIGHLIGHTS IN PROGRAM DEVELOPMENT

1. Consensus Development Conferences

Based upon considerable clinical trial data generated by the Cooperative Groups two cancer therapy related Consensus Development Conferences were sponsored by the Office of Medical Applications of Research of the National Institutes of Health. One dealt with adjuvant therapy for large bowel cancer patients. It endorsed the use of post-op pelvic irradiation and systemic 5-FU for Stage II or III rectal cancer and post op systemic 5-FU and Levamisole for Stage III colon cancer patients. For the first time, a consensus was possible on this subject. The other conference confirmed the value of less extensive surgery for early stage breast cancer and adjuvant systemic therapy for relatively poor prognosis node negative patients. Both these common epithelial malignancies represent major public health problems, and these conferences will affect the care of many Americans.

2. Public Announcements

An important means of disseminating highly relevant clinical data is the public announcement mechanism. Over the past 18 months the NCI has issued two announcements. The first concerned the utility of Tamoxifen or chemotherapy node negative breast cancer patients and of 5-FU + Levamisole for Stage III colon cancer patients. The Colon Cancer Update was particularly widely distributed and according to the American College of Surgeons survey, particularly effective. Both of these NCI announcements promulgated positions reinforced by the National Institutes of Health Consensus Development Conferences and by the Oncologic Drug Advisory Committee of the Food and Drug Administration. In order to explore these complicated issues of investigator prerogatives, ethical responsibility of investigators to patients and issues of scientific credibility a conference was sponsored by Dr. D. Korn and the National Cancer Advisory Board. Guidelines which will affect future announcements were generated and reviewed and approved by the National Cancer Advisory Board.

3. Important New Agents

This year an unusually large number of important therapeutic advances were made. The Food and Drug Administration approved New Drug Applications for Levamisole for colon cancer. Taxol was identified as an active agent in patients with refractory ovarian cancer. Major C efforts were initiated for Levamisole and Fludarabine. Promising data with Verapmil apparently sensitizing myeloma cells to cytotoxic chemotherapy.

STAFF PUBLICATIONS:

1. Friedman, M: If not now, when? Point/Counterpoint. JNCI 1990; 82: 106-109.

2. Friedman, M and Cain, D: National Cancer Institute sponsored cooperative clinical trials. Cancer 1990; 65: 2376-2380.

3. Friedman, M and Dorr, FA: Opportunities in clinical trials -- adjuvant breast cancer. Cancer. In Press.

4. Galloway DJ, Cohen AM, Shank B, and Friedman, MA: Adjuvant multimodality treatment of rectal cancer. Improved results based on randomized clinical trials. British Journal of Surgery 1989; 76: 440-447.

5. Hamilton JM, Nerenstone S, and Friedman, MA: Chemotherapy and radiotherapy interactions in clinical medicine: Liver metastases from GI malignancies and hepatoma. In press.

6. Hamilton JM, Sznol M, and Friedman MA: 5-Fluorouracil plus levamisole: Effective adjuvant treatment for colon cancer. In: Hellman, DeVita, Rosenberg, eds. Important advances in oncology, Philadelphia, J.B. Lippincott Publishing Company, 1990; 115-130.

7. Hohn DC, Stagg RJ, Friedman, MA, Hannigan J, Rayner A, Ignoffo RJ, and Lewis BJ: Randomized trial of continuous infusion versus hepatic Intra-arterial floxuridine in patients with colorectal cancer metastatic to the liver. JCO 1989; 7: 1646-1654.

8. McCabe M and Friedman MA: The impact of 3rd Party reimbursement on cancer clinical investigation: A consensus statement coordinated by the National Cancer Institute. JNCI 1989; 81: 1585-1586.

9. Nerenstone S and Friedman MA: Primary liver cancer. Comprehensive textbook of oncology. Second Edition, Chapter 88. In press.

10. Shoemaker D, Burke G, Dorr A, and Friedman MA: A regulatory perspective. In: Spilker B eds. Quality of life assessment in clinical trials. New York, Raven Press, 1990; 16: 193-201.

11. Simon R and Friedman, MA: The design and interpretation of clinical trials. In: M. Perry eds The Chemotherapy Source Book, Baltimore, Williams and Wilkins, 1989. In press.

12. Valone FH, Friedman MA, Wittlinger, PS, Drakes T, Eisenberg PD, Brown BW, Malec M, Hanningan JF: Treatment of patients with advanced colorectal carcinoma with 5-FU alone, high-dose leucovorin plus 5-FU or sequential methotrexate, 5-FU, leucovorin: A randomized trial of the Northern California Oncology Group trial. JCO 1989; 7: 1427-1436.

BIOMETRIC RESEARCH BRANCH

1. STATISTICAL PLANNING AND REVIEW OF CTEP SPONSORED CLINICAL TRIALS

The Biometric Research Branch collaborates in the development of clinical trials to evaluate new chemotherapeutic and biological agents. The BRB reviews all Cancer Treatment Evaluation Program (CTEP) sponsored extramural 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 participates in 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, including the specification of study designs, endpoints and sample sizes. BRB staff participate in national disease oriented strategy meetings to develop study designs for new generations of clinical trials in a particular type of cancer. BRB staff perform interim analyses of contract supported clinical trials and evaluate reports of promising therapeutic regimes for the planning of possible future clinical trials.

The BRB serves as liaison to extramural statistical centers. BRB staff visit centers, review data management and monitoring procedures and organize national meetings in order to improve statistical and data management practices.

2. PRECLINICAL DRUG DISCOVERY

a. Methods for detection of differential cytotoxicity, related to histology, have been developed for the in vitro human tumor cell line assay in collaboration with Drs. Kenneth Paull and Robert 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 7 histologic subpanels. A primary goal is the identification of agents with marked differential cytotoxicity related to histology. Detection and analysis tools, both graphical and statistical, have been developed and implemented on the DTP VAX computer. Explanatory presentations have been given at the annual Anticancer Drug Discovery and Development Conference (1990, Wayne State University) and the annual joint MRC-EORTC-NCI Conference (1990, Bethesda). An explanatory manuscript has been published and a second is in press. Additional work is ongoing.

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

c. A set of simulations was completed to determine the required number of cell lines per histologic subgroup to enable the in vitro cell line panel to effectively detect differential cytotoxicity. It was determined that a cell line panel including 10 histologic subgroups should have at least 10 cell lines per subgroup.

d. 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.

3. COMPARATIVE STUDIES TO EVALUATE MAGNETIC RESONANCE IMAGING

The BRB has collaborated with the Diagnostic Imaging Branch (Dr. Matti Al-Aish, Acting Chief) of the Radiation Research Program and Dr. Hedvig Hricak of UCSF Medical School in the conduct of a prospective multi-institutional evaluation of MRI in the diagnosis of uterine neoplasms.

The BRB participated in the following ways:

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

b. Supervision of the data management contract.

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

4. NATIONAL CLINICAL TRIALS OF EARLY OVARIAN CANCER

a. BRB staff has served as primary statistician for clinical trials of the staging and treatment of early ovarian cancer. Final analyses of the therapeutic questions have been performed and a manuscript has been published. The results indicated that

adjuvant chemotherapy was not appropriate for patients with very early stage disease (FIGO stages Iai and Ibi). Post-surgical delivery of chromic phosphate (P32) was as effective as chemotherapy for those patients with slightly more advanced disease (FIGO stages Ic, Iaii, Ibii, IIa, IIb).

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

5. PROGNOSTIC FACTORS IN EARLY STAGE EPITHELIAL OVARIAN CANCER

Due to the relatively low incidence of the early forms of epithelial ovarian cancer and the long survival of most patients with this disease, individual clinical trials have limited statistical power for defining the role of therapy and of biological and medical factors in the prognosis of these patients. For this reason, a joint American and an Italian database consisting of a total of 530 patients has been established and is being analyzed. In conjunction with the usual regression models, a new method (recursive partitioning) is being used to identify important prognostic determinants.

6. 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 are attempting to quantify the correlation between response and survival by retrospectively analyzing data from all published randomized clinical trials since 1975 using a meta-analytic approach.

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

b. 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 is in preparation with Dr. Ronald Feld.

c. A protocol comparing lobectomy vs limited resection in T_1N_0 NSCLC patients has completed accrual and is in follow-up under the supervision of Dr. Robert Ginsberg.

d. Analysis of the incidence of second primaries and recurrence among T_1NO patients, across several protocols, has been completed and a paper has been published with Dr. Paul Thomas.

8. TIAZOFURIN TOXICITY

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

9. 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 by the NCI-COP for the use of suramin in the treatment of patients with stage D2 carcinoma of the prostate who have failed hormone treatment. Patients with measurable disease are being enrolled in a phase 2 study. A randomized study is open for patients with non-measurable disease using survival and "quality-of-life" endpoints. BRB serves as statistical center and EMMES as data coordinating center for this study. The suramin dose is determined by blood levels and BRB has collaborated with Dr. Charles Myers on evaluating assay calibration at the participating institutions. Further dosing schedules for suramin are also being investigated.

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

Most success of cancer chemotherapy have required the use of combinations of cytotoxic drugs. This has been true of childhood acute leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, testicular cancer and more recently for the surgical adjuvant treatment of breast cancer and colorectal cancer. The reasons for the necessity of combinations are less clear. In the chemotherapy of bacterial infections, multi-drug regimens are often employed either to cover the spectrum of possible pathogens before culture results are obtained (spectrum coverage), 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 antitumor effect subject to constraints on combined toxicity. This approach does not assume that maximally dose-intense regimens are clinically appropriate in all situations. Whether the identified regimen is superior to standard treatment should be determined by prospective randomized clinical trials. Determining which drugs to combine and in what proportions to combine them offers combinatorially huge numbers of possibilities. The method developed offers one approach to identifying combinations worthy of evaluation in prospective trials. A manuscript has been submitted for publication and invited presentations have been given at two scientific meetings.

11. STATISTICAL ASPECTS OF MEASURING AND COMPARING QUALITY OF LIFE ENDPOINTS

The importance of quality life endpoints for certain types of cancer clinical trials is becoming more evident. To help integrate these endpoints into the trials being planned, DCT and DCPC organized a joint conference for the summer of 1990. Investigators who have experience developing quality of life instruments, as well as using them in clinical trials, and representatives from the cooperative groups and pharmaceutical companies were invited to attend. BRB staff have been involved with the planning of this conference.

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

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BRB staff are working with CIB staff to write a survey paper concerning clinical trials methodology for Head and Neck cancer. Some of the issues that are especially important for this disease are: (1) There are many different primary sites, can they be pooled in clinical trials? (2) Because of the possible disfiguration and loss of voice, quality of life issues are very important. (3) There is a high rate of second malignancies. (4) There are compliance problems with many of these patients who may have alcohol dependence. (5) Metastatic disease is not a major problem.

13. 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 almost 50 such studies. The growth of intergroup studies represents a recognition of the need for larger sample sizes in many clinical trials and a need for groups to collaborate in exploiting the most promising therapeutic opportunities. In the past, intergroup studies have been developed and conducted in an informal manner. Many participants have been frustrated by lack of adequate quality control mechanisms, opportunities for input in study design and inadequate monitoring procedures.

Improving and facilitating the conduct of intergroup studies is an important priority of CTEP. The BRB has taken the lead in this effort by developing guidelines for the conduct of intergroup studies, by organizing and funding a second national workshop on intergroup data management, by developing guidelines for data monitoring committees in intergroup studies and by beginning to critically review the data collection plans for intergroup studies. The intergroup data management workshop was held on April 28-29,1990 in Denver with over 200 cooperative group representatives. The Chief of the BRB gave the opening address and led sessions on streamlining and "debulking" intergroup studies in order to increase patient accrual and reduce costs. A proceedings of the workshop is being published.

14. PLANNING OF MULTI-TREATMENT CLINICAL TRIALS

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

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

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

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

16. STATISTICAL PLANNING OF "PHASE II" STUDIES OF COMBINATION REGIMENS

Phase II clinical trials of new drugs determine whether there is any antitumor activity in the disease studied. The objectives of phase II studies of combinations of active drugs are more ambitious. Such studies generally seek to determine whether the level of activity is sufficient to warrant a randomized phase III trial. Such phase II trials are inherently comparative, with historical experience on standard treatments being the basis for informal comparison. The prevalence of negative phase III trials is indicative of the inadequacy of many phase II trials. We have attempted to improve the planning of phase II trials of active combinations by explicitly incorporating specific historical control data. Planning takes into consideration the finite size of the historical control results. We find that with a substantial historical control experience and little inter-study variability, the conventional phase II trial is appropriate. In other circumstances, however, the conventional approach is found to provide a high probability of false positive and false negative results; larger sample sizes and a proportion of the patients randomized to the standard treatment for combining with historical controls are required. A manuscript describing these results has been published.

17. 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 a more "efficient" if it were truly a surrogate. We are attempting to evaluate the relationship between these endpoints using individual patient data from multi-institution clinical trials with surgery only control arms and adequate follow-up.

18. 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 maximal tolerated dose) and to characterize the statistical properties of these designs. We have been particularly concerned about the adequacy of traditional phase I designs for dose escalation of combinations in the presence of bone marrow growth factors. The maximum tolerated doses defined in such studies may be used directly in phase III trials.

19. DESIGN CONSIDERATIONS FOR AIDS CLINICAL TRIALS

The AIDS epidemic has created new challenges for the process of developing effective new drugs. In collaboration with leading statisticians at NIH, Harvard and in England, we have re-evaluated many aspects of the drug development process and made recommendations for speeding drug evaluation. The recommendations include combining some of the conventional phases of drug evaluation, relaxing entry criteria, carefully examining the need for masking treatments, answering more than one question in a single trial and permitting patients to enter more than one trial at once. A manuscript has been submitted for publication.

20. GROUP C/TREATMENT IND 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.

I88-0018: Fludarabine phosphate for patients with refractory chronic lymphocytic leukemia.

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

I89-0017: Levamisole for use with 5-FU as adjuvant treatment for patients with Dukes C adenocarcinoma of the colon.

21. PROGNOSTIC DETERMINANT OF PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

Follow-up information for the 1175 patients from 4 institutions used to develop the Working Formulation for Non-Hodgkin's Lymphomas was updated. A manuscript was published in the Annals of Internal Medicine on long-term survival results within the Working Formulation histologic subtypes. A second manuscript has been published on prognostic determinants for patients with diffuse large cell and immunoblastic lymphomas and a new staging system was proposed. A manuscript on prognostic determinants and staging of patients with low-grade lymphomas is in preparation. In conjunction with the usual regression methods, two new methods (recursive partitioning and cubic spline regression) have been used and appear to be promising tools for model building.

22. DRUG DEVELOPMENT REVIEWS

The clinical trials conducted in the development of CHIP and CBDCA have been reviewed and an assessment of lessons learned for the development of future analogs has been performed in conjunction with Dr. Brenda Foster. A manuscript has been published. A review of the clinical trials conducted in the development of AMSA has been performed with Dr. Leyland-Jones. A manuscript has been submitted on this case-study in the development of an antileukemia agent.

23. QUANTIFYING PREDICTIVENESS OF MULTIVARIATE SURVIVAL MODELS CONTAINING COVARIATES

Identification of factors that predict the prognosis of cancer patients is important for advising patients, improving the efficiency of clinical trials and for more effectively targeting important therapeutic questions to appropriate subsets of patients. Many prognostic models are "statistically significant" but not very predictive. We have developed measures to quantify the importance of such models for predicting survival or disease-free survival. Such quantification is a useful step in the development of truly accurate predictors. A manuscript describing this work has been published.

24. QUALITATIVE TREATMENT BY PROGNOSTIC FACTOR INTERACTIONS

Qualitative interactions are said to occur in a clinical trial when for some subset of patients, one treatment is superior, while for another subset of treatments the other treatment is superior. While some methodology had existed to test this hypothesis, these methods were appropriate only in situations where the subsets were non-overlapping. We have developed more general methods to allow one to simultaneously examine the data for qualitative interaction with respect to each of several prognostic factors while still using all the information available. This permits one to detect qualitative interactions with smaller sample sizes than the method previously available. A manuscript has been submitted describing these results.

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

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

26. MODEL SELECTION IN STEPWISE REGRESSION

Stepwise regression analysis is one of the most commonly used methods of data analysis in statistics. The commonly used methods for deciding when to terminate the stepwise procedure are ad-hoc, however. In collaboration with Drs. Peter Thall and David Greer of George Washington University, we are evaluating the use of cross validation and generalized cross validation as model selection criteria in stepwise regression. The NCI's Cray XMP supercomputer is being used for this evaluation.

27. WHEN TO RANDOMIZE?

Some clinical trials compare two therapeutic approaches which differ only after a certain time. A typical example is chemotherapy alone compared to chemotherapy followed by radiotherapy. Controversy exists about the optimal time to randomize, whether it should be before starting chemotherapy, or after its completion. Late randomization avoids the loss of power due to drop-outs after randomization. But early randomization is often more comfortable to physicians and results in fewer patients refusing participation. We have developed a model comparing required sample sizes for each design in order to provide guidelines in these situations. A manuscript has been submitted for publication.

28. 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. One paper is in press, two additional papers have been submitted, and a fourth paper is in preparation.

29. EXPLAINED RESIDUAL VARIATION AND GOODNESS-OF-FIT

This work helps define the notion of the predictiveness of a model, and compares it with the notion of goodness-of-fit. It gives a theoretical underpinning to our work last year on quantifying the predictiveness of survival models. (See item 23 on this year's report). A paper has been accepted for publication.

30. PROJECTING STATISTICAL POWER FROM A COMPLETED STUDY

Many clinical trials use previously completed trials to estimate the magnitude of treatment to be expected. It has been suggested (Brown et al., Controlled Clinical Trials 3 (1987), pp 29-44) that the results of these previous trials could be used in a formal Bayesian analysis to do a power calculation for a planned study. A similar approach can be used in the problem of computing conditional power in a partially completed trial (Choi et al., Controlled Clinical Trials 6 (1985), pp 280-288). There are problems with these approaches from a frequentist point of view, however. In particular, the operating characteristics may be poor. A paper has been published describing these problems, and another is in press that describes the similar problems with maximum likelihood approaches.

31. APPLICATIONS OF CRUDE INCIDENCE CURVES

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

32. SURVEY EFFECTS IN LONGITUDINAL STUDIES

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

33. RANDOMIZED CLINICAL TRIALS WITH CLINICIAN-PREFERRED TREATMENT

A new design for a randomized clinical trial has been developed in which clinicians are able to choose for each patient the treatment they believe is most appropriate for that patient. A treatment is randomly assigned to the patient but the patient is treated by a physician who favors that assignment.

This design may have application when conventional randomized designs are rejected by the participants because (1) clinicians believe strongly for some patient that one treatment is better than another, but (2) they disagree on some of these same patients about which is the better treatment. A trial has been begun at the University of Pacific Orthodontic Clinic (San Francisco) using this design. A manuscript, written in collaboration with Dr. S Baumrind of UCSF, has been submitted for publication.

34. EDUCATION IN CLINICAL TRIALS METHODOLOGY

One of the greatest challenges facing the national clinical trials program of the NCI is the severe shortage of biostatisticians. Conducting reliable clinical trials also requires physician investigators with a sound appreciation of clinical trials methodology. Consequently, the BRB participates in activities to facilitate such training. During the past year we hosted two individuals for training in clinical trials methodology; an American statistician and an Italian physician investigator. We also presented two invited papers at a cancer clinical trials meeting in Paris. ("The Phase III Clinical Trial", "The NCI Program of Therapeutic Research"), an invited presentation on subset analysis and multiple endpoints to the Society for Clinical Trials pre-conference workshop, an invited critique of adaptive treatment assignment methods to the American Statistical Association, an invited presentation on selection designs for clinical trials to the national meeting of the AIDS Clinical Trial Treatment Group, an invited presentation on new clinical trial designs to the U. Md. Cancer Center Grand Rounds, an invited presentation on Advances in Clinical Trial Methodology in the 1980's to the American Statistical Association, an invited publication on the importance of confidence intervals for reporting clinical trials for Controlled Clinical Trials and an invited chapter on the design of phase III clinical trials.

35. LONGITUDINAL ANALYSIS OF THE DEVELOPMENT OF THE HUMAN JAWS

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

36. HOW DOES RELATIVE TREATMENT EFFICACY VARY AMONG HOSPITALS

One question that is often important in the evaluation of new treatments is whether and to what extent treatment differences vary among institutions. For cancer clinical trials, this is rarely addressed adequately because of the nature of the endpoints (often censored survival data) and the use of many institutions with limited numbers of patients per institution. We are developing statistical methods to better estimate the inter-institution distribution of treatment differences. We use non-parametric estimates and obtain confidence intervals for percentiles of this distribution using the bootstrap. This computationally intensive method is being evaluated.

37. WHEN TO STOP RANDOMIZING PATIENTS IN CLINICAL TRIALS WITH LONG TERM ENDPOINTS

When treatments are compared with regard to survival or disease-free survival, the number of "events" observed determines statistical power. Number of events is influenced by both number of patients and duration of follow-up. It sometimes happens that accrual rate decreases in the later part of a trial or a new therapeutic opportunity arises. One question then becomes, given the current results, what are the relative merits of continuing accrual versus terminating accrual and extending follow-up to the current study. Also, what are the advantages of delaying the reporting of results compared to reporting more immediately. We have developed statistical tools to address these questions and are evaluating the tools in cooperative group clinical trials.

38. TYPE ONE ERROR AFTER READJUSTING THE SAMPLE SIZE

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

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

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

40. STATISTICAL DESIGN AND ANALYSIS OF A PHASE III STUDY OF HIGH DOSE IL-2 WITH OR WITHOUT ALPHA-INTERFERON FOR PATIENTS WITH METASTATIC MELANOMA

This multi-institution clinical trial will randomize a total of 240 patients over four years. The primary endpoint is survival. BRB staff are the principle statisticians for this study and will perform interim and final analysis.

41. OTHER CONSULTING

The chief of the BRB provides consultation for high ranking officials of NIH, other government agencies and private foundations concerning the design and analysis of medical studies. During the past year, this has included development of recommendations for the issuance of clinical announcements by the NCI and by other institutes, commentary on an article by Pauling in PNAS, response to several published articles concerning cancer risk and cancer treatment, data monitoring and analysis of NCI and VA AIDS clinical trials, planning a prevention trial for type I diabetes, evaluation of results of a

clinical trial of thymosin alpha 1 for non-small cell lung cancer and commentary on an FDA proposal concerning endpoints for drug evaluation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CN 06308-19BRB

PERIOD COVERED
October 1, 1989 through September 30, 1990

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 M. Simon, Ph.D., Chief, Biometric Research Branch, CTEP, DCT, NCI
Others:
Lawrence V. Rubinstein, Ph.D., Statistician, BRB, CTEP, DCT, NCI
Timothy Chen, Ph.D., Statistician, BRB, CTEP, DCT, NCI
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Valter Torri, M.D., Visiting Scientist, BRB, CTEP, DCT, NCI

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

LAB/BRANCH
Biometric Research Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.67	5.67	1

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

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

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

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

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

CLINICAL INVESTIGATIONS BRANCH

GENERAL BACKGROUND

The Clinical Investigations Branch (CIB) is responsible for the administration and coordination of the extramural clinical trials sponsored by the Division of Cancer Treatment. In performing this task, the CIB coordinates its activities with the other Cancer Therapy Evaluation Program Branches and with relevant NCI and NIH components. Utilizing an integrated mix of advisory, informational, and facilitative activities, CIB identifies promising scientific opportunities and stimulates specific multi-institutional trials. The CIB emphasizes rigorous prioritization of research questions leading to clinical trials, as the number of treatment questions requiring answers is greater than can be addressed by the existing clinical trials network. The need to concentrate on key issues and to address them with trials of adequate size done in an expeditious manner is paramount and governs the activities of the CIB.

I. ORGANIZATION

In order to promote efficient collaborative research, the CIB utilizes a comprehensive disease and modality perspective to identify and articulate key research questions. CIB staff actively gather disease and modality information from all available sources, including the published literature, interim data from domestic and foreign cooperative groups, information from scientific meetings, and the pool of research project grants supported by the NCI.

Each individual staff member of CIB is responsible for maintaining information on current and developing research opportunities and serves as an information resource to CTEP, DCT and extramural investigators, as follows:

CIB MODALITY COORDINATORS

MODALITY	STAFF
BONE MARROW TRANSPLANT	CHESON
INFECTIOUS DISEASE	CHESON
QUALITY OF LIFE	MOORE
NUTRITION	MOORE
PSYCHOSOCIAL	MOORE
RADIATION	HAMILTON
SURGERY	FRIEDMAN (interim)

Specific disease responsibilities are divided as follows:

DISEASE	STAFF
AIDS	CHESON
BRAIN	HAMILTON
BREAST	DORR

Disease Responsibilities--Continued

DISEASE	STAFF
ENDOCRINE	HAMILTON
GASTROINTESTINAL	HAMILTON
GENITOURINARY	DORR
GYNECOLOGIC	MOORE
HEAD & NECK	MOORE
LEUKEMIA (ADULT)	CHESON
LUNG	MOORE
LYMPHOMA (ADULT)	CHESON
MELANOMA	DORR
MYELOMA	CHESON
PEDIATRIC (LEUKEMIA + SOLID)	UNGERLEIDER
SARCOMA (ADULT)	HAMILTON

II. COORDINATION AND ADMINISTRATION OF THE COOPERATIVE GROUP SYSTEM

A major responsibility of the Clinical Investigations Branch is to advise and coordinate administrative and scientific aspects of the Clinical Cooperative Groups. This effort is necessary to optimize the productivity of the cooperative agreement mechanism (U10), through which the NCI provides funds for definitive (Phase III) multi-institutional trials. Approximately $60 million is devoted to this mechanism. The CIB is responsible for and responsive to the Cooperative Groups; peer review judges the ultimate product. While the CIB has responsibility for administrative and scientific aspects of the Groups, it is not concerned with their micromanagement.

The Clinical Investigations Branch advises and directs the Cooperative Groups in allocating limited financial, investigator and patient resources. During the past year, particular Group-related administrative activities have included: supervision of the implementation of a per-case reimbursement system by the Radiation Therapy Oncology Group; finalizing the terms of award for cooperative agreements; supervision of the phase-out of the Lung Cancer Study Group and reorganization as part of the SWOG Thoracic Oncology Program; and supervision of the selection of the second generation of high priority clinical trials by the Cooperative Group Chairmen and the DCT Board of Scientific Counselors. These activities were in addition to the more routine administrative 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.

From a scientific point of view, the CIB and the Cooperative Group system identify and prioritize clinical research questions of interest. There is a potential interaction between the CIB and all Group organizational levels at any time during the process of generating a study, as follows: CIB staff regularly attend formal Group meetings to serve as a source of information and to provide guidance in the development of protocols. An effort is made to prevent duplicative protocols and to foster the very best science.

CIB staff organize strategy meetings in selected disease sites in order to help provide an overview of current therapeutic issues whose resolution might be facilitated through collaborative clinical trials. Representatives of cooperative groups participate in these meetings in which a consensus regarding the objectives and design of optimal trials is developed. The likelihood of duplicative trials is reduced and the probability of intergroup trials is enhanced by this process.

A continuum of CIB interaction with the Cooperative Group system exists throughout the evolution of a protocol, from the very earliest idea formation to the review of the finished document.

An increasingly important area of interaction is the Concept Review, an evaluation of the essence of a major Phase III study proposal while still in an early stage of development as it is deemed more efficient and productive to evaluate a concept than to modify a protocol at the final stage of development. A description of the content of a concept submission was developed by CIB staff to ensure uniformity. A brief document outlining the scientific background, objectives, eligibility, treatment schema and statistical section is sent by the investigators to the CIB, which provides relevant criticism in return. During the past year (07-01-89 to 06-30-90), 25 concepts were reviewed, of which 5 went forward to become active studies; one is currently in review. This format invites fruitful early dialogue between the investigators and NCI, at a time when the thrust of the experiment is most easily altered.

The formal Protocol Review process is in itself a major analytic activity. In this forum, a mature study plan that has already undergone considerable Group discussion and assessment is reviewed for safety and scientific issues. CTEP staff critique these protocols and request changes when appropriate. In order to supplement intramural expertise outside reviewers assist as needed. A written consensus review is provided the investigators which outlines required and/or recommended changes in the protocol document. During the past year 361 protocols were reviewed by the committee.

The CIB promotes clinical trials that are sufficiently large to be reliable, and are completed in the briefest possible time. The CIB encourages appropriate intergroup studies. Generally, at any particular moment, there are a number of scientific questions of the highest priority. An intergroup study is deemed appropriate when a study by an individual Cooperative Group would require an inordinately long time for completion and/or might accrue too few patients to permit statistically valid conclusions.

Finally, but with increasing emphasis, the CIB promotes relevant laboratory-clinical correlative investigations which might prove scientifically fruitful. Information concerning the best correlative studies comes not only from Group pilot activities, but also from information gained from the R01/P01 pool of grants which CTEP manages.

The following is a list of the Cooperative Group organizations that were

functioning with NCI support in FY90 and the CIB staff member who was responsible for scientific liaison with that organization.

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

COOPERATIVE GROUP OUTREACH PROGRAM (CGOP)

The Cooperative Group Outreach Program was transferred from DCPC to DCT in FY87, at which time a recompetition for awards was conducted. Five Groups (Eastern Cooperative Oncology Group, Children's Cancer Study Group, Cancer and Leukemia Group B, Southwest Oncology Group and the National Surgical Adjuvant Breast and Bowel Project) were selected for awards in FY88. As planned at the time of recompetition, the CGOP award periods are synchronous with the parent Cooperative Group award and require recompetition at the time the parent Group recompetes. In FY90, the Eastern Cooperative Oncology Group successfully recompeted and had its CGOP component approved for an additional 5 years.

TERMS OF AWARD FOR GROUPS

The formal rules describing the interaction of the NCI and the Cooperative Groups and the expectations for Group performance are known as the Terms of Award. These Terms have been revised by CIB staff to reflect evolving expectations. Under the new Terms, the NCI interacts more closely with each Group to implement clinical studies of the highest quality. New studies are initiated only after close scrutiny to ensure that all relevant scientific issues have been considered and that the study will be completed as quickly as possible. Intergroup collaborations are required where appropriate, and capitation forms of reimbursement authorized to stimulate patient enrollment. The substantial NCI involvement called for by the cooperative agreement mechanism has been enhanced in order to maximize productivity. The new Terms of Award have been accepted by the Cooperative Group Chairmen,

endorsed by the DCT Board of Scientific Counselors, and were accepted by NIH in FY 90.

CLINICAL TRIALS TRACKING SYSTEM

CIB has developed a clinical trials tracking system to follow the progress of ongoing studies and to coordinate subsequent trials. This system draws on designated items of high interest in the current CTEP information system, made more accessible through a data base management system. Major features of this system are the ability to organize and identify studies by the scientific hypothesis being tested, and to accurately assess patient accrual and projected closure date, thus improving the management of Group studies.

III. SCOPE OF GROUP ACTIVITIES

ACCRUAL

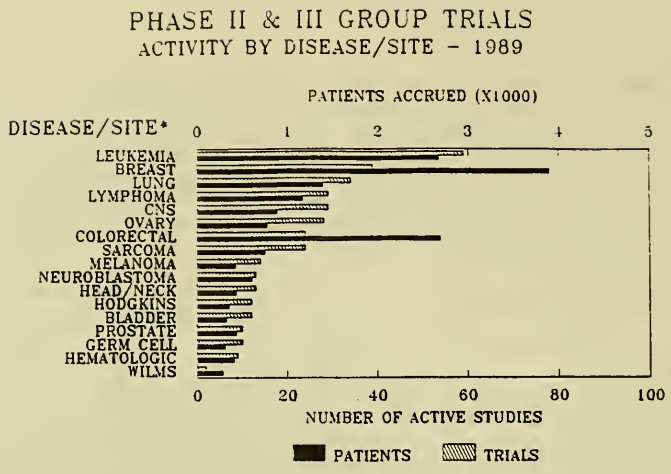
In 1989, over 20,000 new patients were entered on therapeutic studies (Phase I, II and III) in CTEP-sponsored trials conducted by the Cooperative Groups, with most of these patients entering Phase III trials (Figure A). Virtually every type of malignancy is being studied in this collaborative enterprise. 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 B. In addition, over 30,000 patients were entered on Group non-therapeutic/laboratory correlative studies using clinical trials patients/samples.

FIGURE A

NCI CLINICAL COOPERATIVE GROUPS
ACCRUAL SUMMARY
CALENDAR YEAR 1989

	PATIENT ENTRIES	OPEN STUDIES
PHASE I	429	49
PHASE II	4,805	292
PHASE III	14,854	172
NON-THERAPEUTIC/CORREL.	30,241	105
EORTC (1989) (EST)	6,100	205

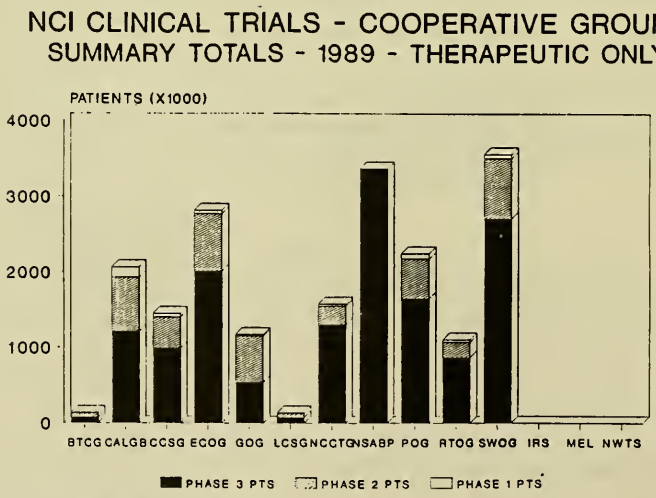
FIGURE B



*SOME MODALITY STUDIES INCLUDE PATIENTS WITH OTHER CANCERS AND SOME PATIENTS ENTER STUDIES SEQUENTIALLY

Accrual to treatment studies by the major Cooperative Groups is shown in Figure C:

FIGURE C



DATA ADJUSTED FOR ACCRUAL TO INTERGROUP TRIALS

In relation to a number of specific causes of cancer deaths, the following table compares impact of disease and clinical research effort.

COOPERATIVE CLINICAL GROUP STUDIES IN SELECTED DISEASE AREAS - PROTOCOLS ACTIVE IN 1989 ACCRUAL TO PHASE II AND PHASE III STUDIES

ORGAN	NEW CASES IN 1989 (ACS DATA)	DEATHS IN 1989	STUDIES OPEN TO ACCRUAL	TOTAL ACCRUAL 1989	% ONTO GROUP STUDIES
Lung	155,000	142,000	34	1,395	0.9
Colon/Rectum	151,000	61,300	24	2,687	1.8
Breast	142,900	43,300	39	3,890	2.7
Prostate	103,000	28,500	10	436	0.4
Bladder	47,100	10,200	12	323	0.7
Lymphoma, Non-H	32,800	17,300	29	1,167	3.6
Leukemia	27,300	18,100	59	2,672	9.8
Pancreas	27,000	25,000	10	185	0.7
Stomach	20,000	13,900	10	180	0.9
Ovary	20,000	12,800	28	774	3.9
Brain/CNS	15,000	11,000	29	884	5.9
Cervix	13,000	6,000	20	398	3.1
Myeloma	11,600	8,600	9	408	3.5
Esophagus	10,100	9,400	4	44	0.4
Lymphoma, Hodgkin's	7,400	1,500	12	355	4.8
Solid Tumors, Peds	2,200	N/A	29	1,402	64.0
TOTAL	785,400	408,900	358	17,200	

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 of an enthusiasm for clinical trials by the general public and health care workers. The trials were selected for this program based on their potential to increase the survival rate for a number of common cancers or for their ability to answer questions of special biological significance. The accelerated patient accrual was intended to speed the resolution of underlying medical questions and to bring successful new cancer treatment procedures to the cancer patient more quickly.

It was recently established, for example, in an intergroup clinical trial of colon cancer that treatment with levamisole in combination with 5-fluorouracil (5-FU) markedly improves patients' survival. As a result, a recent NIH Consensus Development Conference has recommended that this therapy be considered for all Dukes' C colon cancer patients. Other studies suggest that the combination of 5-FU and leucovorin is beneficial

to patients. Further development of the adjuvant treatment of colon cancer is the goal of one of six trials (INT-0089) of the current High-Priority Trials series. This study will evaluate the effectiveness of levamisole plus 5-FU plus leucovorin compared to 5-FU/levamisole or 5-FU/leucovorin.

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

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

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

Thus far, two series (1988 and 1989) of High Priority Trials have been identified by the Group Chairmen, the Division of Cancer Treatment Board of Scientific Counselors, and CTEP staff:

Series I High Priority Trials - Name and NCI Identification Number

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

Series II High Priority Trials - Name and NCI Identification Number

- Chemotherapy Before and After Surgery for Breast Cancer (NSABP-B-18)

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

Figures obtained by CTEP show that 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 Phase III treatment studies. The ten active High Priority Trials now are accruing patients about three times the average rate of the other Phase III trials.

Five Phase III studies originally were identified as High Priority Trials (Series I). Four of the five trials have succeeded in accruing patients more rapidly than initially anticipated. The National Surgical Adjuvant Breast and Bowel Project (NSABP) Study C-03 reached its accrual goal and closed in April 1989. The North Central Cancer Treatment Group (NCCTG) rectal study (864751) is accruing at three times the initially projected rate and will close soon (more than 1.5 years earlier than anticipated); its replacement study is being prepared. The NSABP rectal study (NSABP-R02) and Southwest Oncology Group (SWOG) lymphoma intergroup (INT-0067) trial are entering patients somewhat more quickly than planned, and are expected to complete accrual on or ahead of schedule. The SWOG Bladder intergroup study is an unprecedented intergroup effort (INT-0080) and continues to accrue below the planned rate.

In June 1989, six additional trials were approved by the Chairmen of NCI's Clinical Trials Cooperative Groups and received the High Priority designation (Series II). Although these are mostly very large studies, their planned accrual periods are all less than 3.3 years. Accruing beyond their projected rates after approximately one year are the Eastern Cooperative Oncology Group small cell lung cancer study (INT-0096) and intergroup node-negative breast cancer study (INT-0102). These studies will complete accrual in less than the projected time period if the current rates continue. The Radiation Therapy Oncology Group lung intergroup study (RTOG-8808), which opened in January 1989, also is accruing patients more quickly than anticipated.

The NSABP breast study B-18 addresses a familiar adjuvant population and is accruing at nearly the projected rate, while the NSABP breast study B-21 (Occult Stage I Disease) deals with a unique patient population and is entering patients more slowly than planned. The further study of levamisole in colon cancer (INT-0089), is accruing patients very rapidly, and should be completed ahead of schedule.

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

ACCRUAL TO HIGH PRIORITY TRIALS
SERIES I

STUDY	ACTIVA-TION DATE	ACCRUAL TARGET	1/88	7/88	1/89	7/89	1/90	7/90
Lymphoma (INT 0067)	04/86	1,000	219	336	481	631	755	834
Bladder (INT 0080)	08/87	298	5	12	35	64	96	105
Rectal (NCCTG 86-47-51)	06/87	450	26	49	87	211	403	483
Colon (NSABP C-03)	08/87	855	114	441	830	1,081	(CLOSED)	
Rectal (NSABP R-02)	08/87	750	27	117	191	289	373	429

ACCRUAL TO HIGH PRIORITY TRIALS
SERIES II

STUDY	ACTIVA-TION DATE	ACCRUAL TARGET	1/88	7/88	1/89	7/89	1/90	4/90
Colon Cancer INT-0089	08/88	2,700	-	30	113	382	659	923
Lung (small cell) INT-0096	04/89	250	-	-	-	3	34	65
Breast (node-) INT-0102	07/89	2,600	-	-	-	-	233	542
Breast NSABP-B18	11/88	1,275	-	-	40	211	390	483
Breast NSABP-B21	06/89	1,350	-	-	-	5	41	59
Lung (non-small cell) RTOG-88-08	01/89	360	-	-	-	16	90	132

INITIATIVE FOR INCREASING MINORITY ACCRUALS TO COOPERATIVE GROUP TRIALS

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

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

STRATEGY MEETINGS

Strategy meetings help provide an overview and prioritize national efforts in selected disease sites. Expert oncologists from the Cooperative Groups and Cancer Centers meet at the National Cancer Institute to review ongoing clinical experiments and identify short-term priorities for research. The format of these 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. Three strategy meetings are planned for FY 90:

1. NON-SMALL CELL LUNG CANCER

TOPIC: STRATEGY MEETING FOR LOCALLY ADVANCED NON-SMALL CELL LUNG CANCER

DATE: February 14, 1990

COORDINATOR: Timothy D. Moore, M.D.

Participants	Cooperative Group/Institution
James D. Cox, M.D.	RTOG
Robert Ginsberg, M.D.	SWOG
Mark R. Green, M.D.	CALGB
E. Carmack Holmes, M.D.	SWOG
Waun Ki Hong, M.D.	RTOG
James Jett, M.D.	NCCTG

David H. Johnson, M.D.	ECOG
Larry Kaiser, M.D.	ECOG
Steven M. Keller, M.D.	ECOG
Robert B. Livingston, M.D.	SWOG
Thomas Pajak, M.D.	RTOG
Steven Piantadosi, Ph.D.	Johns Hopkins Oncology Ctr
Kathy Propert, Ph.D.	CALGB
John C. Ruckdeschel, M.D.	Albany Medical College
William T. Sause, M.D.	RTOG
Stephen Seagren, M.D.	CALGB
Edward G. Shaw, M.D.	NCCTG
Frances Shepard, M.D.	NCIC
David J. Sugarbaker, M.D.	CALGB
Victor Trastek, M.D.	NCCTG
Andrew Turrisi, M.D.	SWOG
Henry Wagner, M.D.	ECOG
Bruce A. Chabner, M.D.	NCI
Timothy Chen, Ph.D.	NCI
Bruce Cheson, M.D.	NCI
Eli Glatstein, M.D.	NCI
Michael Grever, M.D.	NCI
John D. Minna, M.D.	NCI
Timothy D. Moore, M.D.	NCI
Harvey Pass, M.D.	NCI
Richard S. Ungerleider, M.D.	NCI
Carolyn Gotay, Ph.D.	EMMES Corporation
Pamela Phillips, M.H.S.	EMMES Corporation
Donald A. Vena, B.S.	EMMES Corporation

These medical, radiation and thoracic-surgical oncologists discussed issues relevant to the treatment of locally advanced non-small cell lung cancer. This session resulted in 3 Cooperative Groups combining their efforts in an adjuvant trial of patients with completely resected Stages II - IIIA disease. In addition, a staging algorithm was developed which should permit precise categorization of patients in future neoadjuvant and adjuvant studies.

2. CUTANEOUS T-CELL LYMPHOMA

A number of new, innovative approaches have been developed for the treatment of patients with cutaneous T-cell lymphoma. These include 13 cis-retinoic acid, alpha-interferon (in combination with phototherapy), gamma interferon, fludarabine, and 2-chlorodeoxyadenosine. CIB feels that it is now appropriate to develop a coordinated strategy to further develop these modalities. The meeting will be attended by experts from the various adult cooperative groups, the NCI and NCI-Navy, as well as dermatologists with an academic interest in clinical trials in cutaneous T-cell malignancies.

3. HIGH DOSE CHEMOTHERAPY FOR BREAST CANCER

There has been a striking increase in the use of high dose chemotherapy with stem cell support (either bone marrow or peripheral blood) for the treatment of women with either metastatic or high risk breast cancer.

Nevertheless, the optimal regimen, the clinical indications, and the benefit of this treatment relative to standard therapies are not completely clear. Moreover, third party reimbursement remains a problem for many patients offered this therapy. It is hoped that this meeting will result in a multi-center protocol which will better characterize the role of high dose chemotherapy in breast cancer. Participants will include representatives from the adult cooperative groups and major cancer centers.

STRATEGY SESSIONS PLANNED FOR 1991

1. Advanced Multiple Myeloma

2. Myelodysplastic Syndrome

3. Gastric Cancer

4. Renal Cell Cancer

5. Adult Acute Lymphoblastic Leukemia

6. Chronic Myelogenous Leukemia

7. Non-Hodgkin's Lymphoma in HIB-positive patients

8. Metastatic colon Cancer

CONSENSUS DEVELOPMENT CONFERENCES

CIB Senior Investigators have been central to the organization of NIH Consensus Development Conferences regarding cancer treatment.

1. Dr. Hamilton was the chairman of the planning committee for the Consensus Development Conference on Adjuvant Therapy for Patients with Colon and Rectal Cancer (April 16 - 18, 1990).

Large bowel adenocarcinoma is a major health problem in the United States. More than 150,000 new cases of colon and rectum cancer will be diagnosed in 1990, and approximately 75 percent will have a primary surgical resection with the hope of complete tumor eradication. Despite this high resectability rate, nearly half of all colorectal cancer patients die of metastatic tumor.

The need for effective adjuvant therapy is obvious. Over the past three decades many studies have failed to identify the benefits of adjuvant therapies, and claims of efficacy have often been viewed with skepticism by the practicing physician. However, more recently, new information has been generated from carefully designed and performed clinical trials. Several studies purport to demonstrate disease-free and overall survival benefits for selected groups of patients.

To judge the relative merits of several adjuvant treatment programs, this

conference brought together surgeons, gastroenterologists, medical oncologists, pathologists, radiation oncologists, statisticians, patients, and the public to examine these issues.

Following a day and a half of presentations and discussions, a consensus panel weighed the scientific evidence and wrote a draft statement in response to the following key questions:

- Who is at risk for recurrence after colon and rectum cancer resection?
- Is there effective adjuvant therapy for patients with colon cancer?
- Is there effective adjuvant therapy for patients with rectum cancer?
- What are the directions for future research?

Conclusion and Recommendations

In answer to the question, "Who is at risk for recurrence after colon and rectal cancer resection?", the consensus panel recommended the following:

- The TNM system based on a complete pathological description can effectively describe risk groups for recurrence and should be used in clinical trials research and clinical practice.
- Patients with colon and rectal cancer should be studied separately when defining adjuvant strategies.
- Patients with Stage III colon cancer or Stage II/III rectal cancer are at high risk for recurrence and warrant adjuvant therapy.
- Anatomic or biologic features may define subsets of patients with Stage II colon cancer at intermediate risk of recurrence sufficient to merit testing of adjuvant treatment compared to observation only controls. These features include:

  -- T4 NO MO.

  -- T3 NO MO plus one or more of the following:

    -- Pre-op CEA > 5ng/ml.

    -- Aneuploid DNA content

    -- High S phase

    -- Colloid, signet ring, or poorly differentiated histology.

    -- 17p or 18q deletion

- Patients with Stage I lesions are at low risk of recurrence and should not receive adjuvant treatment.

- Correlation of laboratory observations and clinical data must be pursued to define the biological and clinical significance of these cellular and molecular characteristics.

In answer to the question, "Is there effective adjuvant therapy for patients with colon cancer?", the consensus panel concluded that:

- Optimal adjuvant therapy for Stage II and III colon cancer has not yet been devised. Continued clinical trials in this disease are essential to discover more active adjuvant therapies.

- Based on current clinical trial data, Stage III patients unable to enter a clinical trial should be offered adjuvant 5-FU and levamisole as administered in the intergroup trial unless medical or psychosocial contraindications exist.

- The panel cannot recommend any specific adjuvant therapy at this time for Stage II patients outside of clinical trials.

In answer to the question, "Is there effective adjuvant therapy for patients with rectal cancer?", the consensus panel concluded the following:

- No adjuvant therapy is recommended for Stage I patients; in contrast to Stage II colon cancer, we recommend adjuvant treatment of Stage II rectal cancer.

- Combined postoperative chemotherapy and radiation therapy improves local control and survival in Stage II and III patients and is recommended.

- At the present time, the most effective combined modality regimen appears to be 5-FU plus methyl-CCNU, and high-dose pelvic irradiation (45 to 55 Gy) but chronic toxicity considerations of methyl-CCNU mitigate against using this regimen outside ongoing clinical trials.

- Current clinical trials of combined modality therapy are designed to improve the prognosis of Stage II and III patients. Entry of patients into these clinical trials is highly encouraged.

The following directions for future research were suggested:

- The highest priority for future adjuvant trials in colon cancer should build on the results achieved with 5-FU/levamisole using modulators of 5-FU, modulators of host response, and new regimens of proven efficacy in advanced disease.
- The highest priority for future adjuvant trials in rectal

cancer will be to integrate radiation therapy with newer 5-FU modulated regimens such as 5-FU/levamisole, 5-FU/leucovorin, or other combinations with demonstrated activity in advanced disease.

- There is a need to identify new determinants of risk to be used to select early stage patients likely to benefit from adjuvant therapy.

- There is a need to incorporate into intergroup trials the appropriate basic laboratory investigations required to define mechanisms of drug action, especially in trials involving modulators of host immune response.

- There is a need to address issues of quality of life and the cost benefit of such therapies.

- There is a need to initiate trials to address questions of differences in disease and outcome observed in various ethnic and socioeconomically disadvantaged groups.

2. Dr. Dorr was chairman of the planning committee for the Consensus Development Conference on Early Stage Breast Cancer (June 18 - 21, 1990).

The management of patients with early stage breast cancer has evolved rapidly over the past 20 years. Strategies to treat the primary tumor as well as distant micrometastases have been the subjects of worldwide clinical investigation. Since 1985, a number of randomized clinical trials have evaluated different approaches to two treatment strategies for patients with early stage breast cancer: breast conservation and systemic adjuvant therapy for patients whose axillary lymph nodes test negative for tumor growth.

Considerable controversy exists, however, as to which patients should be selected for breast conservation and which patients with node negative breast cancer should be selected for adjuvant therapy.

The conference brought together surgical, radiation, and medical oncologists, pathologists and other laboratory scientists, biostatisticians, psychologists, nurses, and other health care professionals as well as representatives of the public.

Following 2 days of presentations and discussions, a consensus panel weighed the scientific evidence and wrote a draft statement in response to the following key questions:

- What are the roles of mastectomy versus breast conservation in the treatment of early stage breast cancer?

- What are the optimal techniques for breast conservation?

- What is the role of adjuvant therapy for patients with node negative breast cancer?

- How should prognostic factors be used in the management of node negative breast cancer?

- What are the directions for future research?

Conclusions and Recommendations:

- Breast conservation treatment is an appropriate method of primary therapy for the majority of women with Stage I and II breast cancer, and is preferable because it provides survival equivalent to total mastectomy and axillary dissection while preserving the breast.

- The recommended technique for breast conservation includes:

  -- Local excision of primary tumor with clear margins

  -- Level I-II axillary node dissection

  -- Breast irradiation to 4,500 - 5,000 cGy with or without a boost.

- The many unanswered questions in the adjuvant systemic treatment of node negative breast cancer make it imperative that all patients who are candidates for clinical trials be offered the opportunity to participate.

- The majority of patients with node-negative breast cancer are cured by breast-conserving treatment or total mastectomy and axillary dissection. The rate of local and distant relapse following local therapy for node-negative breast cancer is decreased by both combination cytotoxic chemotherapy and by tamoxifen. The decision to use adjuvant treatment should follow a thorough discussion with the patient regarding the likely risk of relapse without adjuvant therapy, the expected reduction in risk with adjuvant therapy, toxicities of therapy, and its impact on quality of life.

- While all node-negative patients have some risk for recurrence, patients with tumors less than or equal to 1 centimeter have an excellent prognosis and do not require adjuvant systemic therapy outside of clinical trials.

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 437 active protocols in the CIB hypothesis data base as of April 25, 1990, which includes 206 (47%) phase I/II and II, and 158 (36%) phase II/III and III; and 73 (17%) non-treatment (ancillary) studies. Of these, 123 (28% of all studies, 34% of treatment studies) have scientific correlates. Some examples are:

1. A large intergroup trial for previously untreated patients with CLL

has recently been activated to compare chlorambucil with fludarabine with the combination of the two agents. Companion studies will examine the biology and immunology of this disorder.

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

3. Pediatric neuroblastoma studies are currently stratifying patients on the basis of n-myc amplification.

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

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

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

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

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

9. A workshop, jointly sponsored by CTEP, BRMP, DCPC, and Janssen Pharmaceutica, was held June 11 - 12, 1990 to discuss which approaches should be employed to determine the mechanisms of action of levamisole in cancer therapy. A number of participants will be attempting to integrate their laboratory methods and patient materials that will be available from ongoing clinical trials that employ levamisole in therapy.

10. RTOG is currently evaluating whether flow cytometry can be used as an early predictor of recurrence for patients with bladder cancer, as a potential predictor of disease-free and overall survival for patients with anal cancer, and as a predictor of response in non-small cell lung cancer patients. The RTOG is also attempting to compare flow cytometry

results with computer aided image analysis (CAIA) as a predictor of proliferative activity in prostate patients with paraffin fixed tissues.

SPECIFIC PROGRAM ACCOMPLISHMENTS

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

PEDIATRICS

Accomplishments

1. The Pediatric Oncology Group published the results of a randomized controlled trial to determine whether irradiation of primary sites of involvement could safely be omitted from the treatment of children with localized non-Hodgkin's lymphoma (Stages I and II) who have a favorable prognosis. In addition, the chemotherapy regimen was less intense and shorter (eight months) than usual. The projected disease free survival ($\pm$ S.E.) at four years among patients assigned to chemotherapy alone is 87.9 $\pm$ 8.8%, as compared to 87.3 $\pm$ 9.4% among patients assigned to combined therapy. The investigators conclude that most children with localized non- Hodgkin's lymphoma can be cured by a chemotherapy regimen of reduced intensity and duration. Radiotherapy can be safely omitted from the therapy of such children without substantially jeopardizing their excellent change of cure (N Eng J Med 322:1169-74, 1990).

2. The Children's Cancer Study Group (CCSG) has reported on the role of intensified chemotherapy in the largest subgroup of acute lymphoblastic leukemia of childhood, those with intermediate risk of relapsing. The Group randomized 1558 patients to one of four systemic treatment arms: standard CCSG therapy, Berlin-Frankfurt-Munster (BFM) therapy (the best reported to date), standard induction and consolidation with BFM delayed intensification, and BFM induction and consolidation without delayed intensification, in order to determine whether BFM was truly superior, and whether all segments of BFM strategy were required. Remission was achieved in 99% of patients. For those under ten years of age, results indicate that delayed intensification is critical for improved event-free survival and that intensive induction and consolidation does not improve outcome. For patients ten years or older the differences are not statistically significant but these early results suggest that both intensive induction and consolidation and delayed intensifications may be required for improved outcome (Proc ASCO 9:216, 1990).

3. Clinical and histopathologic features are often inadequate for accurate prediction of survival of individual patients with rhabdomyosarcoma. Investigators of the Intergroup Rhabdomyosarcoma Study have reported preliminary evidence of a relationship of tumor cell ploidy to histologic subtype and treatment outcome in children and adolescents with unresectable rhabdomyosarcoma. Tumor cell ploidy had a significant impact on survival, with hyperdiploidy conferring the best prognosis and diploidy the worst. Of 37 patients studied, all patients with diploid tumors were dead by 18 months. Tumor cell ploidy was the

best predictor of treatment outcome for patients with either embryonal or alveolar histology. This suggests that patients with unresectable diploid rhabdomyosarcoma have an unacceptably high risk of treatment failure, justifying new therapeutic approaches for this distinct subgroup (Proc ASCO 9:290, 1990).

Future Plans

1. Intergroup Rhabdomyosarcoma Study IV is expected to open in 1990, although several of the treatment arms are currently being piloted for feasibility. IRS-IV will use a pre-treatment staging system independent of surgery for the first time. Stage III patients will be entered in a randomized study to determine the benefit of ifosfamide and of ifosfamide/etoposide in combination when added to conventional VAC therapy, as well as the benefit of hyperfractionated versus conventional radiation. Patients with newly diagnosed metastatic disease will be randomized to receive one of four drug pairs which are known to be active in previously treated patients prior to beginning conventional therapy. This design is intended to establish a rank order of activity for the drug pairs for use in future trials; additionally, active drug pairs will be integrated into the conventional therapy for individual responders.

2. The Children's Cancer Study Group has initiated a study (CCG-2891) of newly diagnosed children with ANLL to compare in a randomized fashion the efficacies of a) increased intensity induction therapy (the second course delivered in an obligatory fashion) vs standard intensity induction therapy (the second course delivered upon recovery of marrow function), and b) increased intensity consolidation therapy (requiring bone marrow reinfusion) consolidation therapy. Current induction therapies provide 70-75% remission rate; 75% of these children will not have a related matched bone marrow donor, and with conventional continuation therapy, only 25-50% will achieve long-term remission. This study seeks to test whether intensifying induction therapy (through alterations in the timing of administration), and intensifying consolidation therapy (with the support provided by autologous bone marrow infusion), will improve upon these results.

GU CANCER

Testis Cancer

The Eastern Cooperative Oncology Group conducted an interim analysis of their trial comparing BEP versus etoposide and cisplatin for patients with minimal or moderate risk metastatic disease by Indiana criteria. This study was designed as a toxicity reduction trial, hoping to avoid the toxicities of bleomycin. The relapse rate was found to be significantly higher in the patients receiving the two drug regimen for three cycles compared to the three drug regimen for three cycles. The study has thus been closed with the recommendation that three cycles of BEP should continue to be the standard treatment for these patients. A study from Memorial Sloan Kettering Cancer Center has found 4 cycles of the two drug regimen to be comparable to the five drug regimen (VAB-6) that had been their standard for low risk patients. MSKCC with SWOG is evaluating the 2 drug regimen given for four cycles versus etoposide +

carboplatin to determine whether they are equally effective and if the latter combination is less toxic.

For patients with advanced disease, trials continue to evaluate more intensive therapies (bleomycin, etoposide, cisplatin vs etoposide, ifosfamide, cisplatin) and early institution of high-dose chemotherapy with autologous bone marrow reinfusion. New drugs being studies in patients with refractory disease include: fazarabine, 5-azacytidine, navelbine and taxol when drug supply allows.

Future Plans:

There are no current plans to conduct another randomized study for patients with low risk or moderate risk metastatic testis cancer since most of these patients are cured with standard therapies (>90%).

New trials for patients with advanced disease will not begin within the next fiscal year pending outcome of the current studies.

Prostate Cancer

Accomplishments:

A randomized trial to compare radical prostatectomy versus radiation therapy for patients for Stage A2 and B prostate cancer has been started during this fiscal year. Patients must undergo surgical staging of their pelvic lymph nodes prior to randomization to confirm the absence of nodal metastases. Endpoints for the study are overall survival, cause-specific survival, disease-free survival and several measurements of treatment toxicity and quality of life. The study is designed to enter 1,128 patients in a 7.5 year period.

Another trial initiated this year is comparing orchiectomy + flutamide versus orchiectomy + placebo for patients with newly diagnosed metastatic prostate cancer. A previous intergroup trial demonstrated that flutamide + leuprolide results in significantly superior survival compared to placebo + leuprolide. One interpretation of that study has been that the role of flutamide is primarily to block the initial tumor flare associated with LHRH analog administration, producing the observed differences in time to progression and survival. In order to further evaluate the concept of complete androgen blockade, without the confounding factor of tumor flare, this study will enter 1,200 patients in 2.5 years.

New drug trials continue in prostate cancer. Suramin has nearly completed testing in patients with hormone refractory, measurable metastatic prostate cancer. Results of that study should be forthcoming in the near future. A randomized study comparing suramin with palliative therapy in patients without measurable disease was activated during this fiscal year. This study has a proposed sample size of 200 patients and is using quality of life as well as survival as study endpoints.

A trial of taxol in prostate cancer has recently been approved and will be started in this fiscal year.

Several trials evaluating adjuvant radiation therapy and adjuvant endocrine therapy for patients with Stages B, C and D1 disease are underway.

Future Plans

The combination of 5-FU and alpha interferon will soon be evaluated in a Phase II trial in the Southwest Oncology Group. A number of other biologic therapy studies exploring gamma interferon, alpha interferon and IL-2 will be done in both SWOG and ECOG. SWOG hopes to evaluate the role of nephrectomy in patients with metastasic disease since there are many anecdotal reports that response likelihood to systemic therapy is increased inpatients whose primary tumor has been removed. This study will probably be conducted as an intergroup with all patients receiving alpha interferon with the randomization being to nephrectomy or no nephrectomy.

Bladder Cancer

Accomplishments:

On the basis of clinical trials conducted in the Southwest Oncology Group, the FDA recently approved the use of BCG for intravesical instillation for treatment of superficial bladder cancer. Ongoing trials are comparing the importance of maintenance BCG relative to short course therapy and the relative efficacy of BCG and mitomycin in the treatment of superficial disease.

An intergroup and international trial coordinated by the Eastern Cooperative Oncology Group compared single agent cisplatin with combination chemotherapy with methotrexate, vinblastine, adriamycin and cisplatin (MVAC) in patients with metastatic bladder cancer. The MVAC combination was shown to prolong survival by approximately 50%.

Initial trials with gallium nitrate in the late 1970's found a low level of activity in bladder cancer when given on a bolus schedule. Recent evidence that the drug is more effective when given by continuous infusion has been evaluated in bladder cancer patients. Among the first 4 patients treated, all 4 have responded. This study will be the first priority for the Southwest Oncology Group for the remainder of this year.

One of the NCI-designated High-Priority Trials is the study for patients with locally advanced bladder cancer in which patients are randomized either to radical cystectomy alone or radical cystectomy preceded by three cycles of MVAC chemotherapy. This study continues to accrue patients in the Southwest Oncology Group and the Eastern Cooperative Oncology Group.

The RTOG has recently begun a trial for patients with locally advanced bladder cancer in which the control group is treated with radiotherapy + concurrent cisplatin and the experimental group receives two cycles of cisplatin, methotrexate and vinblastine followed by radiotherapy + concurrent cisplatin.

Future Plans

Several groups will take different approaches to dose intensifying the MVAC regimen by combination with colony stimulating factors either by altering the dose and schedule or simply by increasing the prescribed doses. Once some experience has been gained with dose intensified MVAC, a randomized trial will be conducted comparing standard dose MVAC with intensified MVAC.

Since taxol has been shown to be active in cisplatin resistant diseases and cisplatin is the most active agent in bladder cancer, a Phase II trial of taxol will be carried out as soon as drug supply allows.

MELANOMA

Accomplishments

Two adjuvant trials have been concluded this year:

SWOG 8642 evaluated adjuvant gamma interferon. An interim analysis found that patients receiving gamma interferon had a higher recurrence rate than those receiving no adjuvant therapy. The study has therefore been closed and all patients still on gamma interferon had therapy stopped.

ECOG 1684 evaluated adjuvant alpha interferon. An interim analysis found that patients receiving alpha interferon a significantly lower recurrence rate than those receiving no adjuvant therapy. No difference in survival is yet noted. The study will formally close as of July 6, 1990.

Another study which has been ongoing for 7 years and has now accrued over 700 patients has compared width of excision (2 versus 4) and therapeutic value of regional lymph node dissection in patients with melanoma of 1 to 4 mm thickness. This study will be closed in late 1990.

Future Plans

The North Central Cancer Treatment Group is planning a randomized study of adjuvant megace versus no adjuvant therapy for patients with thick primary melanoma (>1.69 mm) or with regional lymph node metastases. This follows a small randomized study done at the Mayo Clinic in the early 1980's which showed a significant difference in survival for patients receiving adjuvant megace. That study had only 67 patients (33 in one arm, 34 in the other) so the results need to be confirmed in a study of adequate sample size.

The Southwest Oncology Group is planning a randomized trial comparing no adjuvant therapy with adjuvant vaccine using a vaccine developed by Malcolm Mitchell at USC.

ECOG plans to conduct a confirmatory trial of its recent results with alpha interferon trial described above using the same dose and schedule of alpha interferon. A third arm will be added using a lower dose and shorter schedule of alpha interferon which is being investigated by the

World Health Organization. If subsequent analyses of the current trial show a survival difference for patients receiving alpha interferon, the untreated control arm will be dropped from the study.

ECOG also plans a study of adjuvant levamisole in patients with primary tumors 1 - 3 mm in thickness to follow up the trial of the NCI-Canada which found a survival advantage for this group of patients. Since several other trials have found no effect on survival by levamisole, a confirmatory trial using the same dose and schedule is needed.

Recent results from several institutions have found that patients receiving single agent or combination chemotherapy plus tamoxifen appear to have a significantly higher response rate than patients receiving the same chemotherapy without tamoxifen (in non-randomized trials). Laboratory evidence suggests that tamoxifen may be modulating drug resistance. Randomized trials will be started to evaluate this apparent effect of tamoxifen on drug resistance. Other trials combining cytotoxics with biologic therapies in patients with metastatic melanoma are ongoing.

GYNECOLOGIC MALIGNANCIES

OVARIAN CARCINOMA

Accomplishments

1. Two important Phase III trials in advanced, sub-optimally debulked ovarian cancer trials have recently been completed. The SWOG demonstrated that the combination of CBDCA and cyclophosphamide had efficacy similar to cisplatin and cyclophosphamide. However, women receiving CBDCA and cyclophosphamide experienced substantially less nausea, vomiting and neurotoxicity. The GOG randomized women to two different cisplatin-cyclophosphamide regimens differing in dose intensity. The results of this study will help to define the importance of dose-intensity in ovarian cancer.

2. The population of patients with advanced (St III), optimally debulked ovarian tumors is the focus of a Phase III intergroup effort, evaluating intraperitoneal CDDP with systemic CYT, versus intravenous CDDP/CYT. This is a very important trial, involving SWOG and the GOG, given the theoretical advantage of regional therapy in small volume ovarian cancer, and is a large enough study to conclusively determine the role of upfront intraperitoneal therapy in this group of patients.

3. Patients who are NED after second look laparotomy are being enrolled in two separate studies of adjuvant intraperitoneal therapy. SWOG randomizes patients to alpha interferon versus observation, while the GOG is looking at P^{32}.

4. A large number of women with ovarian cancer fail standard induction treatment, or recur after responding to cisplatin based regimens. Several approaches to salvage therapy are being developed For patients with minimal residual disease the GOG is systematically evaluating drugs administered via an intraperitoneal route. For women

with larger amounts of disease, both the GOG and SWOG are studying intravenously administered drugs having novel mechanisms of action in a Phase II setting. This approach was responsible for identifying significant activity for taxol in this disease.

Future Plans

1. Taxol has emerged as the most active new cytotoxic agent against ovarian cancer in the last ten years. The GOG recently initiated a protocol comparing taxol and cisplatin to standard therapy (cyclophosphamide and cisplatin). Additionally, the GOG is defining the optimal intraperitoneal dose of taxol in a Phase I trial, while several systemic Phase I studies are attempting to determine the maximum dose of taxol that can be safely administered by combining it with a hematopoietic colony stimulating factor.

2. The SWOG is building their prior experience with CBDCA and cyclophosphamide by combining these drugs with GM-CSF. A Phase III trial will evaluate whether GM-CSF allows a higher dose of CBDCA to be safely given than could normally be expected, and whether this translates into a higher pathologic complete response rate.

CERVICAL CANCER

1. The Cooperative Groups have continued extensive Phase II screening of drugs in this disease. Recently the GOG has demonstrated a 34% objective response rate with Dibromodulcitol in patients with advanced or metastatic disease.

2. Locally advanced patients (St IIB, III, and IVA) continue to be entered into a GOG study (which SWOG has just joined), comparing the radiosensitizing effect of Hydroxyurea versus a combination of CDDP-5FU.

Future Plans

1. The positive DBD study will be the basis for a randomized Phase III trial, with CDDP versus CDDP-DBD combination. Response rate, and duration of response will be the endpoints of interest. As bone marrow suppression, particularly thrombocytopenia, was the major toxicity encountered in these patients, a future study with escalating doses of DBD using CSF's would be of interest.

2. An adjuvant study of 5-FU + bolus CDDP after radiation therapy for selected early patients (St IA2, IB, and IIA) is under discussion as an Intergroup study with the GOG and SWOG. Since this is a relatively small subset of patients, the feasibility of adequate accrual has not yet been fully explored.

3. The GOG recently initiated a three arm trial comparing cisplatin to either cisplatin and DBD or cisplatin and ifosfamide.

4. The NCCTG demonstrated promising activity for the MVAC regimen, commonly used in bladder cancer, in advanced or recurrent cervical

cancer. However, neutropenia resulted in a substantial number of treatment delays. In an effort to administer more of the drugs on time, and to see if this influences overall outcome, they plan to initiate a comparative trial of the MVAC regimen with or without G-CSF.

5. Novel radiation fractionation schemas will be tested by RTOG and GOG in an attempt to increase control in locally advanced cervical cancer patients.

SARCOMAS

Accomplishments

1. The Phase III trial of doxorubicin with DTIC ± Ifosfamide in advanced soft tissue sarcomas continues to rapidly accrue patients. A new pilot trial using GM-CSF will attempt to dose escalate this regimen in hopes of increasing the response rate and response duration.

2. ECOG continues accruing on its advanced sarcoma protocol (EST-2786) of 3 regimens: single agent doxorubicin, doxorubicin + Ifosphamide, and doxorubicin + mitomycin C + cisplatin.

3. INT-0087 an Intergroup Sarcoma Trial of adjuvant therapy of soft tissue sarcomas is accruing patients using doxorubicin, DTIC, and Ifosfamide.

BREAST CANCER

Accomplishments

Adjuvant Therapy of Breast Cancer

Primary breast cancer is diagnosed in over 100,000 women each year in the United States. Following removal of their breast tumors and resection of the regional axillary lymph nodes, patients are at varying risks of recurrence depending on particular characteristics of their tumor. For patients with node negative breast cancer, five clinical trials are currently active (Table 1). All of these trials were opened in the 1989 fiscal year and continue to accrue patients during 1990, with one study (NSABP B-19) about to complete the accrual phase of the study. A replacement study is now being planned and will be instituted before the end of the 1990 fiscal year. These studies have accrued approximately 3,000 women this year.

For patients with node positive breast cancer, there are six currently active trials testing the most important concepts in adjuvant therapy for breast cancer (Table 2). One of these trials (CALGB 8541) began in 1985 and will be completed later this year. All of the remaining adjuvant therapy trials began in fiscal year 1989 and have been successfully and rapidly entering patients during the current year.

Two studies which were closed to accrual in recent years were analyzed this year and have demonstrated improved survival for different subgroups

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

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

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

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

Future Plans

High dose chemotherapy:

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

TABLE 1

BREAST CANCER CLINICAL TRIALS
ADJUVANT STUDIES - N_0

STUDY	ELIGIBILITY	DESIGN	QUESTIONS BEING ASKED
INT-0102	T_{1-3a}, N_0	CAF → TAM / PLACEBO	Is an adriamycin regimen superior to a non-adriamycin regimen?
		CMF → TAM / PLACEBO	Does Tamoxifen add to chemo-therapy, including ER-, PgR- subset?
NSABP B-18	T_{1-3a}, N_{0-1}	AC 4 --> Surgery Surgery --> AC 4	Is preoperative chemo-therapy superior to post operative chemotherapy?
NSABP B-19	T_{1-3a}, N_0 ER NEG	MFL CMF	Is classical CMF superior to sequential M-->F plus leucovorin as given in NSABP trial B-13?
NSABP B-20	T_{1-3a}, N_0 ER POS	Tamoxifen TAM + MFL TAM + CMF	Does chemotherapy add to tamoxifen in this group of patients? Does classical CMF pro vide superior results to sequential M-->F?
NSABP B-21	T_1, N_0 [occult tumors (<1 cm too small for bio-chemical ER]	Tamoxifen RT + Placebo RT + Tamoxifen	Does tamoxifen improve disease-free and over-all survival?

TABLE 2

NODE POSITIVE BREAST CANCER
ADJUVANT THERAPY TRIALS

STUDY	ELIGIBILITY	DESIGN	QUESTIONS BEING ASKED
CALGB 8541	$T_{1-3a}N,M_0$	CAF(300/30/300) x 4 CAF(600/60/600) x 4 CAF(400/40/400) x 6	Is there a relationship between outcome and amount of drug administered?
INT-0108	$T_{1-3a}N,M_0$ ER NEG	CAF CAF VML	Is there a complex, dose intense regimen superior to conventional CAF?
INT-0100	$T_{1-3a}N,M_0$ ER POS Postmenopausal	Tamoxifen CAF + Tamoxifen CAF -->Tamoxifen	Does chemotherapy add to Tamoxifen? Does concurrent tamoxifen alter efficacy of chemotherapy?
INT-0101	$T_{1-3a}N,M_0$ ER POS Premenopausal	CAF CAF --> Zoladex CAF --> Zoladex + Tamoxifen	Does endocrine therapy add to chemotherapy in this Group? Is "Complete Estrogen Blockade" superior to no endocrine therapy or medical castration alone?
NSABP B-18	$T_{1-3a}N_{0-1},M_0$	AC x 4 --> Surgery Surgery --> AC x 4	Is preoperative superior to postoperative chemotherapy?
NSABP B-22	$T_{1-3a}N,M_0$	AC 60 x 4/600 x 4 AC 60 x 4/1200 x 2 AC 60 x 4/1200 x 4	How do intensity and total dose of cyclophosphamide affect outcome?

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

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

Chemoprevention of breast cancer:

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

Cooperative Human Tissue Network:

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

Drug Development:

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

NEUROENDOCRINE TUMORS

A Phase III intergroup study will soon be opened, randomizing advanced islet cell patients between chlorozoctocin + doxorubicin versus streptozotocin + doxorubicin versus a Phase II drug. The initial Phase II drugs will be interferon gamma + interferon alpha. Relapse patients on the Phase III portion will be randomized to one of the interferons and patients on the Phase II portion will be randomized to one of the nitrosourea - anthracycline combinations. This will permit testing of new agents in untreated patients and provide some data on cross-resistance.

ADULT ACUTE LEUKEMIA

Accomplishments

A large intergroup study comparing alpha-IFN with 2'-deoxycoformycin for patients with hairy cell leukemia completed accrual much faster than projected and the data are currently under analysis.

Elderly patients with AML more often die from myelosuppression than resistant disease. CALGB and SWOG are each evaluating the use of G- or GM-CSF to reduce the chemotherapy-related myelosuppression in this patient population. Both of these Groups are also evaluating the use of hematopoietic growth factors in relapsed AML to "prime" the leukemia cells to be more responsive to chemotherapy.

ADULT MALIGNANT LYMPHOMA

Accomplishments

The SWOG is completing a large, 4-arm trial comparing four chemotherapy regimens for patients with previously untreated intermediate grade NHL. This study should help define the standard of care for these patients.

However, the availability of hematopoietic growth factors should permit an increase in dose intensity which may translate into higher response rates and more durable responses. CALGB has completed a Phase I study of a new CHOPE regimen without GM-CSF and is completing a Phase I study with the growth factor. They will soon begin a trial comparing the two programs. SWOG is developing an intense ProMACE/CytaBOM regimen in previously treated patients, ECOG a similar regimen in previously untreated patients.

BONE MARROW TRANSPLANTATION

Accomplishments

The recent availability of recombinant human hematopoietic growth factors permits the study of a number of important issues in bone marrow transplantation. Whether reducing the period of neutropenia improves response and survival is being evaluated in a number of CTEP-sponsored trials. The ability to generate sufficient numbers of peripheral blood stem cells appears to be enhanced by pretreatment with a growth factor and may make this approach a less expensive and more desirable option to autologous bone marrow transplantation, especially in patients with bone marrow involvement by tumor.

Using high-dose chemotherapy with combinations of alkylators requiring autologous marrow rescue, investigators from the Dana-Farber Cancer Institute have reported exciting response rates in heavily pre-treated patients. For example, 90% of women with metastatic breast cancer achieve a response, 10-20% of which are CR's. Trials will be conducted in women with high-risk breast cancer comparing standard chemotherapy with high dose chemotherapy requiring autologous bone marrow transplantation.

ABMT in Stage III breast. A multi-center trial is ongoing in CALGB to test high-dose, multi-agent chemotherapy with marrow transplant in women with stage III breast cancer. Traditionally most of these women relapse and die after conventional chemotherapy/and or radiation therapy with or without surgery. This protocol offers an innovative approach for those high risk patients.

ECOG, SWOG and CALGB are collaborating on an important prospective, randomized comparison of allogeneic BMT, ABMT, and conventional consolidation chemotherapy in patients with AML in first CR.

ADULT MALIGNANT LYMPHOMA

It is becoming increasingly apparent that there are a number of molecular and cytogenetic factors which may have important prognostic relevance in NHL. For example, the bcl-2 oncogene has been cloned from patients with follicular lymphomas. These molecular studies have, to date, only been evaluated in a limited number of cases. With current technologies, CALGB plans to perform molecular genetic and cytogenetic studies on larger numbers of patients, correlating these findings with response to treatment and survival. SWOG has recently established a lymphoma repository to provide samples for future studies as new probes and markers are developed.

MALIGNANT BRAIN TUMORS

Accomplishments

Interstitial versus External Beam Radiation Therapy for Gliomas--
Interstitial irradiation for malignant tumors in the brain has been administered widely throughout the country. From center to center the isotopes, surgical techniques, and dosimetry are highly variable. The BTOG currently has a Phase III comparison of external beam versus external beam plus interstitial irradiation for malignant gliomas which is more than 1/3 completed.

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

Future Plans

The current standard treatment of primary CNS lymphoma is surgery and/or radiation therapy. Several group studies are testing multiagent chemotherapy with radiation.

GASTROINTESTINAL CANCERS

Accomplishments

ESOPHAGEAL CANCER

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

COLORECTAL CANCER

1. NSABP C02 and NCCTG 79-46-04

Phase III adjuvant trials of postoperative, seven day, 5-FU portal vein infusion versus surgery alone have been completed in both NSABP AND NCCTG. The NCCTG trial was reported in May, 1989 as showing no benefit for adjuvant portal vein 5-FU. The NSABP trial reported in May, 1990 showed an improved survival among the portal vein infusion patients but no improvement in control of liver metastases, the presumed rationale for such therapy.

2. NSABP C03 and C04

C03, an adjuvant trial for colon cancer activated by the NSABP in 1987 employed the previous best chemotherapy arm of MOF (MeCCNU, Vincristine, 5-FU) randomized against chemotherapy with 5-FU/leucovorin. Very rapid accrual to this trial permitted closure with an 1800 patient sample in April, 1989. A replacement (C04) comparing 5-FU/leucovorin/levamisole versus 5-FU/leucovorin versus 5-FU/levamisole was activated in June, 1989 and also is accruing rapidly, now at 2.5 times its predicted rate.

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

The recently closed NCCTG rectal adjuvant trial employed combined radiation + chemotherapy and used a 2 x 2 factorial design. Its analysis will evaluate the contribution of MeCCNU to 5-FU for response and toxicity, compared to 5-FU alone. It will also evaluate the benefit of continuous infusion 5-FU during radiation therapy compared to intermittent bolus 5-FU. High priority status was assigned to this trial and stimulated accrual, such that the trial completed accrual 06-30-90. The replacement trial (INT-0114) is ready for activation. This trial will accrue 1200 patients to a comparison of pelvic irradiation plus one of four chemotherapy regimens, 5-FU, 5-FU/levamisole, 5-FU/leucovorin, or 5-FU/levamisole/leucovorin.

4. Intergroup Colon Adjuvant Trial (INT-0035)

Based on the NCCTG experience with the immune modulator, levamisole, a confirmatory intergroup Phase III trial in adjuvant treatment of colon cancer was started in 1985. Accrual of 1200 patients was accomplished in 2.5 years. The results confirmed a benefit for adjuvant 5-FU/levamisole in Dukes' C colon cancer. These positive results provide the basis for distribution of an NCI clinical update and for provision of levamisole by means of a Group C/Treatment IND protocol. Over 4,000 patients with Dukes' C colon cancer have been treated with 5-FU/levamisole using CTEP/NCI supplied drug.

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

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

6. EST 1290:

A trial of active specific immunotherapy has been started in ECOG, comparing 5-FU/levamisole with or without autologous tumor vaccine.

GASTRIC CANCER

An intergroup gastric adjuvant protocol has been reviewed and will be activated in 1990 testing 5-FU/leucovorin + radiation versus surgery alone.

LUNG CANCER

NON-SMALL CELL

Accomplishments

1. The CALGB reported early closure of a positive trial showing the survival benefit of induction Cisplatin and vinblastine before radiation for locally advanced but unresectable non-small cell lung cancer. (NSCLC). They are currently building on this study with several pilot trials investigating the relative efficacy of simultaneous chemo-radiation therapy and the role of carboplatin. The more promising pilot regimen will be compared to sequential chemo-radiation therapy in their next phase III study.

2. Based on the CALGB experience in locally advanced but unresectable non-small lung cancer, RTOG is currently conducting a confirmatory study in which a third question has been added. This will look at the effectiveness of hyperfractionated radiation therapy, which a previous RTOG study indicated prolonged survival in this patient subset.

3. The NCCTG has recently initiated a phase III adjuvant study in patients with completely resected stage III-A non-small lung cancer. These patients currently are at high risk of early relapse. This ambitious trial will investigate the role of radiation therapy, with or without chemotherapy.

4. Two Cooperative Groups are studying hydrazine in patients with metastatic disease. A prior study indicated that this drug improved survival by ameliorating cancer-associated cachexia. This hypothesis is being tested rigorously by the NCCTG and CALGB in two placebo controlled, randomized clinical trials.

Future Plans

1. On the basis of discussions held at a recent CTEP sponsored strategy meeting, ECOG, RTOG and CALGB plan to conduct an intergroup adjuvant study in patients with completely resected stage II, III-A disease. Combined chemo-radiation will be compared to radiation alone.

2. The RTOG is developing a neo-adjuvant trial in which chemotherapy--> surgery--> chemotherapy--> is compared to "standard treatment of chemotherapy--> RT--> chemotherapy for patients having locally advanced disease. This study will seek to define the role; for surgery in patients with marginally unresectable lung cancer following induction chemotherapy.

SMALL-CELL

Accomplishments

1. In limited stage small cell lung cancer ECOG has taken a regimen which they piloted incorporating Cisplatin VP-16, and concurrent accelerated-hyperfractionated radiation therapy into phase III testing. The median duration of survival has not been reached at 2 years in the limited institution pilot study, which compares favorably with previous results (10-16 month median survival). RTOG will also participate in the phase III trial.

2. ECOG recently published the results of a randomized trial which demonstrated no detrimental effect in survival for patients with extensive stage disease treated with an experimental Phase II agent using a "window of opportunity" approach, compared with those treated initially with a "standard" chemotherapy regimen. These results provided justification for testing promising new drugs, such as taxol, in a more optimal, responsive patient population, rather than testing these drugs in previously treated patients.

Future Plans

Several recently published studies have indicated that VP-16's activity can be enhanced when given orally for 21 days, even in patients who had previously relapsed on intravenous VP-16. This observation will be tested in an upcoming Phase III CALGB trial in which conventionally administered VP-16 will be compared to 21 day oral VP-16, with cisplatin

included in both arms.

HEAD AND NECK CANCER

Accomplishments

1. The head and neck intergroup trial compared postoperative radiation with postoperative radiation plus cisplatin-5-FU in the adjuvant setting (pathologically negative margins). The trial had encountered difficulties with patient compliance on the combined modality arm; however by changing the randomization to the postoperative setting, accrual increased substantially. Accrual goals should be reached by July of this year. When the data matures it will help define what role, if any, is played by adjuvant cytotoxic therapy in head and neck cancer.

2. The Head and Neck Intergroup has recently initiated a phase III trial in nasopharyngeal carcinoma comparing simultaneous Cisplatin/RT followed by maintenance Cisplatin/5-FU to radiation alone.

Future Plans

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

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

IV. CONTRACTS

Extramural Clinical Trials Office--EMMES

EMMES currently serves the operations and data management functions for the extramural IL-2/LAK cell trials and the suramin studies. The IL-2/LAK studies are being conducted at approximately 17 institutions which have accrued over 240 patients. The suramin studies are just being activated, related in part to difficulties in establishing the pharmacologic monitoring at each institution. There are now 2 centers participating, with additional institutions anticipated in the near future.

There are currently four Group C protocols which are actively accruing patients. EMMES was involved in developing the case study forms for several of these and serves the operations and data management functions for all of them. These protocols include levamisole for Stage III colon

cancer, pentostatin for hairy cell leukemia, VM-26/ara-C for relapsed acute lymphocytic leukemia, and fludarabine for refractory chronic lymphocytic leukemia. Accrual to the fludarabine study has been particularly active with greater than 600 cases to date. Over 5,000 patients have been entered on the levamisole study.

V. MANAGING INVESTIGATOR-INITIATED GRANTS AND CONTRACTS (R SERIES, PO1's, SBIR's

The purpose of the CTEP grants and contract management is to integrate relevant research information from all available sources, to disseminate the information contained in the grants and contracts to the Disease Coordinators of CIB and the Drug Monitors of IDB, and to serve as the primary contact for extramural investigators for administrative and scientific advice.

In FY 90, the CTEP managed 130 investigator-initiated funded grants. Grants are assigned to one of three areas within the Program: Clinical Oncology, Surgical Oncology, and Cancer and Nutrition. Clinical Oncology includes clinical studies designed to improve 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. An analysis of the grant expenditures in FY 90 is included in the following table.

CANCER THERAPY EVALUATION PROGRAM
DIVISION OF CANCER TREATMENT

GRANT EXPENDITURES FOR FY90 (ESTIMATED)

TYPE OF GRANT	NUMBER	TOTAL COST AWARDED*
Clinical Oncology (CO)		
Research Projects (R01)	56	9,712
Program Projects (P01)	29	44,394
Small Business Innovative Research (SBIR)	2	530
Small Grants (R03) **	12	750
Conference Grants (R13)	2	13
Merit Awards (R37)	4	496
First Awards (R29)	3	247
Outstanding Investigator Award (R35)	3	1,033
Subtotal	111	57,175
Surgical Oncology (SO)		
Research Projects (R01)	3	595
Program Projects (P01)	4	3,050
Small Business Innovative Research (SBIR)	2	498
Merit Awards (R37)	1	177
First Awards (R29)	1	90
Subtotal	11	4,410
Nutrition (NT)		
Research Projects (R01)	6	773
First Awards (R29)	2	173
Subtotal	8	946
TOTAL AWARDS	130	62,531

* $ Times 1,000
** Estimated for FY90

Most of the money in the grants/contracts pool was spent on P01 grants. CTEP manages one of the largest portfolios of P01 grants both in terms of dollars and numbers of grants within the NCI. During FY 90, the Program Directors attended 13 site visits for the review of P01's and performed 3 formal consultations on P01 submissions. During these formal consulting sessions the applicants bring drafts of their letters of intent, and the Grants Program Director along with other appropriate program staff (Disease Coordinators, Drug Monitors) give scientific as well as logistic

advice. In addition to these formal consultations, five additional consultations and numerous hours were spent on the telephone with both new and recompeting applicants. Approximately 50% of those P01 grants assigned to CTEP were funded, a relatively successful rate.

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's have become major studies in the 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.

CTEP continues to fund research project grants (R01) and FIRST awards (R29) at a level similar to last fiscal year. From 147 applications reviewed, 21 were funded for a rate of approximately 14%. With the declining funding rate in the research project grants (RPG) pool, CTEP is worried that clinicians will be discouraged from continuing in cancer research. It is difficult for clinical research to compete for funds against basic and preclinical studies in the study sections. CTEP is active in trying to increase the number of research grants and to attract applicants into specific areas which need development or are ready for clinical study through the issuance of Requests for Applications (RFA). Two RFAs were issued in FY 89 and have been funded this year. CTEP issued an RFA on "Therapeutic Correlates of Drug Resistance" which invited grant applications involving the correlation of drug resistance to clinical response and development of clinical treatment to overcome drug resistance. Recent basic research efforts have uncovered numerous molecular and cellular mechanisms that may be operative in resistance to chemotherapy. Thirty-two applications were received in response to this RFA and seven were funded at a total cost of $1,500,000. All of the successful applications combined the measurement of drug resistance in human tumors with clinical trials designed to overcome or reverse clinical drug resistance. They are listed below:

1. Dr. Charles Erlichman, Ontario Cancer Institute
   Modulation of Multidrug Resistance by Cyclosporin A

2. Dr. Charles A. Schiffer, University of Maryland
   Mechanisms of Drug Resistance in Acute Myeloid Leukemia

3. Dr. Ellin Berman, Memorial Hospital
   Effect of Tamoxifen on Reversing the MDR Phenotype

4. Dr. Marc Ernstoff, University of Pittsburgh
   Metallothionein & Human Tumor Resistance to Chemotherapy

5. Dr. Edward McClay, University of California, San Diego
   Modulation of Cisplatinum Resistance in Malignant Melanoma

6. Dr. Branimir Sikic, Stanford University
   Expression & Modulation of Multidrug Resistance

7. Dr. Robert Ozols, Fox Chase Cancer Center
   Detection and Reversal of Ovarian Cancer Drug Resistance

In collaboration with the Biological Response Modifiers Program, a RFA on studies of chronobiological effects in cancer treatment was issued and funded. By understanding the differences in the circadian dependence of the response of normal and tumor cells to therapeutic agents, antitumor effects may be optimized while toxicity to normal tissues may be minimized during cancer therapy.

CTEP has issued two new RFAs in FY 90. One of them involves the initiation of a new funding mechanism, the R03 small grants program. The other RFA involves AIDS-lymphoma. The R03 small grants RFA is entitled "DCT Small Grants to Stimulate Correlative Laboratory Studies and Innovative Clinical Trials". This RFA had 2 aims: (1) to provide a mechanism for accelerated funding of innovative correlative studies relevant to clinical trials and (2) to stimulate pilot clinical studies with novel laboratory correlations so as to foster the development of interactions between basic science laboratories and clinicians performing clinical trials. Over 170 letters of intent were submitted by potential applicants and a total of 162 applications were received and reviewed. The overwhelming response to this RFA indicates a tremendous need for such a funding mechanism. The total set aside of $750,000 will be spent on approximately twelve grants this fiscal year.

The AIDS-lymphoma RFA is a cross divisional effort involving DCE as well as DCBDC. This RFA entitled "Biologic and Therapeutic Insights into the Management of AIDS-lymphoma Patients" solicits applications that would bring laboratory research efforts to the clinic to attack the problem of management of AIDS-lymphoma patients. Proposals involving etiology, diagnosis and treatment of this disease will be funded.

CTEP Program Directors have been active in encouraging investigators to apply for minority research supplements to their grants. NCI, through its Comprehensive Minority Biomedical Program, provides support to minority scientists and students in order to influence a greater number of minority individuals to develop their research capabilities and pursue independent careers as cancer research investigators. Principal investigators were contacted about the program resulting in six applications for minority supplements (out of a possible 65 grants that qualified). Four applications have been approved at this time and CTEP hopes to fund at least three supplements.

SBIR grants and contracts continue to be an important component of the CTEP program. One phase I grant is funded at the present time and three SBIR grants have reached phase II. These include Information Analysis Corporation's grant on the creation of new software for a remote data entry system access for cancer clinical trials. A database on questionable cancer therapies that will be useful to insurance companies and may eventually become part of PDQ is being developed in another

grant. In addition, two software packages for cancer clinical trials are being developed. A SBIR contract topic on the development of new assays to measure drug resistance of human tumors was issued and received two contract proposals. One contract proposal received a high technical score and has been recommended for funding.

Highlights of Investigator Initiated Grants/Contracts

Several significant discoveries/leads with potentially important clinical applications/implications were made in FY90 by PI's who were supported by grants managed by CIB. They have been described below:

Dr. Charles Moertel, Mayo Foundation (5 P01 CA31224-06) has been extremely productive during the past year. A large confirmatory trial establishing 5FU and levamisole as an effective surgical adjuvant therapy has been published this year (NEJM 322:352, 1990). In patients with advanced colorectal cancer, a clear demonstration of improvement in survival, tumor response, and quality of life with leucovorin combined with 5FU compared to 5FU alone has been established (J Clin Oncol 7:1407, 1989). Dr. Moertel also completed a controlled evaluation of intra-arterial FUDR compared to systemic 5FU for the treatment of colorectal cancer metastatic to the liver. Significant improvement in objective tumor response rates and time-to-hepatic-progression were documented with intra-arterial FUDR.

Dr. Moertel's group has been active in analyzing large numbers of colorectal tumors for cytogenetic and molecular differences. A large group was analyzed for DNA ploidy demonstrating that DNA diploid patients have longer survival than non-diploid patients. When 101 colorectal tumors were analyzed for allelic loss, the percentage of allelic loss on chromosomes 5, 17, and 18 varied significantly between tumor sites (sigmoid colon and rectum showed greater amounts of allelic loss). K-ras mutations were analyzed in 170 patients and a number of mutations were found in both adenomas and carcinomas while no mutations were found in normal tissue form the same patient.

Dr. William Beck, St. Judes Children's Research Hospital (R01 CA30103-10) has characterized a form of MDR which may occur in significant numbers of leukemias that is not due to expression of the mdr1 gene, but rather appears due to an altered topoisomerase II mechanism (at-mdr). Dr. Beck has shown that nuclear matrix topo II activity is decreased by 6- to 8-fold in his at-mdr resistant cell lines (CEM/VM-1 and CEM/VM-1-5). Using cell fusion techniques, he has found that VM-26 resistance is expressed recessively (Cancer Res 49:2422, 1989). In addition, a mutation has been identified in the at-mdr cell lines that may account for changes in resistance.

Dr. Victor Ling, Ontario Cancer Institute (R01 CA37130-07) continues to work at the forefront in the field of mdr drug resistance. To improve immunological detection of p-glycoprotein (pgp), he has developed a number of monoclonal antibodies. His laboratory has characterized three pgp antibodies (C219, C494, C32) by high resolution epitope mapping using a series of 250 hexapeptides. His laboratory has developed sensitive immunohistochemical techniques for analysis of pgp expression in

formalin-fixed, paraffin-imbedded archival tumor specimens using C219 as primary antibody. In a retrospective study with Drs. Helen Chan and Paul Thorner, Hospital for Sick Children, pgp expression in pediatric sarcomas appears to be a significant prognostic marker of durable response. This group has shown in recent studies that cyclosporin A, a compound that has been shown previously to reverse MDR phenotype, can bind to pgp suggesting that the mechanism of reversal is an interaction with this drug efflux pump.

Dr. Emil Frei III (P01 CA38493) at Dana Farber Institute heads the Solid Tumor Autologous Marrow Program. In Project 1, Dr. Karen Antman has just completed a series of laboratory and clinical studies focused on developing an active preparative regimen for breast cancer. In an initial evaluation of a combination of cyclophosphamide, cisplatin, and carmustine, considerable activity in metastatic breast cancer was demonstrated but with substantial toxicity. In laboratory studies, simultaneous exposure to thiotepa and 4-HC was synergistic resulting in a Phase I study of high dose cyclophosphamide, thiotepa, and melphalan. Cyclophosphamide and thiotepa proved active with acceptable toxicity, but the addition of melphalan resulted in prolonged life threatening mucositis.

In Dr. Beverly Teicher's Project 4 (Preclinical Studies of Combined Alkylating Agents), Emt-6 murine mammary tumors were made resistant to CDDP, carboplatin, cyclophosphamide (CTX), or thiotepa in vivo by treatment of tumor bearing animals with the drug during a 6-month period. In spite of high levels of in vivo resistance, no significant resistance was observed when the cells from these tumors were exposed to the drugs in vitro. These results indicate that very high levels of resistance to anticancer drugs can develop through mechanisms that are expressed only in vivo (Science 247:1457 1990).

Dr. William Dalton, University of Arizona College (R29 CA43043-05) has been studying mechanisms of drug resistance in multiple myeloma. His laboratory has established that p-glycoprotein is an acquired form of resistance in multiple myeloma and occurs only rarely in newly diagnosed patients. Verapamil at high doses can reverse resistance and his preclinical and clinical studies have served as a basis for the design of a Cooperative Group study investigating the effectiveness of oral verapamil (Blood 74:53a, 1989). In addition, his laboratory has determined that resistance to melphalan in human myeloma cells is likely due to elevated levels of intracellular glutathione.

Dr. William Ensminger, University of Michigan (P01 CA42761) is examining the radiobiology of halogenated pyrimidines. His laboratory has found that the modulation of IUdR and BUdR incorporation into DNA occurs at low levels of exposure to FUdR and FU in colorectal cell lines and in vivo in the VX2 rabbit tumor. The modulatory abilities of FUdR and FU uniformly increase incorporation in "resistant" lines up to levels in "sensitive" lines. This implies that FUdR and FU modulation can assist in overcoming resistance to incorporation of the analogs in heterogeneous tumor cell populations. Preliminary data indicate that leucovorin is a potent sensitizer of FUdR mediated radiosensitization and that FUdR appears to decrease the repair of sublethal radiation damage.

Dr. Sydney Salmon, University of Arizona (P01 CA17094) has made significant progress in both preclinical therapeutic studies and clinical pharmacology studies. Dr. Alberts and his laboratory has recently published on the association of lysosomal activity with sensitivity and resistance to Tumor Necrosis Factor (TNF) in a murine L929 cell system. This study showed that specific lysosomal enzymes are increased in cells developed for resistance to TNF, while the opposite pattern is true for cells developed for resistance to doxorubicin or multidrug resistance (Cancer Res 49:2722-28, 1989). These results may explain the earlier observation that cells which develop chemotherapy-induced multidrug resistance may have enhanced sensitivity to biologic agents such as TNF.

Dr. Salmon's group has completed Phase I clinical trials with Sulfonylurea (LY186641) with definite evidence of antitumor activity in ovarian cancer patients. The major toxicities of this drug were methemoglobinemia and hemolytic anemia. Phase II trials have recently begun due to the high activity seen in the human tumor clonogenic assay (HTCA) against ovarian, colon and melanoma cells. Working together with Drs. Grogan and Dalton, Dr. Salmon recently published a study showing high correlation between p-glycoprotein expression and doxorubicin resistance with HTCA in myeloma, lymphoma, and breast cancer (JNCI 81:696-701, 1989).

Dr. William McGuire, University of Texas Health Science Center at San Antonio (P01 CA30195) has evaluated a panel of prognostic factors for human breast cancer. In studies of the HER-2/neu oncogene, expression was most prominent in the earliest detectable malignant lesions (duct carcinoma in situ) and progressively declined to low levels as the lesions evolved into mature invasive carcinomas, suggesting that HER-2/neu expression is an early event in malignant transformation. In node-negative cancer, HER-2/neu expression identifies a group of patients with a high recurrence rate who otherwise would be considered good risk (small, ER+ tumors) (J Clin Oncol 7:1120-28, 1989). Another prognostic factor, cathepsin-D overexpression, was associated with decreased disease-free and overall survival for patients with node-negative disease (N Engl J Med 322:297-302, 1990). The 323/A3 surface glycoprotein was examined for possible associations with tumor characteristics and behavior. 322/A3 was found to be related to increased recurrence ($p=0.003$) and mortality ($p=0.036$).

Dr. Hillard Seigler, Duke Medical School (5P01CA32672-07) and his colleague, Dr. Darryl Bigner reported progress in the use of intrathecal (IT) administration of monoclonal antibodies for clinical therapy of leptominigeal tumor dissemination. They have the only approved investigational new drug (IND) in the United States for IT therapeutic administration of radiolabeled MoAB fragments to patients with neoplastic meningitis. The development of this technique would represent a significant therapeutic achievement as current therapy is poor and leukoencephalopathy is often seen in long term survivors treated with external radiation and IT chemotherapy.

Dr. Emil Freireich, M. D. Anderson Cancer Center (5R35 CA39809-06) and his colleagues summarized their experience with fludarabine in the

treatment of chronic lymphocytic leukemia (CLL). Fludarabine was used to treat 68 patients with previously treated CLL. Nine (13%) patients achieved a complete remission an 30 (44%) a partial remission. The response to fludarabine was rapid, with 36 (92%) of the 39 responders having achieve at least a partial response following the first three courses. Survival was strongly correlated with the final Rai stage achieved. These data verified the previous encouraging report by Grever et al. on the use of fludarabine for the treatment of CLL.

Dr. **Donald Morton**, UCLA Medical School (5P01CA29605-09) has developed a new approach for treatment and management of patients with clinical stage I melanoma. His new surgical technique would involve intraoperative mapping of the regional lymphatic basin and identifying the "sentinel node(s)" in the lymphatic basin which drains the primary melanoma. He then performs selective lymphadenectomy of the sentinel node(s) to determine the presence or absence of micrometastasis. If micrometastasis are found in these sentinel lymph node(s) then a therapeutic lymph node dissection will be necessary. If micrometastasis are not found in these sentinel lymph node(s) the patient will be spared from additional unnecessary lymph node dissections. Using this new surgical approach he has shown that 47 (18.1%) of the 259 sentinel nodes dissected contained metastases while only 15 (0.5%) of the 3,079 non-sentinel nodes had metastases, a 36-fold difference in frequency. Success in identifying the sentinel nodes varied with different surgeons. The surgeon with the most experience with this technique identified the sentinel nodes in 96% of the cases whereas the surgeon with the least experience identified the sentinel nodes in only 61% of the cases. Dr. Morton will be teaching this new surgical technique to other surgeons in the USA so that intraoperative mapping of the regional lymphatics and selective lymphadenectomy become a standard step in the protocol to treat patients with clinical stage I disease. Furthermore, he has initiated a new multicenter trial composed of 7 institutions to validate his findings.

Dr. **Joseph Bertino**, Sloan-Kettering Institute for Cancer Research (1P01CA47997-01) attempted to improve the response rate and the number of complete responses in patients with colorectal carcinoma metastatic to the liver. Twenty four patients were entered into a trial using intrahepatic infusion of FUdR +/- leucovorin. The response rate for the FUdR + leucovorin arm was 72% vs 50% with FUdR alone. No complete responders were observed and hepatic toxicity increased with the use of leucovorin. Continued follow up of these patients have revealed an excellent survival; the median survival has not been reached but it will be greater than 27 months versus a median survival of 17 months for FUdR. Dr. Bertino is currently trying to reduce the hepatic toxicity of this regimen.

Dr. **Lawrence Einhorn**, Indiana University School of Medicine (5R35CA39844-07) addressed the issue of cisplatin-induced nausea and vomiting. He conducted a phase III randomized double-blind study in 45 chemotherapy-naive testicular cancer patients receiving cisplatin plus VP-16 plus bleomycin. Antiemetic results with ondansetron (a selective 5-HT3 receptor antagonist) were compared to metoclopramide. Ondansetron provided similar antiemetic protection to that achieved in the prior phase II study and was statistically superior to metoclopramide producing

fewer side effects as well. Sedation and extrapyramidal side effects common with other antiemetic regimens occurred rarely with ondansetron.

Dr. John Kersey, University of Minnesota Medical School (1R35CA49721-01) has continued to study the ontogeny of rearrangement and expression of T cell receptor genes and immunoglobulin genes in leukemias. These studies have demonstrated a very high incidence of T cell receptor delta gene recombinational events in leukemias; 91% of acute T-cell lymphoblastic leukemias and 68% of non T, non B lymphoid precursor ALL and 80% of mixed lineage leukemias were shown to have rearranged T cell receptor delta. Mature B lineage leukemias and non lymphocytic leukemias retained delta gene in germline. The evidence also suggests that T cell receptor delta precedes T cell receptor gamma in lymphoid ontogeny. In lymphoid precursor ALL most of the rearrangements represented DD or VD rearrangements of the T cell receptor delta locus. V delta 3, D delta 2, J delta 1 rearrangements are found to predominate. Using PCR technology, Dr. Kersey has been able to demonstrate the heterogeneity of these predominant rearrangements in leukemias.

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 FY88 and FY89 annual reports 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 is sponsoring 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.

Conflict of Interest and Scientific Fraud Investigations:

CTEP program staff served on various committees to investigate conflict of interest and scientific fraud. The Head of the Quality and Assurance Section, DCT was detailed to the Office of the Director, NIH to participate in the formation of the Office of Scientific Integrity.

Grantsmanship Seminars/Workshops:

The Grants Program Director chaired and other CTEP and NIH program staff participated in a session at the 3rd International Congress of the Society of Chinese Bioscientist in America on NIH grants and contracts. The aim of this session was to offer a didactic explanation of the general organization, process and functions of the grant and contract processes.

V. FOREIGN INTERACTIONS

1. Dr. Ungerleider is a member of the International Society of Pediatrics (SIOP) and serves on that organization's subcommittee on treatment-related toxicities. In this capacity, he provides the Society with information collected by CTEP's Regulatory Affairs Branch regarding unexpected toxicities of anticancer agents used in children, for dissemination to the members of the Society, both in the USA and abroad.

2. US-USSR: CIB has developed with the Clinical Oncology Program, DCT a clinical trial in testicular cancer in the All University Cancer Center in Moscow. The trial compares cisplatin/VP-16 with CBDCA/VP-16 in limited stage testicular cancer. Dr. Hamilton completed a two month visit to cancer centers in the U.S.S.R. in early 1990. At that time, additional clinical trials were initiated, including an evaluation of Ftorafur plus leucovorin for adjuvant therapy of colon cancer. Additional exchange of U.S. and Soviet scientists were discussed, resulting immediately in a visit by Dr. V. Gorbunova, who spent a month at CALGB Headquarters and a month at CTEP studying U.S. Cooperative Group clinical trials organization.

3. US-India: CIB is coordinating development of an esophageal cancer trial with two cancer centers in India.

4. US-Japan: Dr. Friedman is the coordinator for the treatment area of this important scientific agreement for the exchange of research information.

5. US-Hungary: Dr. Friedman is the coordinator for this joint scientific agreement.

6. European Organization for the Treatment of Cancer (EORTC): Dr. Cheson is the liaison between the EORTC and CIB for clinical protocols.

7. National Cancer Institute of Canada (NCIC): Several CIB staff interact with Canadian investigators; Dr. Dorr is the official CIB liaison.

8. US-France: Dr. Cheson is the liaison between CIB and these investigators.

9. World Health Organization (WHO)/European Organization for the Treatment of Cancer (EORTC) Melanoma Activities: Dr. Dorr coordinates these activities with CIB.

10. US-Italy: Dr. Ungerleider is the liaison between CIB and Italian investigators.

VI. ANTICIPATED ACTIVITIES (FY 91) FOR THE CLINICAL INVESTIGATIONS BRANCH

The challenge for the upcoming year will be to maintain CIB's current activities while expanding into new areas during a period of serious fiscal constraints. Attention will be paid toward increasing the participation of minority patients in the clinical trials process, improving upon the striking increased cancer mortality seen among Blacks, native Americans, native Hawaiians and other minorities; new clinical trials will be sought in those diseases with a disproportionate incidence and mortality in minorities (e.g., multiple myeloma, esophageal carcinoma); inappropriate obstacles to the participation of the elderly in clinical trials will be removed and the accrual of elderly patients will be encouraged; the Cooperative Groups will be encouraged to become a resource for the entire National Cancer Institute for the conduct of trials in cancer prevention, etiology, and biology; and a Five-Year Plan for the Clinical Trials Cooperative Groups will be developed.

STAFF PUBLICATIONS:

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INVESTIGATIONAL DRUG BRANCH

DESCRIPTION

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

The Investigational Drug Branch is divided into three sections: two medical sections, one for cytotoxic agents and one for biologic response modifiers, which are concerned with the clinical aspects of the drug development process, and the Drug Management and Authorization Section, which regulates the distribution of investigational new drugs to all NCI-sponsored investigators. The professional staff of the Branch includes 10 physicians, 5 pharmacists, 1 Ph.D. and 1 registered nurse.

BIOLOGICS EVALUATION SECTION

IMMUNOREGULATORY CYTOKINES

INTERLEUKIN-2 (IL-2)

Phase II studies of IL-2 administered as a single agent by continuous infusion to patients with lymphoma, melanoma, colon, pancreas, kidney, breast, ovarian, and lung cancer continued to accrue patients. Accrual was slow secondary to the toxicity of the regimen and the requirement for inpatient administration, which caused closure of the lung studies prior to their completion. A Phase II study in patients with head and neck cancer was also initiated.

In addition, IL-2 was combined with a number of biologic and cytotoxic agents. Preliminary results from the Surgery Branch, NCI indicated the combination of high dose IL-2 and interferon-alpha may have response rates of 30-35% or higher in patients with renal cell carcinoma and melanoma. Therefore randomized trials of high dose IL-2 versus IL-2/interferon-alpha were started by the IL-2 Extramural Working Group (ILWG). An outpatient regimen of IL-2/interferon alpha using subcutaneous administration was also developed, and will be tested in Phase II trials to determine if it has a similar level of activity with less toxicity.

Phase II trials of IL-2 in combination with tumor necrosis factor were initiated in melanoma, lung cancer, and renal cell carcinoma. These trials will use concurrent administration since preliminary results using a sequential schedule were not promising. Phase II studies of an outpatient IL-2/interferon gamma combination will be started in renal cell carcinoma and melanoma.

Several trials of IL-2 (plus/minus LAK cells) in combination with monoclonal antibodies were also opened. Monoclonal antibodies being utilized in these initial trials were directed against colon cancer (L6, 17-1A), melanoma (R24), and lymphoma (LYM-1). A monoclonal antibody which non-specifically activates T cells (anti-CD3) was also combined with IL-2 in a Phase I trial.

Several Phase I trials focused on the combination of IL-2 with cytotoxic agents such as doxorubicin, cisplatin, or cyclophosphamide. An alternating regimen of high dose cisplatin sequenced with high

dose IL-2 produced responses in 40% of melanoma patients. Patients responding to both agents appeared to have the most objective benefit from treatment as measured by durability of response. Other trials sequencing combination chemotherapy with IL-2 were initiated in small cell lung cancer and melanoma. In addition, IL-2 is currently being tested in three trials with high dose chemotherapy. One trial alternates IL-2 with Thiotepa/ABMT for patients with melanoma. A second study uses IL-2 before and after high dose cisplatin, cyclophosphamide, and etoposide to test for improved immunologic and hematologic recovery following chemotherapy. Finally IL-2 is being used to activate tumor killer cells in marrow used to rescue hematopoietic function in patients receiving marrow-ablative chemotherapy. A trial of IL-2 in patients with AML in second remission is ongoing. This is the only current NCI-sponsored use of IL-2 in the adjuvant treatment of acute leukemia.

Regional administration of IL-2 remains an interesting area of investigation. Local administration of IL-2 to a head and neck cancer patient in a Phase I study produced a partial response at a relatively low dose of IL-2. A Phase II trial of IL-2 (and LAK cells) administered directly intracavitary to brain tumor patients is planned, after preliminary survival data in the Phase I study appeared superior to historical controls. Following reports of anti-tumor activity using intraperitoneal IL-2 and IL-2/LAK in ovarian carcinoma patients this strategy is being pursued in a Phase II. Responses have been noted in a Phase I trial of intravesical IL-2 for patients with bladder cancer, and a similar trial using direct intralesional IL-2 administration of local bladder recurrence is accruing patients.

IL-4

The NCI launched its initial studies of the immunoregulatory cytokine IL-4 this year. Based on laboratory studies, IL-4 was added in vitro to IL-2/tumor infiltrating lymphocytes (TIL) cell cultures for the purpose of enhancing the generation of tumor-specific cytotoxic cells. Phase I studies of systemically administered IL-4 were also initiated in the Surgery Branch, NCI. An MTD was established using thrice daily administration and evidence of anti-tumor activity was seen. Formal Phase II trials are being conducted in patients with melanoma and renal cell carcinoma by the IL-2 Extramural Working Group. Based on in vitro evidence of synergy when combined with IL-2, Phase I studies of IL-4/IL-2 are ongoing in the Surgery Branch and in several extramural sites. Phase II studies and comparisons to IL-2 alone will follow.

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

The development of these molecules is in an early phase. Trials to define the toxicity of both types of IL-1 are underway. Based on data already gleaned from these efforts, studies have been designed to evaluate the effects of these agents to protect against the toxicity of high-dose chemotherapy and also to assess the anti-tumor activity of interleukin-1 against melanoma following intravenous or intralesional administration.

TUMOR NECROSIS FACTOR (TNF)

Phase II trials designed to define the anti-tumor activity of TNF when given as a single agent are generally complete or nearing completion. Though the effectiveness of TNF when given alone has been limited, promising leads for benefit when TNF is given together with topoisomerase II inhibitors (e.g., VP-16 and actinomycin-D) are being pursued. Recent data suggesting synergy between TNF and the topoisomerase I inhibitor, hycamptamine, have stimulated interest in the clinical evaluation of the concurrent administration of these agents. Trials to examine the combined activity of TNF when administered with other cytokines such as interleukin-2 and interferon-alpha have also recently been initiated. An important role for TNF could also be as a protector against the toxicity of radiation therapy or chemotherapy. Studies to evaluate these actions are currently being formulated.

COLONY STIMULATING FACTORS

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

A great deal of information regarding the toxicity and effectiveness of these agents when used to reduce the side effects of chemotherapy or to allow higher doses of chemotherapy has now been developed. Both drugs appear to reduce the duration of neutropenia following chemotherapy, and as a result may reduce the frequency of neutropenic sepsis. Little effect to counter the chemotherapy-induced thrombocytopenia has been observed with G- or GM-CSF. However, newer agents such as IL-1, IL-3 or IL-6, when used in conjunction with G or GM-CSF may provide protection against thrombocytopenia. Preliminary information has also implied that the CSF's might reduce mucositis and diarrhea which occur after some forms of chemotherapy. Clinical trials to definitively evaluate the true ability of the growth factors to moderate these toxicities are being designed.

Administration of CSFs following chemotherapy has permitted in increased dose intensity with certain single agents, such as cyclophosphamide and adriamycin, and with combination 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 at shorter intervals. However, the use of G or GM-CSF alone did not result in increased dose intensity for other chemotherapeutic agents such as thiotepa, melphalan and carboplatin. These data are important because they also point to the need to develop combination protective regimens involving the CSF's and IL-1, IL-3, and IL-6 as well as TNF and non-biological myeloprotective agents like WR-2721.

In addition to raising white blood counts, G-CSF and GM-CSF appear to stimulate the release of stem cells into the peripheral blood. Peripheral blood stem cell harvest may offer an outpatient alternative to bone marrow transplantation following 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 to reconstitute the bone marrow. Use of the growth factors may also allow ex vivo expansion of stem cell populations which would permit reinfusion of greater numbers of cells.

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

Beyond their use in increasing white blood cell numbers the CSF's may have important utility as activators of macrophages and neutrophils. Studies to examine the ability of the growth factors to facilitate therapy of infection in cancer patients have been proposed and are likely to be approved shortly. The use of prophylactic antibiotic treatment in concert with CSF therapy is also a subject for further scrutiny. Protocols have been approved to study the ability of these factors to augment the anti-tumor activities of macrophages and neutrophils, especially 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 to increase the number of early bone marrow progenitor cells. Trials to better define the side effects of this important molecule and to evaluate its ability to protect against chemotherapy-

induced myelosuppression are being planned and should begin shortly. Particularly important will be a determination of how best to use IL-3 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 enhancing peripheral blood progenitor cell collection, and treating myelodysplasia and leukemia. Protocols to evaluate these uses based on pre-clinical information and the experience acquired with the better studied colony stimulating factors are now being formulated.

INTERFERONS

INTERFERONS (INTERFERON-ALPHA AND INTERFERON-GAMMA)

Interferon-alpha is licensed in this country as treatment for hairy cell leukemia and Kaposi's sarcoma. Clinical research investigations in a wide variety of other cancers are also being pursued; these include chronic myelogenous leukemia (CML), renal cell cancer and malignant melanoma. Pre-clinical studies have also indicated that interferon-alpha may synergize with certain biologicals, most notably the anti-tumor activity of IL-2. Phase I studies have documented the anti-tumor activity of the combination of IL-2 and interferon-alpha in renal cell carcinoma and malignant melanoma; multiple trials exploring this combination in other disease states and different administration regimens are ongoing. Interferon also demonstrates some synergistic activity when combined with certain cytotoxic chemotherapeutic agents, most notably 5FU. Recent trials have indicated that 5FU plus interferon-alpha has substantial activity in the advanced colon cancer setting. Additional studies in other malignancies and/or utilizing other administration schemes for this combination are being explored.

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

ACTIVATED CELLS

ADOPTIVE IMMUNOTHERAPY

LAK cell studies are currently focused on combinations with monoclonal antibodies. Phase I and II trials with other non-specific, and perhaps more potent, tumor cytotoxic cells, such as expanded LAK cells, adherent LAK cells, or anti-CD3 activated cells are ongoing or in the process of initiation. The Modified Group C therapy protocols utilizing LAK cells administered together with IL-2 will be discontinued since alternative IL-2 regimens are now under study.

Studies with TIL continue in the Surgery Branch, NCI. After the initial promising results noted in patients with metastatic melanoma, these investigators have initiated trials with gene-transfected effector cells. TIL cell adoptive immunotherapy studies are currently ongoing in extramural sites both in melanoma and renal cell carcinoma, with studies planned in breast cancer and ovarian cancer. Alternative methods of generating TIL cells, including in vivo immunization followed by harvest and in vitro expansion of sensitized cells, are under investigation.

ANTIGEN-DIRECTED THERAPIES

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

UNCONJUGATED MONOCLONAL ANTIBODIES

The binding characteristics, biological activities, and pharmacokinetics of murine antibodies to a wide range of hematopoietic and solid tumor-associated antigens have been studied over the last several years. Some of the most interesting of these antibodies have been chimerized, and these chimeric antibodies, with their promise of longer circulating half-lives, reduced immunogenicity, and increased immunobiological activity, are now being introduced into NCI-sponsored trials. Phase I trials have begun with one of these chimerics directed against the TAG-72 adenocarcinoma-associated antigen. At the same time, trials investigating the combination of monoclonal antibodies together with immunostimulatory cytokines, including IL-2, interferons, GM-CSF, and M-CSF, have been initiated.

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

RADIOLABELLED MONOCLONAL ANTIBODIES

Through a series of NCI-sponsored contracts, clinical trials are under way to establish optimal means of utilizing radioimmunoconjugates in therapy directed against adenocarcinomas. The antibody targets initially being studied include both the TAG-72 and CEA antigens; radiolocalization and pharmacokinetic studies will compare the characteristics of radioiodine-labelled murine antibodies of different affinities, of antibody fragments as compared to whole antibody, and of chimeric antibody in relation to the parent murine antibody. The maximally-tolerated doses of these immunoconjugates administered as single doses, or alternatively in multiple dose fractions, are being determined. In other trials the relative characteristics of alternative isotopes, including Yttrium-90, are under study.

Trials conducted over the last year have demonstrated that the target antigen TAG-72 can be upregulated by therapy with interferon gamma. The combined strategies of: (i) utilizing complementary antibodies which each recognize a subset of malignant cells within an adenocarcinoma but which together recognize all of the cells in a tumor; (ii) increasing antigenic density with concomitant interferon treatment; (iii) using improved chelation chemistry and isotopes with superior radiotherapeutic characteristics; (iv) development of less antigenic antibodies of higher affinities; and (v) fractionated dosage schedules supported by hematopoietic growth factor and/or transfused stem cell support, may allow the development of active radioimmunotherapy against human solid tumors. All of these approaches are now either planned or under active clinical investigation in NCI-sponsored trials.

IMMUNOTOXINS

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

NON-SPECIFIC IMMUNOSTIMULANTS

LEVAMISOLE

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

DIFFERENTIATING AGENTS

TRANS-RETINOIC ACID

Trans retinoic acid has recently been shown in separate Chinese and French trials to have activity in the treatment of acute promyelocytic leukemia (APL). The National Cancer Institute will investigate tRA in a new oral formulation. Phase I trials to determine the maximum tolerated dose of tRA (alone and in combination with other differentiating agents) are now underway. An Intergroup confirmatory Phase II trial of tRA activity in APL is currently in the design phase; a Special Exception Protocol for American APL patients requiring tRA (while the Phase I evaluations of this agent are being completed) is currently being implemented.

COMBINATIONS OF BIOLOGICALS AND CYTOTOXICS

FLAVONE ACETIC ACID

In vitro studies conducted at the BRMP have shown that alkalinization abrogates the anti-tumor activity of this compound. Preliminary results also indicate that stimulation of cytokine production by human PBL requires higher concentrations than that which induces cytokine production by murine cells. Therefore the BRMP has shortened the infusion duration to one hour and eliminated concurrent alkalinization in its ongoing trial of FAA/IL-2. Immune parameters are being monitored to determine if this change in schedule can produce the marked immunologic and anti-tumor effects seen in animals.

VACCINES

The Phase III trial of autologous tumor vaccine in patients with colorectal cancer was halted temporarily when 5FU/levamisole was found to be effective treatment for Dukes' C patients, and the no-treatment control arm in this subgroup was no longer considered ethical. Since accrual of Dukes' C patients was almost complete, the study was closed to this subgroup and continued accruing Dukes' B2 patients. A replacement study for the Dukes' C patients was proposed comparing 5FU/levamisole to 5FU/levamisole in combination with vaccine.

In separate studies, autologous tumor vaccines (designed to elicit immune responses to host tumor in patients with renal cell carcinoma and melanoma) have been combined with IL-2, an agent capable of expanding and intensifying the host anti-tumor immune response. These preliminary trials, which have laboratory endpoints as a primary goal, will be expanded to Phase II studies in patients with advanced disease.

DRUG MANAGEMENT AND AUTHORIZATION SECTION

GROUP C DRUGS

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

The current drugs in Group C status are Amsacrine in refractory AML, Azacytidine in refractory AML, Pentostatin in Hairy Cell Leukemia, Erwinia Asparaginase in ALL for patients sensitive to E. Coli Asparaginase, Hexamethylmelamine in refractory Ovarian Carcinoma, Levamisole in adjuvant Dukes' C Colon Carcinoma, Teniposide in refractory ALL and Fludarabine in refractory CLL.

This year extensive section resources were directed toward registering physicians and patients for the use of Levamisole in Dukes' C Colon Cancer and Fludarabine in refractory CLL. Approximately 1,500 physicians and more than 4,400 patients have been entered into the Levamisole Group C Protocol and 7,090 bottles of drug were shipped. Additionally, more than 1,400 patient were entered on Special Exception (see below) protocols for Levamisole and 600 patients were determined to be ineligible for treatment. Eight hundred and five patients have been entered into the Fludarabine protocol and approximately 10,000 vials of drug have been shipped. Numerous additional patients were entered on Fludarabine Special Exception protocols for such indications as refractory lymphoma and prolymphocytic leukemia.

It is worthy to note some of the unique features of each of these drugs that impact on effective management procedures. For example, Levamisole's adjuvant therapy requires that treatment of the patient be started soon after surgery, preferably within 30 days. This therefore requires prompt patient registration. The entry criteria for the Levamisole protocol are specific but relatively few in number, hence a patient's eligibility can be determined quickly and drug provided to physicians for treatment. With Fludarabine, in contrast, treatment of the patient does not have to begin as soon as Levamisole. However, the entry criteria is more extensive and thus, often requires further laboratory tests and subsequent return phone calls by the registering physician. Gathering and reporting this additional entry data can take several days.

A typical Group C registration involves the investigator first calling the DMAS to discuss a patient's case with a pharmacist. Attempts are made to refer the patient to an existing clinical trial, however if this is not possible and the patient qualifies for the Group C protocol, the entry request is approved. If the physician is not already actively receiving drugs from NCI, an FDA 1572 form (Statement of Investigator) must be completed and returned. A patient registration letter, a copy of the Group C protocol and an adverse drug reaction reporting form are sent. The quantity of the initial drug shipment is also calculated and drug is shipped. Shipment records are maintained and reorder requests are considered as long as the investigator remains in active status and in good compliance.

During this year's American Society for Clinical Oncology Annual Meeting the DMAS pharmacists had an exhibit explaining the procedures for obtaining Group C drugs. The exhibit was visited by a large number of the physicians attending the meeting many of whom completed a survey about their experiences with the Group C procedure. The DMAS processed 314 requests for copies of the Group C protocols. The DMAS's first Group C exhibit was a success.

Over the past year, for all Group C drugs, nearly 5,200 new patients were approved to receive treatment and nearly 3,600 drug reorders were honored by the DMAS.

SPECIAL EXCEPTION PROTOCOLS

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

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

In the past year the DMAS registered numerous Special Exception patients for Levamisole and Fludarabine. This treatment mechanism was used for Levamisole in instances when patients exceeded 30 days but less than about 60 days from colon surgery. Fludarabine Special Exceptions were used when patients were diagnosed with diseases other than CLL for which there is data to support treatment, most commonly in refractory lymphoma and prolymphocytic leukemia.

Over the past year more than 2,400 Special Exception protocols were approved and nearly 1,900 requests for Special Exception reorders were honored by the DMAS.

NEW COMPUTER SYSTEM DEVELOPMENT

The DMAS has embarked on the first major redesign of their computer system in 15 years. A thorough systems analysis was conducted by the DMAS computer support contractor and the findings were presented to and approved by the CTEP Computer Committee. A modification was written for the support contract, a Delegated Procurement Authority was obtained from the Department, funds were allocated for equipment purchase and staffing and the DMAS began a

lengthy detailed self scrutiny of its computer needs. The DMAS is developing a Local Area Network using a relational data base management system, ORACLE, which is compatible with a somewhat similar system being designed for CTEP's Protocol Office. The redesign of the DMAS Drug Computer System (DDCS) began in October, 1989 and the LAN is scheduled to be in place at Executive Plaza by August 1990.

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 90 institutions authorized to order drugs using the ECDR and more are being added. The system has been extended to include international locations in Canada and Brussels, Belgium (EORTC). The system has simplified the drug ordering procedure and has reduced overall drug distribution time from weeks to days. It has been well received and has helped to minimize the need for investigators to maintain large drug inventories and it has thus helped reduce drug costs to NCI.

ENHANCED PROTOCOL REVIEW PARTICIPATION

The DMAS has expanded it involvement with the CTEP Protocol Review Committee. For several years it has been providing weekly drug cost estimates for all protocols being considered by the committee to assist in determining the drug cost consequences of proposed treatment regimens. This drug cost activity continues.

In the past year the section has taken on an additional responsibility of reviewing the drug information, treatment plan, dose modifications and drug supply sections of each protocol and advising the committee, and subsequently the study chairmen, of the findings. This has helped to maintain consistency and accuracy within each protocol and between protocols. This effort has proven to a significant contribution to protocol review.

INVESTIGATOR REGISTRATION

The administration of the primary investigator registrations and annual reregistrations continues to be an important function of the DMAS. There are currently 7,279 clinical investigators registered to receive investigational drugs and compliance with registration requirements is 100%.

DRUG COST EXPENDITURES

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

Drug Distribution Data for the Past Year

Number of Drug Orders (Line Items)	*New Group C Orders (Reorders)*	*New Special Exception Protocols (Reorders)*	*Total Containers (Vials, Ampules, Btls) Distributed*
24,043 (34,086)	*5,173 (3,592)*	*2,417 (1,856)*	*1,046,478*

DEVELOPMENTAL CHEMOTHERAPY SECTION

TOPOISOMERASE I INHIBITORS

HYCAMPTAMINE

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

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 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. Additional preclinical data suggest that fostriecin enters cells by the reduced folate carrier 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. Clinical trials will begin in the near future, as soon as formulated drug is available.

TENIPOSIDE

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol has been approved by the FDA for VM-26 in combination with Ara-C for the treatment of patients with refractory or first relapse acute lymphoblastic leukemia. Two confirmatory trials in small cell lung cancer have been initiated based on data from the Finsen Institute which demonstrated extraordinary single agent activity of VM-26 in small cell lung cancer (J Clin Oncol 4:524, 1986). These trials are ongoing and are too early for meaningful data interpretation.

MERBARONE

The precise mechanism of merbarone cytotoxicity is unknown, although some preclinical data suggest that it functions as a novel topoisomerase II inhibitor. Preclinical data indicated that efficacy was best using an intermittent infusion schedule. Two Phase I studies were conducted to evaluate a 5-day continuous infusion schedule. Because of severe phlebitis, the drug could not be administered via peripheral vein and administration via a central venous catheter is required. To further assess the problem, both trials also evaluated the feasibility of a daily 2 hour infusion for 5 days. Although this was also a tolerable regimen the maximum dose delivered was less than half the dose which could be delivered as a continuous infusion. A broad phase II screening program is planned utilizing the 5-day continuous intravenous regimen.

MITOTIC SPINDLE TOXINS

TAXOL

This unique natural product derived from the bark of Taxus brevifolia has shown promising antitumor activity. Results of a phase II study reported in the Annals of Internal Medicine (August 1989) indicated that 30% of women with relapsed ovarian cancer who had been treated with multiple regimens (radiation, chemotherapy) responded to taxol. Responses, including complete responses, were seen in women who were refractory to conventional therapy. Two additional phase II trials in ovarian cancer were recently completed and preliminary results confirm activity of taxol in this disease site. To pursue this activity, a Phase III comparison of standard therapy (cytoxan + cisplatin) against the combination of taxol + cisplatin is underway in newly diagnosed patients.

Although a broad Phase II evaluation of taxol in most diseases is planned, this is being implemented in phases as the drug becomes available. Current disease sites include common malignancies and those diseases in which taxol demonstrated activity preclinically and in Phase I clinical trials. Combination studies with growth factors are also being conducted to determine whether the single agent dose of taxol can be intensified.

INTERCALATORS

PIROXANTRONE (OXANTRAZOLE)

Piroxantrone is one of a new class of intercalating agents, the anthrapyrazoles. Of the three most active agents in this class developed by Warner-Lambert, piroxantrone is the one currently in development through the NCI. Piroxantrone was the first agent to undergo development utilizing pharmacologically guided dose escalations according to the Blood Level Working Group method. It was estimated that 9-12 fewer patients were required for the phase I evaluation. The regimen currently being evaluated in broad Phase II testing is 150mg/m^2 IV bolus every 3 weeks with potential for dose escalation.

LIPOSOMAL DOXORUBICIN

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

Retrospective analysis of dose-response data from doxorubicin clinical trials suggests that increasing the dose, while limiting the cardiotoxicity, may substantially improve the efficacy of this very useful agent.

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. This agent has been selected for evaluation using a pharmacologically guided dose escalation scheme in Phase I. Once IND submission has been reviewed and accepted by the FDA, Phase I trials will ensue.

REVERSAL OF MULTIDRUG RESISTANCE

Overexpression of p-glycoprotein, the product of the multidrug resistance gene, mdr-I, 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, which specifically and competitively bind to p-glycoprotein, have successfully reversed multidrug resistance in vitro. The evaluation of these agents in clinical trials is planned.

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.

10-EdAM

Data suggests that, while a DHFR inhibitor like MTX, EDAM has more efficient intracellular transport and concentrates in tumor > normal tissue; undergoes more extensive polyglutamylation; is more active than MTX against a number of murine tumors and human tumor xenografts; is clinically active in some tumors where MTX is inactive such as NSCLC, and shows preclinical in vivo synergy with alkylators. A broad Phase II evaluation of EdAM is underway and a comprehensive development plan has been designed, including early evaluation of leucovorin rescue, and combination studies based on preclinical synergy has been discussed.

FAZARABINE (Ara-AC)

Phase II studies in patients with solid tumors have been opened. One Phase II study, in patients with head and neck cancer, demonstrated no responses and has been closed. A Phase I study in patients with leukemia is ongoing.

TIAZOFURIN (TCAR)

Investigators at Indiana University have obtained responses with TCAR using a 15-day schedule in patients in the myeloid blast crisis phase of chronic myelogenous leukemia. At the AACR (196:1166) meeting in May 1990, they reported a complete response in five of nine evaluable

patients with one patient developing tumor lysis syndrome. Three others reverted to the chronic phase of CML. A good correlation between biochemical parameters and hematologic effect was seen and it may be possible to predict responders by evaluating the sensitivity of their peripheral leukocytes to TCAR in vitro. An identical trial is ongoing at M.D. Anderson in an effort to confirm this activity.Allopurinol was required to prevent hyperuricemia. However, substantial toxicity, including fatal pericardial tamponade, seizures and severe hypertension has been encountered.

TRIMETREXATE (TMTX)

A broad Phase II evaluation of the daily x 5 bolus schedule has failed to identify clinically significant activity for this antifol. However, some of the pharmacokinetic data suggests a shorter half life for TMTX than previously thought. The preclinical activity appeared to be strongly schedule dependent. Therefore, a trial of continuous infusion TMTX was initiated. As the most sensitive histology in the bolus studies was soft tissue sarcoma, this single-agent Phase I study will be confined to these patients.

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 has been filed.

FLUDARABINE PHOSPHATE

This agent is the halogenated phosphate derivative of vidarabine, which has the advantage of resistance to deamination by adenosine 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 the potential for severe neurotoxicity, recent clinical investigations revealed significant activity against lymphoproliferative malignancies particularly chronic lymphocytic leukemia (CLL) and low-grade non-Hodgkin lymphoma (NHL). The single agent activity in previously treated CLL appears very promising, with an aggregate response rate of 56% (83 objective responses among 149 evaluable patients). Based on its activity and tolerability, the Food and Drug Administration (FDA) recently granted Group C status to the drug and Fludarabine has become available for the treatment of individual patients with refractory CLL outside the clinical trial settings. A New Drug Application for the treatment of refractory CLL is under review by the FDA. Phase III randomized trials comparing fludarabine with other standard agents (e.g., chlorambucil, cyclophosphamide) in CLL and NHL are planned.

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. The results to date indicate that dCF is one of the most effective agents against hairy cell leukemia. Two randomized Phase III trials comparing dCF with alpha-interferon in HCL have been undertaken: one in previously untreated (unsplenectomized) population and another in patients who have relapsed after splenectomy. The patient accrual for the former trial has been completed. Currently, dCF is available outside clinical trials for the treatment of hairy cell leukemia patients who have failed or are intolerant to alpha-interferon.

MECHANISMS TO OVERCOME ANTIMETABOLITE RESISTANCE

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

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

The combination of Leucovorin and 5-FU has now become the standard regimen for patients with advanced colorectal adenocarcinoma. Leucovorin was the first biochemical modulator of 5-FU proven to have clinically relevant effects and is being evaluated by the FDA for this indication. Many of the compounds referred to above are being evaluated in many different combinations and schedules, against different cancers and both in advanced disease and in the adjuvant setting. Clinical trials using combinations of modulators (e.g. 5-FU/PALA/Dip or 5-FU/LV/PALA) are also underway. The most impressive results remain in colorectal cancer patients. The most active combinations to date are 5-FU/PALA and 5-FU/IFNa in advanced disease and, 5-FU/Levamisole in the adjuvant stage. Large multicenter Phase III studies to confirm the positive Phase II trials are active or are planned.

ALKYLATING AGENTS

HEPSULFAM

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

CARBOPLATIN

An NDA for this agent for the second-line treatment of ovarian carcinoma was approved in the past year and CBDCA is now commercially available. The Investigational Drug Branch is continuing to sponsor trials of high-dose CBDCA with colony stimulating factor or bone marrow support as part of its dose-intensification effort.

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 were initiated recently.

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 or 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. Effects of BSO administration on tumor cell GSH levels are being investigated. In addition, preclinical toxicology studies are underway for a combination of BSO and carboplatin.

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.

NOVEL COMPOUNDS

IPOMEANOL

Three Phase I trials are currently active: single bolus q 21 days at Johns Hopkins and daily x 5 bolus q 21 days at NCI-Navy and Ohio State. The latter trials have only recently begun accrual. Early pharmacokinetic data indicated that, while the dose in mg/m^2 represented approximately 70% of the LD_{10} in female mice, the AUCs at that dose represented only 4% of the AUC at the LD_{10} in female mice and 10% of the AUC at the lowest non-toxic dose in dogs which suggests that human handling of the compound may be somewhat different. The daily x 5 trials have been amended to allow a higher starting dose based on the daily x I experience and will use more aggressive dose escalations and intrapatient dose escalations until a dose which produces biologic activity is identified. After some initial experience is gained with the daily x 5 schedule, trials designed to induce tolerance will be initiated.

SURAMIN

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

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

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

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

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

PYRAZINE DIAZOHYDROXIDE (PZDH)

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

TEREPHTHALAMIDINE

This 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 animal 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 its toxicity. The drug will begin 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 two partial responses in lung and colon cancers being recently reported. Once the MTD and dose limiting toxicities are defined, the drug will undergo broad Phase II testing against solid tumors. The IDB is initiating preclinical synergy studies in collaboration with the DTP to explore possible combinations 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 SR 2508 given with brachytherapy based on preclinical data which suggest that SR 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 beginning 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 being designed that will test the application of these combinations to ovarian cancer and autologous bone marrow transplantation.

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.

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REGULATORY AFFAIRS BRANCH

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

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

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

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

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

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

5. Compliance with adverse drug reaction regulations;

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

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

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

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

The Quality Assurance and Compliance Section is responsible for:

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

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

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

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

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

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

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

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

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

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

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

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

Dale Shoemaker, Ph.D., Chief

Drug Regulatory Affairs Section -
Jay Greenblatt, Ph.D., Head
Maryellen Franko, Ph.D.
Paul Hiranaka, R.Ph.

Quality Assurance and Compliance Section -
Dorothy Macfarlane, M.D., Head
Joan Mauer, B.S., M.T.
Gary Smith, B.S., M.T.

A summary of the activities for FY '90 includes:

1. Thirty 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 IND for one agent was inactivated.

3. One Group C application was submitted to FDA.

4. During CY '89 297 adverse drug reactions were reported to FDA. An additional 815 adverse drug reactions were received, reviewed and held for reporting to FDA through the Annual Reports.

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

6. On-site audits were made to 16 Cancer Centers or other single institutions which are conducting trials with DCT-sponsored investigational agents.

7. Reviewed the reports from Cooperative Group on-site audits at 152 member institutions, 160 affiliates and 29 CCOPs (or CCOP components).

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

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

10. Procedures were implemented for the monitoring of limited multi-institutional Phase I studies carried out by the Cooperative Groups. Five Cooperative Groups are currently conducting Phase I studies.

DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY '90, 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
AZT (Cancer)	NSC 602670
10-EDAM	NSC 626715
Hycamptamine (SK&F 104864)	NSC 609699
Mafosfamide	NSC 626122
Ormaplatin (Tetraplatin)	NSC 363812
Pentosan	NSC 626201
Pyrazine Diazohydroxide	NSC 361456
Pyrazoloacridine	NSC 366140
R-Verapamil	NSC 632821
Terephthalamidine	NSC 57155
Trans-Retinoic Acid	NSC 122758
Zoladex (Breast Cancer)	NSC 606864

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

Agent	NSC Number
Bryostatin 1	NSC 339555
Erythropoietin	Not Assigned
IL-1 Alpha	Not Assigned
IL-1 Beta	NSC 628282
IL-2/IL-4	NSC 620211
IL-2/TIL (Bioreactor Expanded)	NSC 373364
IL-3	NSC 623127
IL-6	Not Assigned
Monoclonal Antibody	NSC 624341
IMMU-4 and IMMU-4 (F(ab')2	NSC 624344
Monoclonal Antibody B72.3 Chimeric Gamma-1	NSC 624342
Monoclonal Antibody 14.18 Chimeric	NSC 623408
Monoclonal Antibody RG83852 Anti-EGF Receptor	NSC 633416
Monoclonal Antibody FVB72.3	Not Assigned
Monoclonal Antibody CC49 to TAG-72	NSC 623111
Monoclonal Antibody Yttrium-labelled Anti-TAC	NSC 631937
MTP-PE	NSC 628280
Murine Monoclonal Antibody Chimeric CC-49 Gamma-1	Not Assigned
PEG-IL-2	NSC 625376

INDs Inactivated.

The IND for the following agent was inactivated:

Agent	NSC Number
5-Methyltetrahydrohomofolate	NSC 139490

A Group C Application was submitted to the Center for Drug Evaluation and Research, FDA for the following compound:

Agent	NSC Number
Fludarabine Phosphate	NSC 312887

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

Adverse Drug Reaction Reporting.

The Section is responsible for reporting adverse drug reactions to FDA. During CY '89 297 adverse drug reactions were reported to FDA. An additional 815 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 all DCT investigators. The data from these reports are being entered into a data base on a personal computer.

Additional Activities.

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

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

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

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

The Section's professional staff continues to participate in discussions concerning the expansion of the IL-2/TIL cell trials and validate the IL-2/TIL cell process at each new institution. The staff also inspects TIL cell laboratories.

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 staff prepared the following guidelines to assist extramural and intramural investigators in meeting FDA requirements:

1. Guidelines for Antigen-Specific Immunotherapy (ASI),

2. Revised guidelines for the manufacture and testing of monoclonal antibodies, and

3. Revised guidelines on requirements for IND submissions for IL-2 in combination with adoptive transfer of cytotoxic cells.

The Section's staff reviews all new Biologic Response Modifiers Program monoclonal antibody contracts for compliance with FDA requirements.

The staff advises NCI Monoclonal Antibody contractors on the production, purification and testing of monoclonal antibodies.

The staff continues to work closely with Dr. Anderson, Dr. Blaese, and Dr. Rosenberg in getting FDA approval for the treatments involving retroviral gene insertion into human cells.

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

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

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

Alternative procedures for implementing Material Transfer Agreements with pharmaceutical companies have been developed and utilized.

QUALITY ASSURANCE AND COMPLIANCE SECTION

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

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

Cooperative Group On-Site Monitoring.

In the case of the Cooperative Groups, DCT has delegated the responsibility for organizing and conducting the monitoring program to each group. Each institution is to be monitored at least once every three years. During the past year, the Cooperative Groups site visited 152 member institutions, 160 affiliates and 29 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.

Seven special audits were carried out to examine the data and verify response determinations in promising Phase II trials. These included: 5-FU/PALA in colorectal and pancreatic cancer, Taxol in ovarian cancer,

DDP/DTIC/BCNU/Tamoxifen in melanoma, Ricin/Immunotoxins in lymphoma/leukemia, Suramin in prostate cancer, and PACE/g-IFN in small cell lung cancer.

Protocol and Information Office.

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

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

The New Drug Study Group application is included with the LOI approval letter for any institution wishing to do independent studies which is not an NCI-supported Cancer Center.

Applications are reviewed and approved by Section staff in cooperation with Investigational Drug Branch staff.

Additional Activities.

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

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

In June 1990, the Section Head was invited to speak at the FDA Center for Drugs Seminar Series on "Assuring the Quality of Clinical Trials Data: Lessons from the NCI Experience." She also gave presentations to the Pediatric Oncology Group data managers about regulatory concerns in clinical trials, at the Intergroup Data Managers Meeting in April 1990.

. SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1989 - September 30, 1990

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

PROGRAM ACCOMPLISHMENTS

OFFICE OF THE ASSOCIATE DIRECTOR

Laboratory Investigator-initiated Research Activities

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

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

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

A more complete discussion of various agents and therapeutic strategies will be taken up in the laboratory project report section. In summary, Dr. Broder's laboratory has focused on the development of certain anti-retroviral nucleoside analogues, their biochemical pharmacology, and their

application to the therapy of patients with AIDS and related disorders. In addition, certain targeted therapies designed to inhibit HIV binding to cells or to the suppression at the genomic level have been explored. Finally, a Retroviral Disease Fellowship has been initiated within the COP for individuals interested in pursuing laboratory and clinical research on HIV and other human retroviruses. This program will be integrated with that of the Medicine Branch.

One family of anti-retroviral nucleosides is referred to as dideoxynucleosides. The first in vitro assessment of these drugs against HIV was undertaken in this laboratory about six years ago and has been discussed in previous annual reports. About five years ago, one member of this family, AZT, was used by COP for the first time to treat patients with AIDS. This drug is the first and, at present, the only anti-retroviral chemotherapeutic agent approved for prescription status.

During the past year, new studies of AZT have been undertaken. One of the primary toxicities of AZT is bone marrow suppression. In an attempt to obviate this problem, a feasibility study was initiated to test whether AZT's bone marrow suppressive activity could be ameliorated by the administration of GM-CSF. GM-CSF is a bone marrow stimulant that can promote the regeneration of cells belonging to the granulocyte and macrophage series. AZT was initially given in an alternating manner with GM-CSF to patients with AIDS. Ten patients were studied extensively. The results suggest: (1) that GM-CSF can potentiate bone marrow function in AIDS patients and (2) that an alternating regimen of GM-CSF and AZT is clinically active against HIV.

At the same time, these studies showed that GM-CSF, if given by itself (that is, without AZT), actually could increase the replication of HIV in patients with AIDS. This clinical finding is in accordance with the laboratory finding made by this laboratory that GM-CSF can enhance HIV replication in human monocytes. However, as will be discussed below, GM-CSF can increase the anti-HIV activity of AZT in vitro in such cells. Because of the concern that GM-CSF may enhance HIV replication if used alone and the evidence that it can enhance the activity of AZT in macrophages, we have started a study of simultaneous AZT and GM-CSF administration in patients with severe HIV infection and neutropenia. Preliminary reports from this study indicate that this combination is well tolerated and has anti-HIV activity. Several patients who had not previously received AZT have had striking increases in their T4 counts on this regimen.

The Program also has continued a feasibility study of AZT alternating with dideoxycytidine (ddC), another dideoxynucleoside, in patients with AIDS or AIDS-related complex (ARC). In 1987, a phase I study of ddC was completed. This drug was shown to exert a virustatic effect in vivo in patients with AIDS and related disorders. The major dose-limiting toxicity was not bone marrow suppression (as with AZT), but rather a peripheral neuropathy. AZT does cause peripheral neuropathy. Because of these non-overlapping toxicities, a pilot study testing a regimen of weekly AZT, alternating with weekly ddC, was initiated. This regimen was found to be clinically active.

Some patients have been enrolled in this regimen for up to three years without significant toxicities. The combination of AZT and ddC seems to have reduced the level of bone marrow suppression observed with the administration of only oral AZT. The risk of peripheral neuropathy associated with single-agent ddC at high-dose continuous administration also seems to have been reduced significantly. The patients who developed a peripheral neuropathy usually had a mild, reversible form. Generally, the peripheral neuropathy did not occur during the first six months of the regimen. These results have lead to the initiation of a large-scale, multi-center study to determine whether an alternating regimen of AZT and ddC is more active and less toxic than single-agent therapy.

During the past year, we have investigated the anti-HIV activity of dideoxyinosine (ddI). Our group showed that this compound along with the related compound dideoxyadenosine (ddA) was a potent inhibitor of HIV replication in vitro, but exerted little toxicity to T cells. ddA is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase, and, for many purposes, both may be considered alternate forms of the same drug. Both drugs are cleaved into dideoxyribose and the free base under acid conditions (as in the stomach). However, while adenine, the free bass of ddA, can cause renal damage, the free base of ddI, hypoxanthine, is relatively well handled by the body. For this reason, ddI is probably the preferred form for oral administration.

In the beginning of 1988, we began a small clinical study of ddA, and in July of that year, we initiated a Phase I study of ddI in patients with AIDS or AIDS-related complex. This study demonstrated that at doses which were well tolerated, patients had increases in T4 cells and total lymphocytes, decreases in HIV p24 antigen (a measure of the viral load), and other evidence of immunologic, virologic, and clinical improvement. In addition, some patients had a reversal of HIV-dementia. At very high doses, the limiting toxicities of ddI were found to be painful peripheral neuropathy, occasional pancreatitis, and occasional hepatitis. Doses of 200 to 750 mg/day of ddI, however, are associated with activity but rare toxicity, and these are the doses being used in the Phase II/III trials.

As a result of this Phase study (with supporting data from two other studies), three Phase II/III studies of ddI were initiated in October of 1990. In addition, ddI is being made available to patients who cannot tolerate AZT or who have deteriorating disease while on AZT under the regulatory mechanisms of a "treatment IND" and "Expanded Access Program." At the same time, we are continuing to investigate the clinical use of this drug, both alone and with other agents. We have found, for example, that the T4 rises on ddI are most consistent in patients who have previously received AZT for less than one year. Even patients with long-term prior AZT therapy, however, generally have virologic responses to ddI as measured by HIV p24 antigen. We have also observed that the survival of patients receiving ddI is quite good: overall, the survival of our patients with AIDS or severe AIDS-related complex was 88% at 20 months. This is quite striking considering that the median T4 count of the patients at entry was 44/mm3. For those patients who had a diagnosis of AIDS at entry, the 20 month survival was 80%. Again, this is substantially better than the 6 to

12 month median survival in untreated AIDS, and it suggests that the drug will be found to be efficacious in the controlled Phase II/III trials.

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

We have recently been investigating the development of tumors in patients with AIDS or AIDS-related complex on long-term HIV therapy. We have recently observed that 8 or 55 patients on long-term AZT containing regimens developed non-Hodgkin's lymphomas. When the development of the lymphomas was plotted by the methods of Kaplan and Meier, the chance of developing a non-Hodgkin's lymphoma was 46% in patients with AIDS or severe ARC who were maintained on AZT-based therapy for 3 years. We believe that these most likely represent "opportunistic" lymphomas which have arisen as profoundly immunosuppressed patients remain alive longer. This situation is analogous to certain childhood immunodeficiency diseases such as Wiskott-Aldrich syndrome in which the cumulative incidence of lymphomas has increased as patients have remained alive longer. AIDS-related lymphomas are typically high grade, occur in extranodal sites, and are difficult to treat. In collaboration with Dr. Dwight Kaufman and members of the Medicine Branch, we are exploring a regimen of combination chemotherapy, AZT and GM-CSF for the treatment of AIDS-related non-Hodgkin's lymphomas.

In collaboration with Genentech, Inc., we are exploring means of inhibiting HIV binding to target cells. Starting in 1988, a study of recombinant CD4 in patients with AIDS was conducted, perhaps representing the first time that a specifically-designed, targeted therapy has been used in the treatment of AIDS. CD4 is a glycoprotein on the surface of T cells and certain other cells and acts as the receptor for HIV. Recombinant CD4 (rCD4) is a glycoprotein produced by genetic engineering technology which contains the extracellular domains of CD4. It can bind to HIV and, therefore, block its binding to, and infection of, human lymphocytes and monocytes. The rCD4 clinical study involved continuous infusion to patients with severe HIV infection at doses up to 1000/ug/kg/day. The half life of rCD4 is about 40 minutes. Essentially no drug toxicity was observed, and no patient developed anti-CD4 antibodies. Some patients had decreases in their HIV p24 antigen or increases in their T4 cells, but this was not consistently observed.

At the same time, <u>in vitro</u> studies to explore new hybrid compounds of CD4 and immunoglobulins have been conducted in collaboration with Genentech. CD4 is in the same supergene family, and hybrid proteins combining the first two domains of CD4 (which bind to gp120) with the Fc portion of IgG heavy chain retain certain desirable properties of both moieties. The term "immunoadhesin" is sometimes used to describe such hybrid proteins. These immunoadhesins bind avidly to gp120 and, at the same time, will stay in the

circulation much longer than rCD4. In addition, it may preserve certain immunologic functions of immunoglobulin. The studies in the laboratory showed that these proteins effectively inhibited the HIV infection of T cells and monocytes. Starting in August of 1989, a clinical trial of CD4-IgG immunoadhesin was conducted. This compound was found to be well tolerated at doses up to 1000 mg/kg/day intravenously. Essentially no toxicity has been observed. However, it is too early to determine if it has activity against HIV.

It has been observed that low levels of anti-HIV antibody can enhance the infection of monocytes and certain other cells by HIV. The question has been raised whether this may occur by a CD4-independent mechanism, and if so, whether infection under such conditions might not be inhibited by agents such as soluble CD4. A study in the laboratory showed that while such enhanced infection could be observed in vitro, it was still inhibited by both recombinant CD4 and by anti-CD4 antibodies. These results indicate that even in the presence of such enhancing antibodies, infection still occurs by a CD4-independent mechanism. In other studies, it has been observed that HIV-2 is relatively resistant to the inhibitory effect of recombinant CD4. This appears to occur because HIV-2 has a lower affinity for CD4 than does HIV-1.

A number of structure-activity relationships involving nucleoside analogues have been explored. For example, an epoxy congener of cytidine has been found to possess considerable activity. This drug, 1-(2′,3′-anhydro-beta-D-lyxofuranosyl)cytosine, was prepared in collaboration with Dr. Tom Webb at Genentech, Inc. It is one of the few epoxy analogues known to possess potent anti-retroviral activity. Also, during the past year, in collaboration with Dr. Jiri Zemlicka at the Michigan Cancer Foundation, a new class of acyclic compound activity against HIV-1 and HIV-2 was discovered. Adenallene and cytallene, represent two newly-synthesized compounds. A study of structural activity relationships revealed that the presence of two cumulated double bonds between the 2′ and 3′-carbons conferred anti-retroviral activity in certain pyrimidine and purine derivatives containing a four-carbon side chain. These compounds are now being evaluated for possible further development.

We are now also studying several lipophilic nucleoside analogues which may have improved central nervous system penetration as compared to the prototype drugs -as such, these compounds may be particularly useful in patients with dementia.

Finally, a major effort is underway to deal with viral replication and expression in cells that are already infected. Virtually all the major drugs, which now are used in clinical studies, affect viral replication by protecting uninfected target cells from becoming infected by HIV. In collaboration with members of the Medicine Branch and Dr. Jerry Zon at Applied Biosystems, Inc., certain novel oligodeoxynucleotides have been studied for capacity to inhibit viral replication. Special attention has been focused on phosphorothioate analogues, compounds in which one of the non-bridging oxygens has been replaced by a sulfur atom. Such constructs are quite stable and resistant to enzymatic attack. These compounds have a sequence-specific and non-specific activity. The sequence non-specific

activity appears to be mediated by a competitive inhibition of template-primer interactions within the viral reverse transcriptase.

More recently, we have focused on the sequence-specific inhibition. The mechanism appears to be translation arrest. We have observed that a 28-mer phosphorothioate analogue of an oligodeoxynucleotide, which is in an anti-sense configuration to the art/trs gene of HIV, can block the expression of virus even in chronically infected target cells. Full length (genomic) viral mRNA seems to undergo the greatest reduction in infected cells that are exposed to this construct. Envelope expression is affected, but to a lesser extent, while smaller transcripts such as 3'orf seem to be unaffected. This may provide the first proof that one can affect viral expression in a chronically infected target cell without necessarily killing the cell. A continuation of this study has shown that combining intercalating compounds with such antisense constructs selectively can increase their anti-HIV activity. We are now exploring the possible clinical application of this approach.

We have recently found that certain agents which inhibit reverse transcriptase, such as ddI or dideoxyguanosine (ddG) can inhibit the replication of hepatitis B virus. This may occur because hepatitis B virus, although a DNA virus, replicates through an RNA intermediate using a reverse-transcriptase. Hepatitis is an important cause of morbidity and mortality throughout the world, and is an important cause of hepatocellular carcinoma. Pekin ducks with hepatitis showed a decrease in the load of hepatitis virus when given either ddI or ddG. During the past year, we have been able to demonstrate a similar effect in human T cell lines infected with hepatitis B virus. In collaboration with Dr. Jay Hoofnagel, we are now conducting a pilot study to determine whether ddI will have a beneficial effect in patients with chronic hepatitis B infection.

In other studies, we are exploring new methods for monitoring the effect of anti-retroviral therapy on HIV replication in AIDS patients. During the past year, a technique of quantitative polymerase chain reaction was developed to assess the load of HIV DNA in HIV-infected patients. It was found that in the peripheral blood, most of the HIV DNA was in T cells, and little was in B cells or macrophages. Decreases in HIV DNA could be detected in lymphocytes from patients started on ddI therapy. We are developing a technique for assessing the HIV RNA in the plasma of patients with HIV infection. This technique may more accurately reflect viral replication than analysis of DNA.

Another topic of interest is HIV resistance to drugs. During the past year, several viral isolates have been obtained from patients on long-term dideoxynucleoside therapy, and the patterns of resistance are being analyzed. Several isolates from patients on long term AZT therapy which are resistant to AZT have been identified, and the mutational changes in these isolates are being examined. Such isolates have preserved their sensitivity to ddC, ddI and most other dideoxynucleosides. We have found that several isolates from patients on long-term alternating AZT and ddC therapy have preserved their sensitivity to both drugs, and we are exploring whether such combination therapy will indeed delay the development of resistance.

With some tools at hand, the Program has tried to make an impact on AIDS and the retrovirus which causes this disease. Many of these tools were discovered or identified in Program research performed in previous fiscal years. At the same time, the Program has embarked on a campaign to find new tools for future interventions against this disease.

Biostatistics and Data Management Section

BDMS is the statistical and data management component of the COP. The Section provides statistical leadership and data management consultation for the Program's major activities. It is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials for experimental cancer treatments. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors; to evaluate diagnostic procedures; to develop improved staging systems; and to assist investigators in the design, execution, and analyses of major *in vitro* drug testing.

BDMS develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols; works closely with interested branches to improve data recording and retrieval; and is working to develop specialized clinical data bases for the COP branches.
The Section works with the Clinical Center Medical Information System (MIS) team, enabling Program input for decisions directly impacting patient care and protocol management. The Section assists the NCI Deputy Clinical Director with proper monitoring of protocols through the MIS Toxicity screens and other mechanisms.

MEDICINE BRANCH

There have been a number of important clinical and laboratory changes over the past year.

1. Major personnel changes:

Dr. Robert Wittes, Vice President for Cancer Research at Bristol-Myers Squibb, formerly Associate Director for the NCI's Cancer Therapy Evaluation Program, was recruited as Chief, Medicine Branch. Dr. Charles Myers will reformulate the Clinical Pharmacology Branch and continue to test novel therapeutic targets as described below.

Jean Jenkins, who was head of our protocol office, left to take over as head of Cancer Nursing. She was replaced by Diane Howser, who came to NCI from Medical College of Wisconsin.

Edward Sausville was recruited to the branch as a section head from Georgetown University and will be setting up a program in cancer cell biology.

Maribeth Weinberger and Donna Headlee have been recruited to the protocol office as new research nurses.

2. Major clinical advances:

Suramin has proved to be quite effective in the treatment of patients with prostate cancer who have failed previous hormonal therapy with a median survival of 60 weeks compared with a historical control of 32 weeks. The drug has also caused PRs in 5 out of 10 patients with nodular lymphoma. These results are sufficiently promising to support further development of suramin and similar analogs with alternate site of action in these diseases.

In a cohort of patients receiving single agent cisplatin or carboplatin therapy, the level of platinium-DNA adduct in WBC DNA correlated well the prognosis. In fact, a measure of platinum adducts at the end of cycle one may be sufficient to accurately predict response.

A clinical trial of carboplatin-GM-CSF in heavily pretreated ovarian carcinoma gave an overall objective response rate approaching 40%. This is much better than historical experience.

A dose-intense combination of cytoxan, adriamycin, 5-FU and leucovorin has resulted in close to a 100% response rate in metastatic breast carcinoma.

3. Major laboratory advances:

The synthesis of thymidylate synthase is regulated at the level of translation by the amount of active enzyme. Thus, treatment with MTX or 5-FU results in the synthesis of new enzyme. Since inhibition of thymidylate synthase is important to the action of 5-FU, new synthesis of thymidylate synthase may significantly limit the action of 5-FU. Alpha-interferon has been shown to block this increase in thymidylate synthetase by acting at the level of translation. In the process of these studies provocative information has been obtained on the ability of functional thymidylate synthetase to cause feedback inhibition of synthesis of new enzyme by a translational block. A clinical trail in GI malignancies is testing the activity of drug-biologic combinations.

IL-6 has been shown to mediate growth of human myeloma cells by an autocrine mechanism. In addition, as with IL-2, IL-6 appears to have a complex receptor composed of multiple proteins associated to form the receptor complex.

Previous studies had shown that in all Burkitt's lymphoma cell lines, deregulation of c-myc occurs. In addition, this work demonstrated the existence of a DNA binding protein (MIF-1) which associates with the first intron of the c-myc gene. During the present year, the DNA binding sequence of MIF-1 has been identified and a single point mutation shown to block that binding. In addition, a second protein (MIF-2), binding to the first intron of c-myc has been identified. Also, the phosphorylation state of these two proteins have been shown to alter the action of either protein. Finally, suramin has been shown to decrease the expression of c-myc 24-48 hr after treatment followed by a parallel decline in rate of proliferation. This has been shown to correlate with alterations in formation of the MIF-DNA complex. These results suggest that suramin may

represent a prototype of drugs which might have antitumor activity by modulating MIF-1 activity.

Prostate Tumor Cells have been shown to express a purinergic type 2 (P2) receptor. P2 agonists were shown in prostate tumor cells to trigger calcium influx, inositol phosphate synthesis and to cause terminal neuroendocrine differentiation. Several P2 agonists have been identified which are potential drug candidates.

Suramin-treated patients have been shown to secrete a heparan sulfate in their urine. This material has been purified and has been shown to have antitumor activity. This heparan sulfate is more active, on a molar basis, than suramin against several human tumor lines. This suggests that a portion of the antitumor activity of suramin may be due to the formation of this heparan sulfate.

NCI-NAVY MEDICAL ONCOLOGY BRANCH

Study of Mutations in Human Cancers

Tumor Suppressor Genes (Recessive Oncogenes) Mutations in Lung Cancer and Breast Cancer

Chromosome and restriction fragment length polymorphism studies have identified lesions on multiple chromosomes including chromosome regions 3p, 1p, 1q, 5q, 11P and 22. Because such tumor suppressor genes usually required inactivation of both the maternal and paternal chromosomes this would indicate that as many as 10-15 different genetic lesions have occurred in clinically evident lung cancer. These results have direct bearing on future prevention and prognostic studies and direct the search for early molecular detection of lung cancer and/or the detection of patients exhibiting some of these abnormalities in a premalignant phase. Studies are ongoing to try to "correct" malignancy in lung cancer cells by reintroducing the suppressor genes into lung cancer cells through transfection and retroviral vectors. (Minna, Kaye, Birrer, Takahashi, Rosenberg, Johnson, Gazdar in collaboration with J. Whang-Peng).
Lung cancer cells over the past 2 years have been found to exhibit genetic abnormalities of many potential recessive oncogenes. These ongoing studies have identified abnormalities in the rb gene (chromosome region 13q14) p53 and 3p genes in potentially all small cell lung cancers and several non-small cell lung cancers. Recent studies have identified point mutations in the p53 gene (chromosome region 17p13) in 50% of primary non-small cell lung cancers and preliminary breast cancers. (Minna, Takahashi, Chiba, Osborne, Curiel).

Most recently, small cell lung cancer mutants of the rb gene have been described that give aberrant protein forms. (Kaye, Kratzke, in collaboration with Dr. Horowitz and Weinberg, MIT).

The Causes and Consequences of Chromosomal Aberrations During Normal, Premalignant and Malignant Hematopoiesis

The scl Gene

We recently reported the discovery of a new gene, scl, so named because it was identified via the cloning and characterization of a chromosomal translocation associated with the development of a "stem cell" leukemia. We have completed the analysis of the genomic structure of the scl gene, its normal transcript unit, and its aberrant transcript related to its disruption by a t(1:14) chromosomal translocation. We know that the 5′ end of the gene is complex with cell-lineage specific differential exon utilization and alternative transcription initiation start sites. These studies are providing insight into normal hematopoiesis as well as aspects of malignant transformation for we have determined that the scl gene is a member of a recently recognized and critical family of genes, each one of which is known or believed to play a role in either the growth or development of the cell system in which it is active. Members of this family, which include c-,N-, and L-myc, Myo D, myogenin, and the Ig enhancer binding proteins E12/47 all share a structural basic domain-helix-loop-helix motif which is now known to function as a DNA binding region and a region of protein-protein interaction. Thus important features of both function and regulation are contained within this motif. Inferential but compelling data suggest that scl is a gene that plays a role in a critical and early stage of hematopoietic cell-lineage determination. Our current focus is on what scl regulates and what regulates it. These investigations are proceeding through studies of the 5′ regulatory regions of the gene, protein analyses, and the development of vectors for transgenic and retroviral mediated transfection experiments. (Kirsch, Aplan, Bertness, Nakahara, Reynolds, Tchorz).

Abnormalities in T-Cell Receptor Rearrangement

We had previously described the basis for an inv(7) chromosomal abnormality found in the peripheral T-cell population of normal individuals. This abnormality is caused by site-specific recombination between a T-cell receptor gamma variable segment and a T-cell receptor beta joining segment resulting in a hybrid T-cell receptor gene. We have demonstrated that this abnormality occurs at a 70-100 fold increased frequency in the peripheral blood of patients with the autosomal recessive disease, ataxia-telangiectasia (AT). Furthermore, we have evidence that the increased frequency is due to multiple independent events and not monoclonal proliferation of a single inv(7) containing cell. Many of these hybrid genes may be functional in so far as they increase the repertoire of the immune response. Their role, if any, in contributing to the increased frequency of lymphoid malignancies seen in patients with AT is being explored. Preliminary data suggest that the occurrence of these "innocent" inv(7) abnormalities may be a marker for the occurrence of chromosomal aberrations clearly associated with the development of lymphoid malignancy. This hypothesis is being tested in a molecular epidemiological analysis. (Kirsch, Lipkowitz).

Substractive cDNA Cloning to Identify B Cell Lymphoma Genes

We have developed a general method for preparing substractive cDNA (in principle, this approach will also work for genomic DNA) by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNAS libraries. We have used this novel technology to identify seven genes which are expressed in murine plasmacytomas but not B or pre-B lymphomas. The first of these genes to be analyzed encodes an intrinsic membrane protein which has been shown by others to be expressed as a "tumor antigen" in human colon and lung carcinomas. (Kuehl, Timblin, Bergsagel, and Brents).

We found mutations of the p53 gene in 1/8 gastric tumors (6%) and 3/7 (43%) gastric cell lines. Because the tumors were primary lesions while most of the cell lines were from metastatic lesions, mutations of p53 may be associated with metastasis in gastric cancer. (Kim, Takahashi, Minna, Gazdar).

Dominant Oncogenes

Mutations of ras genes in primary lung cancers (mainly K-ras at codon 12) have been associated with a subset of adenocarcinomas having a poor prognosis. We investigated 105 lung cancer cell lines, and found codon 12 ras mutations (all K-ras) in about 20% of lung cancer lines. Unlike other studies, the mutations were not limited to adenocarcinoma, but occurred with equal frequency in all forms of adenocarcinoma. There were similar incidences in cell lines initiated from primary or metastatic tumors. No mutations were found in any of 37 small cell lung cancer cell lines. Mutation of K-ras at codon 12 define a subset of non-small lung cancer, but not small cell cancer. (Mitsudomi, Viallet, Minna, Gazdar).

The myc proteins and their Biological Activities

We have characterized the protein products of the L-myc gene: 1) they arise from alternative translational initiation site) and then undergo post-translational phosphorylation. These various proteins have been shown to cotransform rat embryo cells with an activated ras gene and presently are being examined for differences in biologic activities. In addition, truncated forms of L-myc are being characterized as to their ability to cotransform or inhibit transformation of rat embryo cells. (Dosaka and Birrer).

Deregulation expression of the L-myc gene, although not expressed in mouse erythroleukemia cells, can substitute for c-myc for blocking differentiation. (Segal, Bar-Ner).

Transfection of a c-myc cDNA into an IL3-dependent murine pregranulocyte leukemia cell line results in cells which have increased survival in medium containing C-CSF-without IL3, but have lost the ability to terminally differentiate in this medium. (Foss, Kuehl).

The Transforming Activity of the c-jun Proto-oncogene

The transforming activity for the c-jun proto-oncogene was established by demonstrating the cotransforming activity of this gene in conjunction with an activated ras gene in rat embryo cells. Further, it was shown that c-jun can transform an immortalized rat fibroblast cell line Rat-1a as a single gene. This demonstrates that no mutational activating event is required for c-jun to transform mammalian cells. (Birrer in collaboration with Schutte and Minna).

In addition, the biologic effects of the c-jun proto-oncogene as a single gene expressed in primary cells (rat embryo cells) are being examined by delivery of the gene by retrovirus. This allows for high expression of the gene in many different cell types. These experiments reveal that jun provides a clonal expansion of these cells but not transformation. Further, these effects appear to be amplified in the presence of a tumor promotor TPA. (Birrer and Rosenberg).

To elucidate the mechanism of transforming activity of c-jun we have undertaken a mutation/deletion study of the gene. Presently, the transforming activity of the gene maps to two highly conserved regions in the gene, one of which contains the DNA binding and dimerization domains. The other domain is located at the N-terminus of the gene and is conserved in all jun family members. These same regions are also required jun's ability to transactivate an AP-1 containing reporter gene. These results suggest that jun transforms cells by transactivating AP1 containing genes. (Alani, Brown, Rosenberg, Dosaka and Birrer).

In isolating such jun mutants, some non-transforming ones have been found to inhibit the transforming activity of the full length gene, displaying a 'dominant-negative' phenotype. We are presently characterizing the structural requirements and mechanisms for this phenotype. Once characterized, these mutants will be invaluable for the characterization of the biologic and biochemical pathways by which jun functions. (Alani, Brown, Rosenberg, and Birrer).

Study of Peptide Hormones, Growth Factors, Receptors, Signal Transduction Pathways, and Other Markers Important in the Pathogenesis of Human Cancers

Atrial Natriuretic Peptide Studies

High pressure liquid chromatography (PLC) studies of atrial natriuretic peptide in small cell lung cancer tumors and tumor cell lines have shown that the peptide is similar to that secreted into the plasma by the right atria. The prospective studies of ectopic production of atrial natriuretic peptide in small cell and non-small cell lung cancer patients are underway. These studies have potentially identified the natriuretic factor previously described in the Syndrome of Inappropriate Antidiuretic Hormone (SIADH) present in patients with small cell lung cancer. These studies have lead to a new understanding of the pathogenesis of SIADH and may lead to a new approach to treating hyponatremia in patients with small cell lung cancer. (Johnson).

An insulin like growth factor (most similar to IGF-1) has been found to function as an autocrine growth factor for all types of lung and colon cancer, providing a new potential target for antitumor therapy and diagnosis. (Cuttitta and Mulshine).

GRP gene related peptides (GGAPs) have been identified and characterized in lung cancer cell lines and humor specimens. Specific receptors for some of these peptides have been discovered and they have been found to stimulate the growth of lung cancer cells in vitro providing another target for diagnosis and therapy. (Cuttitta, Mulshine, Linnoila).

Enzymes (PAM) involved in the final cleavage and activation of amidated neuropeptide in lung cancer have been identified and characterized. (Treston, Scott, Cuttitta, Mulshine).

Monoclonal antibodies developed against lung cancer have shown dramatic ability in early detection studies. These have led to the design and implementation of prospective cooperative studies (with Johns Hopkins, and the Lung Cancer Study Group) to validate these findings. The results could have potentially tremendous impact on the early diagnosis of lung cancer. (Mulshine, in collaboration with Johns Hopkins Investigators and the Lung Cancer Study Group. The proposed trial is slated for beginning in later 1990).

New Drugs to Treat Lung Cancer and Studies of Drug Resistance in Human Tumors

Cholera toxin was found to dramatically inhibit the in vitro growth of many lung cancer cell lines. This was related to the expression of specific glycolipid membrane receptors. Further preclinical studies of this toxin are underway to attempt to bring it to clinical trial in the treatment of lung cancer. (Viallet, Minna, in collaboration with E. Sausville of the Medicine Branch and J. Plowman, DCT, DTP).

Studies of drug resistance and sensitivity in lung and colon cancer cell lines has revealed: 1) excellent correlation between in vitro chemosensitivity and resistance and response in patients; 2) that the MDR1 gene does not appear to play a role in multiple drug resistance in lung cancer; and 3) provides the first good evidence that no true synergism exists between etoposide and cis-platin in the response of lung cancer; 4) proof of schedule dependency of drug interaction in vitro between methotrexate and 5-FU; 5) proof of leucovorin enhancement of 5-FU cytoxicity in human lung as well as colorectal cell lines; 6) enhancer cytotoxicity of 10-EDAM in human lung cell lines using persantine. (Gazdar, Kramer, Dearing, Mulshine, Lai, in collaboration with Goldstein and Gottesman, DCBD).

NMOB investigators have correlated expression of topoisomerase I and II genes and drug resistance in lung cancer cell lines. Approximately 10% of the lines had rearrangements of one of the genes. In 7/8 lines studied in greater detail, there was an excellent correlation between in vitro chemosensitivity and topoII expression, but not with topoI expression. (Giaccone, Gazdar).

Studies of Tumor Cell Biology

N-CAM, an important neural adhesion molecule, is expressed concordantly in lung cancer cell lines with neuroendocrine properties. All N-CAM positive lines lack substrate adherence, and grow as floating cell aggregates, a feature characteristic of several neuroendocrine and neural cell lines. (Gazdar, Linnoila, Carbone).

NMOB investigators have studied lung cancer tumors and cell lines for expression of CEA and the related genes NCA and BGP. Normal lung has abundant NCA, but relatively little CEA or BGP. All three genes are expressed, but discordantly, in lung cancer cell lines. Cell lines expressing neuroendocrine features have a much higher expression of CEA RNA and protein than other lung cancers. Identification of the precise family member expressed in lung cancers may be of diagnostic importance. (Kim, Kaye, Gazdar).

Immunohistochemical detection of the SAP-35 protein (surfactant associated protein 35 kd) has been shown to identify a much higher than previously recognized number of non-small cell lung cancers with bronchoalveolar properties. These finds have important implications for studying the epidemiology of lung cancer in the United States. (Linnoila, Gazdar, in collaboration with the Lung Cancer Study Group, and Eastern Cooperative Oncology Group).

Synaptophysin has been discovered to be a new neuroendocrine marker of lung cancer differentiation. The expression of such neuroendocrine markers has been found to correlate with drug sensitivity and potentially with prognosis. (Linnoila, Mulshine, Gazdar, in collaboration with the Lung Cancer Study Group and the Eastern Cooperative Oncology Group).

A unique human cell line has been established from a patient with adrenocortical carcinoma. Mass spectrometry studies indicate that even after 7-10 years in culture the cell lines continues to secrete about 35 steroids (glucocorticoids, mineralocorticoids and sex hormones). These studies indicate that all of the important p450 enzymes involved in adrenal steroid synthesis are present. The line, which is being patented, should be invaluable for studying steroid hormone synthesis and its regulation. (Gazdar, Oie).

Initiation of AIDS-related Studies

Evidence for a potential new retrovirus in patients with T-cell lymphomas, separate from HTLV-1 and HIV, has come from newly-established cultured cells from patients with cutaneous T-cell lymphomas. This virus is being molecularly cloned and characterized. (Foss, Turner, Lynch).

Development of clinical tests of drugs inhibiting HIV replication as a treatment of human cutaneous T-cell lymphomas. With evidence for retrovirus infection in cutaneous T-cell lymphomas and their role as a possible stimulation for the development of T-cell proliferation, we are developing protocols to test new anti-HIV drugs in this disease. (Foss, Kramer).

Hematopoietic cell lines have been initiated from the bone marrows of several patients with mycosis fungoides. Preliminary analysis suggests that these have a non-lymphoid (?stromal cell, ?monocytoid cell) phenotype. Preliminary results indicate that the medium from one of these lines contains reverse transcriptase activity, suggesting the presence of a retrovirus. (Foss).

Search for retroviruses related to HIV as a causative agent in human bronchoalveolar and other types of lung cancer and attempts to clone and characterize the retrovirus causing lung cancer in sheep. These include epidemiologic studies in collaboration with Epidemiology Branch (Madigan and Mulvihill), as well as pathologic review of lung cancer incidence and the changing epidemiology of bronchoalveolar lung cancer. (Minna, Buchhagen, Linnoila, Gazdar).

Limited State Small Cell Lung Cancer

The concurrent treatment of limited state small cell lung cancer using twice daily chest radiotherapy and etoposide/cisplatin has approximately doubled the median survival of patients from approximately 15 months for historical controls to 28 months in the current trial. In addition, more than 50% of patients are alive at 2 years. The results of this study have been adopted in cooperative group trials that are currently underway. (Johnson).

Major Staff and Administrative Changes in FY 1990

Dr. Bruce Johnson has been appointed head of the newly established Molecular Biology of Oncopeptides Section of the Branch.

Dr. Herbert Holmes, Captain, PHS, head of Pharmacy Operations, retired after 20 years of service in the PHS to work for the Lombardi Cancer Center, Georgetown University.

Ms. Patricia Schettino assumed the position f Chief of Pharmacy Operations for the Branch.

Dr. Michael Anderson, CDR, USN, Chief of the Oncology Division for the Naval Hospital, retired after 20 years service in the USN to relocate to Massachusetts.

Ms. Gail Gray left the position of Office Manager to set up the Pediatric AIDS outpatient facility.

Ms. Nanci Brice assumed the position of Office Manager for the Branch.

Dr. Mary Pat Dearing is leaving the NCI to relocate in Ohio.

Paul Lin and Leslie Reynolds, two members of Howard Hughes Medical Institute Research Scholars Program, completed their term of research training.

Dr. Richard Rosenberg completed his medical staff fellowship training and will join the faculty of the University of Arizona Cancer Center.

Dr. Jean Viallet completed his medical staff fellowship training and will be joining the faculty of McGill University, Montreal General Hospital.

Dr. John Brennan, completed his medical staff fellowship training and will join CTEP, NCI.

Postdoctoral fellows completing their research training include: Dr. Jochim Schuette (Essen, Germany); Dr. Itsuo Chiba (Sapporo, Japan); Dr. Joohang Kim (Seoul, Korea); Dr. Shinn-Liang Lai (Taipei, Taiwan); Dr. Jos Broers (Nijmegan, Netherlands); Dr. Philip Kasprzyk (Molecular Oncology, Inc.); Dr. Giuseppe Giaccone (Torino, Italy); Dr. Chikabumi Kadayoma (Chiba, Japan); Dr. Chung-Ming Tsai (Taipei, Taiwan); Dr. Richard Osborne (Cambridge, England) and Dr. Hiro Dosaka (Sapporo, Japan).

PEDIATRIC BRANCH

Clinical Studies

1. Newly diagnosed patients with acute lymphoblastic leukemia:
NCI 77.02-CCG 191 - This protocol tested, in a randomized study, whether central nervous system preventive therapy using systemic high-dose methotrexate infusions alone (without cranial radiation) is equally effective and less toxic than 2400 cGy of cranial radiation and intrathecal methotrexate. 181 patients were randomized on this study. The overall remission rate was 98% with an event free survival of approximately 70% at three years from the entire study group. With a median duration on study of 76 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term follow-up evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and psychometric testing) is in progress. A recent analysis of patients longitudinally assessed with a periodic neuropsychological test battery demonstrated a striking decrease in verbal and full scale IQ in patients treated with cranial radiation and intrathecal chemotherapy. In addition, patients treated with cranial radiation and intrathecal therapy manifested significant impairment of academic achievement. No such declines were seen in the high-dose methotrexate groups. These data thus indicate that use of combined cranial radiation and intrathecal therapy can be avoided in nearly 60% of children with ALL, reducing the potential long-term neurotoxicity associated with such combined therapy. In contrast, this study has demonstrated no apparent adverse effects of high-dose methotrexate on cognitive functioning and academic achievement, confirming the value of high-dose methotrexate as central nervous system preventive therapy for children with ALL.

NCI 83P-CCG 134P - Treatment of newly diagnosed acute lymphoblastic leukemia in high-risk patients. The major aim of this study is to demonstrate that high-risk patients can be effectively treated on a regimen which uses CNS preventive therapy devoid of cranial radiation. An additional objective is to determine whether there is a difference in the outcome of patients at high risk for early treatment failure according to

whether they do or do not have features consistent with "lymphoma leukemia syndrome." The protocol involves the use of an aggressive, early intensification phase of therapy and intensive systemic maintenance therapy, together with CNS specific treatment. The latter consists of periodic administration of systemic high-dose methotrexate, systemic high-dose cytosine arabinoside and intrathecal cytosine arabinoside and methotrexate. With a median potential duration of study of 3.7 years, the event-free survival is projected at 65 percent at two 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.

NCI 84A-CCG 144 - This protocol treats newly diagnosed patients in the "average-risk" category, randomizing them to one of two forms of CNS preventive therapy, either high-dose systemic methotrexate infusion or intrathecal methotrexate alone. The median potential duration on study is 30 months. A total of 176 patients have been randomized. There is no significant difference in either the CNS or bone marrow relapse rate in either treatment arm. At the present time the event-free survival at 24 months is approximately 80 percent. These data demonstrate that average-risk patients can receive effective CNS preventive therapy with intrathecal methotrexate alone and do not require high-dose methotrexate.

2. Intrathecal Diaziquone (AZQ) - A newly clinically useful agent for the treatment of meningeal neoplasia -- AZQ is a lipid soluble aziridinyl benzoquinone designed for enhanced CNS penetration of the CNS to treat CNS neoplasms. Despite evidence of clinical activity demonstrated in Phase I and Phase II trials, systemic administration has been limited by severe and prolonged myelosuppression. To circumvent this problem, we are evaluating the feasibility of intrathecal AZQ in a Phase I-II trial in patients with refractory meningeal malignancy. Two schedules of administration are being examined: twice a week for four weeks and "CxT", every 6 hours for three doses, weekly x 4. A total of 38 patients have been treated, 27 of whom had acute lymphoblastic leukemia. Demonstrable antineoplastic activity has been observed on both schedules of administration. Eight of the 18 courses delivered on the twice weekly schedule have resulted in complete responses. On the "CxT" schedule, 7 of 19 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.

3. Intrathecal 6-Mercaptopurine (6-MP) - 6-MP is an active antileukemic agent which has never previously been administered into the cerebrospinal fluid. Preclinical studies of intrathecal 6-MP, performed in a primate model indicated that 6-MP could be safely administered by the intrathecal route. Based on these studies we have initiated a Phase I study of intrathecal 6-MP in children with refractory meningeal malignancy. Both a twice weekly and a concentration x time (CxT) schedule (q12h x 6 doses) are been evaluated. To date, 6 of 9 patients with ALL treated on the twice weekly schedule have responded, 4 are complete responses. No significant

toxicity has been observed. These results indicate that intrathecal 6-MP is safe and active against meningeal leukemia.

4. Thiotepa is an active alkylating agent with a steeper dose response curve than cyclophosphamide. This compound has been used with only marginal success via the intra-CSF route of administration. Our studies demonstrated, for the first time, that substantial amounts of both thiotepa and its metabolite Tepa are present in CSF following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent. As a result of these studies, Phase I study of intravenous thiotepa in pediatric patients has been developed and completed. Thiotepa was administered as an IV bolus on a q/3 week schedule; 65 mg/m^2 was identified as a safe dose for future Phase II trials. As a logical extension of this study we recently embarked on a multi-institutional Phase II study designed to assess the therapeutic efficacy of Thiotepa against brain tumors. Forty-seven patients have been entered (40 are evaluable for response). Two of 13 PNET tumors have had a partial response, 1 had had stable disease. Nine of the patients in the remaining tumor categories have had stable disease; no other responses have been noted.

Thiotepa has a very steep dose response curve and its major toxicity is myelosuppression. In a recently instituted Phase I study, we are attempting to determine whether adjunctive administration of the hematopoietic growth factor GM-CSF will safely permit higher, potentially more effective, doses of systemic thiotepa to be administered.

5. Ongoing Phase I systemic studies including Piritrexim and Thiotepa-GM-CSF. A Phase I intrathecal trial of mafosfamide was recently initiated.

6. We have conducted a number of studies to evaluate the biochemical pharmacology, pharmacodynamics, and pharmacokinetics of those antileukemic agents used for maintenance treatment.

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

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

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

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

The major goal of the new protocol is to determine whether GM-CSF administration will result in increased dose intensity (i.e., dose rate) in high-risk patients, particularly those with bulky disease and/or bone marrow involvement, while at the same time decreasing toxicity. Patients will be randomized to receive either four cycles of CODOX-M/IVAC, or the same treatment accompanied by subcutaneously administered GM-CSF. In both therapy arms, sequential therapy cycles will be initiated as soon as the peripheral blood granulocyte count reaches 1000 per cu.mm. If GM-CSF treatment results in earlier bone marrow recovery, the interval between therapy cycles will be shortened and dose rate will therefore be correspondingly increased. The inclusion of a new regimen as well as GM-CSF in this protocol is justified since, apart from the uncertainty as to whether the strategy will be successful, control arm patients would otherwise be receiving a known ineffective therapy. A small number of patients have been treated to date, but all patients have achieved sustained complete remission. At present, we are taking steps to increase accrual to this promising study.

10. 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.

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

The pilot protocol for the treatment of high-risk sarcomas, 86-C-169, continues to accrue patients. There have been 56 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, 17 patients have been enrolled in this study.

12. To determine the role of new beta-lactam antibiotics in providing simpler, safer and effective therapy for neutropenic cancer patients who become febrile, we have conducted a randomized trial comparing a third-generation cephalosporin (ceftazidime) to a carbapenem (imipenem/cilastatin) for initial empirical therapy. The goal of this study is to both evaluate the role of these agents in providing safe initial therapy as well as determining whether the numbers of modifications of the primary antibiotic varies in patients with defined infection or prolonged granulocytopenia. From March, 1986 - June, 1990, we enrolled 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.

13. A Phase I study of fluconazole has been initiated in pediatric patients. This study is designed to determine the tolerable dose for children and will serve as the basis for a study to evaluate its efficacy in preventing invasive mycoses in cancer patients.

14. We have continued our Phase I-II studies of children with symptomatic HIV infection. Since beginning this project in December, 1986, we have evaluated approximately 170 children, enrolling the majority into clinical trials.

Our initial study of AZT, administered either by continuous intravenous infusion or on an intermittent schedule, are completed. Both routes of therapy appeared to offer benefit, particularly for children with neurodevelopmental deficits. However, the extent of this benefit appears 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. In addition, a third arm in this protocol randomizes patients to receive oral dideoxyinosine (ddI). 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, 11 patients have been randomized.

Our prior studies with AZT demonstrated that the dose-limiting toxicity was myelosuppression related to both dosage and duration. Consequently, we have evaluated two schedules to spare AZT-induced myelosuppression. The first alternates AZT with ddC, and the second combines it with rH-GM-CSF or more recently, with rH-G-CSF. In the study with ddC, we first evaluated this newer dideoxynucleoside in a limited phase I trial, studying four dosage levels (0.015, 0.020, 0.030, and 0.040 mg/kg/q/6h) administered over an 8 week period. Fifteen patients were treated. We observed decreases in P24 antigen in 5/9, increments in CD4 counts in 8/15 during the 8-week trial of ddC as a single agent. We also treated 13 of these 15 patients with an alternating schedule of ddC and AZT and found this to be non-toxic and tolerable during a minimum follow-up of 18 months.

We also initiated a protocol to evaluate the combination of AZT with colony stimulating factor in order to overcome the myelosuppression of AZT. One patient was treated with rGM-CSF plus AZT, and although the leukocyte count rose, more than 99% were eosinophils. More recently, we have begun to treat patients with rG-CSF and AZT. The preliminary results are encouraging.

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, 78 children have been enrolled at several dosage levels (20, 40, 60, 90, 120 mg/m^2/ every 8 hours). This protocol enrolls 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. ddI was rapidly absorbed after oral administration, however, there was significant variability in its bioavailability. Pancreatitis occurred in two patients, one at each of the two highest dose levels. Median CD4 cell count increased from 218/mm^3 at baseline to 327/mm^3 at 24 weeks (P=0.001). Patients with baseline CD4 cell counts greater than 100/mm3 were significantly more likely to show an increase in this parameter. Median p24 antigen declined from baseline to 24 weeks (p=0.005), and there was a significant correlation between ddI plasma concentration and decline in p24 antigen level. A significant correlation was also found between ddI plasma concentration and improvement in cognitive function. Improvements in clinical and immunological parameters occurred in previously untreated patients and in prior AZT recipients. Dideoxyinosine was well tolerated and shows promising antiretroviral activity in HIV-infected children. The correlation between response and plasma ddI concentration indicates that bioavailability is an essential consideration for optimizing ddI activity in the treatment of HIV infection.

As part of our efforts to evaluate new antiretroviral agents in children, we initiated a Phase I study of recombinant soluble CD4 (rCD4) administered steroid by continuous infusion. To date, 10 patients have been enrolled. In addition to evaluating the safety, toxicity and antiviral activity of rCD4 as a single agent, we are evaluating the combination therapy of rCD4 and ddI in this study, by administering oral ddI, in addition to intravenous rCD4, to patients on this study after an initial 12 weeks of rCD4 alone. No significant toxicity has been observed among patients receiving CD4, or rCD4 in combination with ddI.

Pre-Clinical Studies

1. In the unique primate model for CSF pharmacokinetic studies, we have identified several approaches of potential utility in treating meningeal malignancy.

Pediatric Branch investigators have evaluated the feasibility of intrathecal administration of mafosfamide, a pre-activated analog of cyclophosphamide, which does not require hepatic activation, and thus is potentially feasible for regional administration. We have evaluated the pharmacokinetics of mafosfamide in the CSF following intraventricular administration. Our results indicate that therapeutic levels of this compound can be achieved in cerebrospinal fluid following intraventricular dosing. In addition, we have demonstrated that intrathecal therapy with this compound is feasible and safe. Further studies of this approach are being pursued in an effort to introduce this approach into clinical studies as rapidly as possible. A phase I study is now open.

An innovative system which permits assessment of continuous intra-CSF drug administration has been developed in the nonhuman primate. A unique lateral ventricular catheter attached to a continuous infusion portable pump, together with the 4th ventricular Ommaya reservoir enables us to deliver and monitor drug administration by continuous intra-CSF administration.

Using methotrexate as a model drug we have studied continuous intra-CSF administration and have demonstrated that 1) when compared to intraventricular bolus therapy continuous intraventricular infusion maintains target CSF methotrexate concentrations longer with a lower total dose, 2) continuous intra-CSF infusion avoids the high-peak methotrexate concentrations associated with bolus intrathecal dosing and thus may be less neurotoxic. Based on these studies a clinical trial evaluating this approach is now being pursued.

Lymphoblasts from 28 patients were studied for evidence of mdr-1/ P-170, the gene encoding for the plasma membrane glycoprotein associated with multidrug resistance, using RNase protection, RNA in situ hybridization and immunohistochemistry. Overexpression with gene amplification was identified in the cells of three relapsed patients and from one patient at diagnosis (this patient failed to achieve a completed remission with induction therapy.) In situ hybridization, immunohistochemistry, and drug uptake studies demonstrate that this overexpression is heterogeneous. It appears from these studies that

overexpression of mdr-1/P-170 is one mechanism of drug resistance in ALL. A protocol for relapsed ALL patients who express the MDR phenotype is being initiated which will attempt to reverse this form of drug resistance.

2. In previous studies, we demonstrated heterogeneous, though generally distinctive, molecular genotypes for each of three leukemias, B-cell precursor ALL of childhood, T-cell ALL of childhood, and ALL of infancy. Within each group, a spectrum of developmentally pre-committed lymphoid precursors from all Ig and T-cell receptor (TcR) genes germline, to more mature cells with multiple rearrangements have been identified. A recent study we performed suggested that genotypically less mature leukemias may manifest a more difficult course, and that genotypic heterogeneity may be of clinical relevance. This will be the topic of a prospective investigation in a new NCI protocol for the treatment of high-risk patients with ALL. The utility of immune receptor gene rearrangements as markers for preclinical disease detection and the sensitivity and specificity of the PCR reaction using primers which amplify the hypervariable region of Ig heavy chain also is being evaluated.

3. 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.

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Methotrexate (MTX) is the most widely used intrathecal (IT) antineoplastic agent. Accidental IT overdose can produce severe and frequently lethal toxicity. Despite currently recommended interventions, the outcome is often fatal. The carboxypeptidase G class of enzymes rapidly hydrolyze MTX into the inactive metabolite 4-deoxy-4-amino-N^{10}-methylpteroic acid and glutamate. The gene for one member of this class of enzymes, carboxypeptidase-G_2 ($CPDG_2$), has been cloned, and the enzyme purified on a large scale. We evaluated $CPDG_2$ as a potential IT rescue agent for IT MTX overdose in our primate model and studied the CSF pharmacokinetics of MTX with and without $CPDG_2$ rescue. The CSF MTX half-life of 2.3+0.2 hours was decreased to 33.2+6.2 seconds by $CPDG_2$ resulting in a greater than 400-fold decrease in CSF MTX concentration 5 minutes after enzyme administration. Subsequently, groups of three monkeys received either
25 mg IT MTX (equivalent to 250 mg in humans) followed by 150 U IT $CPDG_2$ or
50 mg IT MTX (equivalent to 500 mg in humans) followed by 300 U IT $CPDG_2$.
All animals survived without neurotoxicity. Our studies suggest that $CPDG_2$ may prove to be an important addition to the currently recommended strategy for the management of IT MTX overdose.

We demonstrated that $CPDG_2$ rapidly and dramatically reduces the CSF MTX half-life within minutes of administration and that this approach will rescue animals from IT MTX overdose without producing toxicity.

4. We have recently demonstrated that the polymerase chain reaction (PCR) can be used to identify chromosomal breakpoint locations in the small non-cleaved lymphomas. Making use of the repeat sequences in the u switch region, we have developed three different sets of oligonucleotide amplimers which are capable of amplifying fragments containing portions of both chromosomes 8 and 14 (i.e., contain the breakpoint itself) and are also able to distinguish between three separate breakpoints in the regimen of the c-myc gene- breaks within the first c-myc intron, the first c-myc exon, or in the immediate c-myc 5′ flanking sequences.

The clinical importance of these findings stems from the extremely high degree of sensitivity inheritant in PCR. Pediatric Branch investigators have been able to detect, in artificially created cell mixtures, the presence of one cell in a million containing the breakpoint location in one of the regions that we are able to detect by PCR. Thus, this technique is not only capable of providing definitive diagnosis, but should enable us to identify very small numbers of cells in tissues not recognized microscopically as being involved. In addition, this technique can be used to follow the presence of minimal residual disease in the bone marrow after therapy, and could be of value in predicting which patients will relapse.

5. Pediatric Branch investigators have been able to specifically inhibit the expression of the c-myc gene in a subset of Burkitt's lymphomas. This has been accomplished by using an antisense oligonucleotide directed against intron sequences which are present in the mature messenger RNA

species in Burkitt's lymphomas with c-myc first intron breakpoints on chromosome 8. Both cellular proliferation and c-myc protein expression were inhibited in the experiments. These findings demonstrate that the molecular abnormalities in tumors may also provide a target for specific therapeutic endeavors. Because only Burkitt's lymphoma cells, and not normal cells, contain the genetic abnormalities, such therapeutic approaches may be highly selective. We are pursuing pre-clinical studies with anti-sense oligonucleotides using Burkitt's lymphoma xenografts in a nude mouse model.

6. Dr. Lee Hellman has extended his studies of insulin-like growth factor II in neuroblastoma and 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.

7. These investigators have identified IGF-II as an autocrine growth and motility factor IV rhabdomyosarcomas (RMS). We have recently 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.

8. Since IGF-II acts as a mitogen in rhabdomyosarcoma cells via the type I IGF receptor, we have created an IGF-I-PE40 oncotoxin in collaboration with the Laboratory of Molecular Biology, DCBD. This toxin specifically bind type I IGF receptors and is capable of killing cells bearing such receptors on the cell surface.

This toxin molecule has been demonstrated to inhibit the growth of three separate RMS cell lines. We are currently working to improve the binding of the IGF-I-PE40 toxin molecule to type I receptors by structurally modifying this protein. In addition, we are also attempting to fuse the PE40 toxin molecule to a monoclonal antibody that alone is capable of inhibiting the growth of RMS cell lines.

9. 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.

10. In our unique laboratory model of candidiasis we have developed methods in which to evaluate promising antifungal agents in various clinical settings. These include acute disseminated infection, chronic infection (e.g., hepatosplenic candidiasis), subacute or local infection and prophylaxis. This permits a more reliable assessment of antifungal strategies and has enabled us to determine that a new triazole, fluconazole, may offer benefit for early (e.g., prophylactic) use in neutropenic hosts. Accordingly, we are about to initiate a randomized

clinical trial to assess the utility of prophylactic fluconazole in pediatric and adult cancer patients.

We have also studied the tissue and plasma pharmacokinetics of a number of other new antifungal agents, including itraconazole, Sch 39304 and cilofungin. We have also tested all of these agents with fungal challenges and are assessing their role for clinical study.

We have also demonstrated important phenotypic alterations and biochemical changes in Trichosporon beigelii, an emerging pathogen in cancer patients.

11. Drs. Pizzo and Walsh have studied the effects of antifungal agents (amphotericin, SFC, fluconazole, ketoconazole, cilofungin, Sch 39304), antiretroviral dideoxynucleosides (AZT, ddC, ddI) and cytokines (GM-CSF, G-CSF) on neutrophil function. Within therapeutic concentrations, the antifungal agents do not adversely effect neutrophil function. Of the dideoxynucleosides, ddI has a stimulating effect as do the cytokines. G and GM-CSF stimulate both normal and abnormal neutrophils.

12. These same investigators have demonstrated the superior efficacy of cilofungin, the first clinically studied cell wall active agent, against disseminated candidiasis in persistently granulocytopenic rabbits when administered by continuous infusion compared to intermittent infusion, representing for the first time, an experimental rationale for continuous infusion of a systemic antifungal agent.

13. In our experimental rabbit model, we demonstrated that recombinant G-CSF was able to shorten the duration of neutropenia and induce superoxide production. G-CSF was more effective for prevention than treatment of candidiasis.

14. Following extensive preclinical assessment, we completed a multicenter trial demonstrating the utility of measuring antigens to Candida cytoplasmic enclose (a 48 kD) as a new marker of invasive candidiasis.

15. PMN from HIV-infected children were demonstrated to have significant impairment in their bactericidal capacity against S. aureus. In vitro incubation of defective PMN with GM-CSF corrected the bactericidal impairment. These findings may help explain the increased incidence of bacterial infections in this population, and suggest a potential therapeutic role for GM-CSF.

16. To better understand the humoral deficiency of HIV+ children, we measured IgG subclasses and correlated their levels with the frequency of bacterial infections. No association was found between low levels of specific IgG subclasses and increased susceptibility to bacterial infections. We concluded that other functional parameters than quantities of antibodies are more important in humoral deficiency of these patients.

17. Because T helper cells are the critically involved immune cells in HIV infection, we investigated their function in a group of HIV+ children and compared it to that of HIV- adults and healthy control children. Different patterns of unresponsiveness of T helper cells to recall and allogeneic

antigens as well as 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.

RADIATION ONCOLOGY BRANCH

The Radiation Oncology Branch continues to meet its three major goals: 1) major emphasis on clinical trials of a combined modality nature, collaborative with other clinical branches; 2) a strong radiation biology program, with heavy emphasis on basic science, radiologic physics, and questions of clinical relevance; and 3) a training program in radiation oncology, equivalent to the stature of the programs of training in the Medical, Surgical, and Pediatric Branches within the NCI.

On-going clinical work focuses on small cell carcinoma of the lung, mycosis fungoides, soft tissue sarcomas, pediatric sarcomas, lymphomas, and Hodgkin's disease. These will be presented by other respective Branches, under whose aegis the protocols are carried out.

A primary ROB study centers on Stage I and II breast cancer. Patients with such carcinomas are randomized to receive either modified radical mastectomy or definitive radiation with preservation of the breast following a lumpectomy. This study is now completed, with approximately 250 patients randomized. There is no obvious superiority of either arm, suggesting that the long-term results (10 years) will be comparable. This study differs from studies carried out by Fisher of NSABP in that the surgical excision makes no attempt to have the margins surgically negative, but simply removes the gross lump and also calls for an implant into the tumor bed. Cosmesis is a major end-point in addition to survival and freedom from relapse. This study is open to women who have masses up to 5 cm, with or without nodes, thereby making breast preservation applicable to the vast majority of women who present with breast cancer in this country. Patients with positive nodes receive adjuvant chemotherapy. Psychosocial aspects are under investigation. The next series of breast cancer studies will be focusing on adjuvant chemotherapy issues, and as such, they will be coordinated through the Medicine Branch, rather than ROB. Our next primary effort for clinical investigation will be focusing on bladder cancer. These will be studies carried out in conjunction with Surgery and Medicine. Superficial bladder cancer will be studied with photodynamic therapy, whereas invasive bladder cancer will receive both chemotherapy and radiation, with a heavy emphasis on Cesium implants. It's hard to know how much difficulty we will run into this time in terms of recruiting patients with bladder cancer for our studies. Nonetheless, we will attempt to do so in conjunction with Dr. Linehan of Urology.

Halogenated pyrimidines have been investigated as radiosensitizers, especially IUdR. Our first phase I study of IUdR was completed with special attention to unresectable sarcomas and gliomas. Glioma information

compares favorably to other reports, with a median survival of approximately 14 months in high-grade patients, and several patients alive well beyond three years. In unresectable sarcomas, there have been striking regressions of unresectable masses, and have several patients whose masses have gone away completely with IUdR and radiation of a Phase I study. The probability of local control in unresectable sarcomas has been over 60%, despite the fact that these masses are generally considered radioresistant and have typically been huge in size. We have just begun to take patients with unresectable sarcomas and randomized them to receive IUdR, "yes" or "no", in conjunction with radiation therapy. We've also begun a broad protocol for IUdR in a wide variety of cancers. We hope to investigate intra-arterial IUdR for glioma, before deciding whether or not to randomize patients to intraaterial or intravenous IUdR, but this project has allowed because of the departure of a key neurosurgeon.

Photodynamic therapy trials continue and have been expanded into other diseases. Branch scientists have utilized hematoporphyrin derivative, and laser-controlled sources of light in the treatment of superficial cancers of the skin and mucous membranes. We've also used this for occluded bronchi and to peritoneal surfaces by means of intracavitary administration of the light. Clinical studies in the thorax have begun in collaboration with Dr. Harvey Pass of the Surgery Branch. We also hope to use such treatment in the thorax, but again have not yet been allowed by the FDA to do so. The preliminary results on the peritoneal cases with ovarian cancer appear quite promising, as part of a Phase I study in patients with recurrent disease. In addition to peritoneal investigation, we have begun to investigate the role of photodynamic therapy of the pleura, with a Phase I study that is focusing on mesothelioma.

In the laboratory, we continue to focus on the mechanisms of sensitization or protection, resulting from radiation modifiers, and the investigation of mechanism of action of several different cytotoxic agents. We have studied sulfhydryl compounds heavily, especially glutathione, and its relationship to cell killing or protection by either radiation or chemotherapy. Additional work has gone on in heat shock proteins and in the characterization of human tumor cell lines in conjunction with other Branches. The laboratory has demonstrated conclusively that cells, which are pleiotropically drug resistant, are not necessarily resistant to radiation therapy; moreover, such cells are definitely not resistant to photodynamic therapy. In addition, it synthesized whole new array of nitroxides, which appear to have definite radiation protection associated with them. This protection has been shown to protect against hair loss in animals, and appears to be effective at whole body radiation protection, which has definite implications for radiation safety, as well as the Defense Department. This work is early in its infancy, but appears highly interesting, and it will continue heavily throughout the next year.

Under the direction of Dr. Gansow, we've been able to label a variety of monoclonal antibodies by means of newly synthesized chelates to various isotopes. We've actually treated patients with radiolabeled antibody, but, at the moment, the only antibodies that have been studied in patients are B72.3 labeled with iodine for intraperitoneal use, and the T_{101} labeled with Yttrium. Before the end of this fiscal year, we will be doing studies

imaging patients with B1 antibody against the B-cell lymphoma, in preparation for treatment studies with special intention of treatment of B-cell lymphoma.

Surgery Branch

Laboratory and clinical efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients.

Significant laboratory accomplishments of the Surgery Branch in the last year:

1. Prior experimental studies in mice showed that the adoptive transfer of tumor infiltrating lymphocytes plus IL-2 was from 50-100 times more effective than the adoptive transfer of LAK cells in mediating the regression of established lung and liver metastases in a variety of murine models. We have now shown that murine TIL with enhanced in vivo activity can be grown from tumors by prior separation with immunomagnetic beads and growth in low levels of interleukin-2.

2. Significant therapeutic synergies have been seen in animal models using TIL in combination with local radiation therapy or with alpha interferon. These combined treatments utilizing TIL can result in the elimination of tumor burdens greater than those successfully treated by TIL and IL-2 alone. These studies have important implications for the treatment of human cancer.

3. Techniques have been developed for inserting foreign genes into human tumor infiltrating lymphocytes using retroviral mediated gene transduction. Tumor infiltrating lymphocytes have been successfully transduced with the gene coding for neomycin resistance and these cells can grow in high concentrations of the neomycin analog G418 that is lethal to all other eukaryotic cells.

4. Genes coding for tumor necrosis factor have been successfully introduced into human TIL. These TIL produce over 100 times the normal level of tumor necrosis factor and have significant concentrations of membrane-bound TNF as well. Plans are underway to utilize these TNF-modified TIL for the treatment of human cancer.

5. Tumor infiltrating lymphocytes have been isolated from patients with melanoma. These TIL exhibit unique lytic specificity from the tumor from which they were derived and not for other normal tissues or other allogeneic tumors. These TIL have now been defined as oligoclonal populations of T lymphocytes exhibiting MHC restricted lysis of autologous tumor.

6. Using repetitive selection with lytic cells, tumor cell lines resistant to TIL lysis have been identified. These lines have been used to demonstrate that multiple tumor specific antigens exist on single melanoma cells. These sensitive and resistant lines are now being used to clone the gene that codes for tumor-associated antigens in patients with melanoma.

7. Using both mouse and human TIL, we have shown that cytokines can be specifically released from TIL populations when stimulated by specific tumor. This technique represents an alternate method for identifying specific immune reactions in patients with cancer. The specific cytokines produced by human and murine TIL include gamma interferon, GM-CSF, TNF-alpha and interleukin-3.

8. By examining human TIL lysis of autologous as well as HLA-matched allogeneic melanomas, it has been shown that human melanomas can possess shared antigens that can be recognized in patients sharing specific HLA antigens.

9. Human TIL on patients with melanoma have been shown to have an increased frequency of V-alpha-2 expression of their T cell receptors. This selective utilization of the V-alpha-2 subregion implies a commonality of recognition by human TIL.

10. Significant therapeutic synergies have been seen in animal models using combination cytokine treatment. The most effective combinations studied are interleukin-2 and alpha interferon as well as the use of interleukin-2 and tumor necrosis factor. Recently the combined administration of interleukin-2, tumor necrosis factor and alpha interferon have been shown to be more effective than any of the two cytokines alone. These studies are forming the foundation for combination cytokine therapy in man.

11. Interleukin-6 administration has been shown to mediate the regression of established weekly immunogenic sarcomas in mice. These antitumor effects were found in the absence of apparent toxicity. The combined administration of IL-6 and tumor necrosis factor was more effective than either alone. The anti-tumor effect of IL-6 was mediated through a radiosensitive host component. These were the first studies to identify an antitumor effect of interleukin-6 administration.

12. Studies of toxicity resulting from tumor necrosis factor administration have revealed that free radical scavengers can abrogate some of the toxicity of TNF administration without interfering with its antitumor activity.

13. Pre-clinical studies using polyethylene glycol modified interleukin-2 (PEG-IL-2) showed that it has in vivo antitumor activity and can promote the survival of TIL in vivo in mice.

14. Interleukin-7 has been shown to be a T cell growth factor for human cells and can induce LAK activity from human cells as well.

15. Interleukin-4 has been shown to increase the expression of certain Fc receptors and significantly alter monocyte function in vitro and in vivo.

16. Triple immunodeficient beige/nude/xid mice can be used as models of human melanoma. Fresh human melanoma cells widely disseminate following injection into these triple immunodeficient mice. This mouse model is being used to develop assays for the in vivo effectiveness of TIL and LAK cells.

17. Polymorphic probes localized to the short arm of chromosome 3 have been used to detect the loss of heterozygosity at this locus in tumor tissue from 51 of 58 patients with sporadic renal cell carcinoma. Deletion analysis has shown that this is in the same region as the gene for the familial form of renal cell carcinoma associated with Von Hippel Lindau disease.

18. Studies of the molecular genetics of renal cell cancer have demonstrated abnormalities at recessive gene loci on chromosome 11 near the Wilms locus, and on chromosome 13 at the retinoblastoma locus, and on chromosome 17 at the NM 23 locus. The presence of these abnormalities from patients with advanced renal cancer suggest that these abnormalities may be associated with progression or metastases of renal cell cancer.

19. DNA sequence deletions have been demonstrated in the short arm of chromosome 3 in the familial form of renal cell carcinoma associated with Von Hippel Lindau disease and in patients with pheochromocytoma and the spinal and cerebellar are hemangioblastomas from patients with this disease. Twenty-five families with Von Hippel Lindau disease have been studied to more precisely locate the VHL disease gene on chromosome 3.

20. Suramin, in clinically achievable doses, can inhibit the proliferation of prostate carcinoma in vitro and in vivo.

21. Tolerance to recombinant human tumor necrosis factor can be induced in tumor-bearing animals by repetitive exposure of the animals to subletha1 bolus doses of TNF. Tolerance reduces the toxicity but also the therapeutic efficacy of subsequent doses of TNF.

22. Macrophages exposed to either lactic acidosis or photodynamic therapy in vitro induced the gene for TNF and secrete bioactive TNF.

23. Recent studies have demonstrated significant interactions between the immune and endocrine systems via the release of cytokines. In vitro studies suggested a direct action of TNF on both the pituitary gland with the secretion of ACTH in the adrenal cortex with the secretion of cortisol.

24. A variety of lung cancer cell lines have been shown to be sensitive in vitro to photodynamic therapy. The amount of sensitizer uptake may be a factor in individual cell line susceptibility to photodynamic therapy.

25. Pre-clinical studies in large animals have demonstrated that animals can tolerate as high as 40 jules/cm^2 of photodynamic therapy to intrathoracic organs such as the esophagus, diaphragm, heart, chest wall and lung. These pre-clinical studies have led to the development of clinical protocols to study photodynamic therapy in man.

26. Interleukin-1-alpha and tumor necrosis factor can inhibit the growth of MCF-7 breast cancer cells in a dose-dependent manner and can induce the expression of TNF mRNA in MCF-7 breast cancer cells.

27. Interleukin-1 alpha can down-regulate the estrogen receptor by acting at the post-transcriptional level and can act synergistically with

tamoxifen to antagonize the estradiol stimulation of growth of MCF-7 breast cancer cells in vitro.

Significant clinical accomplishments of the Surgery Branch include the following:

1. Clinical trials with lymphokine activated killer cells and high-dose interleukin-2 or the administration of high-dose interleukin-2 alone have demonstrated, in over 300 patients, that approximately 10% of patients with metastatic melanoma and renal cell cancer can undergo a complete regression of all cancer and approximately 1/3 of patients will undergo at a 50% regression of malignancy.

2. 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. Further follow-up is necessary. This study has shown a tendency towards increased complete response rates and survival in patients receiving LAK cells and IL-2 ($p2 = .07$).

3. Pilot trials utilizing tumor infiltrating lymphocytes plus IL-2 in patients with advanced melanoma have shown that approximately 40% of all patients with advanced melanoma will show objective regression of malignancy. Responses are also seen in patients that have previously failed other regimens utilizing high dose interleukin-2.

4. The first gene transfer trials conducted in man have begun. Eight patients with advanced melanoma have received treatment with autologous TIL modified with the gene coding for neomycin phosphotranferase, which confers resistance to the antibiotic, neomycin. These studies have shown that gene-modified cells can survive for up to 189 days in the circulation and for up to 64 days at the tumor site. No toxicity has been seen in patients due to the gene transfer. Clinical trials utilizing TIL modified by the gene for tumor necrosis factor are planned.

5. Clinical protocols have been performed using increasing doses of alpha interferon and interleukin-2 in the treatment of patients with advanced cancer. These studies suggest that the combination of these two cytokines provides higher response rate in advanced cancer patients than the administration of each cytokine administered alone.

6. A Phase 1 clinical trial of polyethylene glycol-modified interleukin-2 (PEG-IL-2) has been conducted. The maximum tolerated dose of PEG-IL-2 in patients with metastatic cancer is 18 million I.U. m^2/wk by i.v. bolus. Currently, a prospective randomized trial is being conducted comparing repeated cycles of high dose interleukin-2 versus an induction cycle of interleukin-2 followed by low dose maintenance PEG-IL-2.

7. Extensive trials in humans with advanced cancer have been conducted using the administration of interleukin-4. Interleukin-4 causes a dose-related increase in the vascular leak syndrome, nasal congestion and gastritis. In studies using interleukin-4 alone, antitumor effects have not been seen.

8. Trials have been initiated using combinations of interleukin-4 and interleukin-2. Responses have now been seen in patients with renal cell cancer, melanoma and in one patient with breast cancer. These studies are in progress.

9. A combination regimen of 5-FU and leucovorin and IL-2 has undergone Phase I and II testing. This regimen has shown a 55% incidence of complete and partial responses in patients with metastatic colon cancer and is undergoing an evaluation of predictors and correlates of response.

10. A Phase I trial, studying the combination of radiation therapy and interleukin-2 has been completed. This trial is a prelude to the introduction of TIL in conjunction with irradiation and IL-2. Pre-clinical animal models have predicted that irradiation will synergize with TIL and IL-2 administration in inducing tumor regression.

11. Phase II trials of combinations of interleukin-2 with monoclonal antibodies to melanoma and colorectal cancer have been conducted in patients with advanced cancer. Clinical trials using combinations of IL-2, monoclonal antibodies and the administration of LAK cells are now in progress.

12. A prospective, randomized trial comparing breast preservation surgery plus radiation therapy to treatment with modified radical mastectomy has been completed and follow-up is continuing. Preliminary results indicate that breast preservation surgery is associated with the same long-term survival rates as patients receiving modified radical mastectomy.

13. A prospective randomized trial has been initiated comparing intensive pre-operative versus post-operative chemotherapy (5-FU, adriamycin, cytoxan, leucovorin, GM-CSF) for the treatment of clinical stage II breast cancer in women.

14. Photodynamic therapy has been successfully used for the bronchoscopic treatment of endobronchial malignancies in phase II clinical trial.

15. Clinical trials have begun using photodynamic therapy in the treatment of thoracic tumors in man. Phase I studies have shown that up to 15 joules/cm^2 of photodynamic therapy can be safely delivered intrathoracically to patients following debulking of pleural malignancies.

16. Techniques have been developed for the delivery of photodynamic therapy to the peritoneal cavity of patients with disseminated intra-abdominal malignancies. Phase I clinical trials of patients refractory to conventional treatment with ovarian cancer, gastrointestinal cancer and sarcoma in the abdomen using photodynamic therapy have begun.

17. Prospective randomized trials of intraoperative radiation therapy have been conducted on patients with gastric cancer, pancreatic cancer and retroperitoneal sarcomas. At the present time, although a suggestion of improved local control exists, there has been no improvement in disease-free or overall survival in patients receiving intraoperative radiation therapy. These important studies are the only randomized test of the

hypothesis that intraoperative radiation therapy improves treatment outcome when compared to external beam therapy.

18. A prospective trial has been conducted treating patients with high grade extremity sarcomas with chemotherapy and randomizing these patients to receive either post-operative radiation therapy or no radiation therapy. After a median follow-up of four years, there is no difference in disease-free or overall survival between these groups but the incidence of local recurrence is significantly higher in the group that has no radiation therapy.

19. Aggressive surgical resection has been explored in the management of patients suffering from MEN-1 syndrome. Clinical trials suggest that these patients can be cured of insulinoma and VIPoma but not gastrinoma using aggressive surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07209-02 CO

PERIOD COVERED

October 1, 1989 through September 30, 1990

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

Administration of 2', 3'-dideoxyinosine (ddI) for severe HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

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 of 10 mg/kg were associated with anti-HIV activity; 3) dose-limiting toxicities were painful peripheral neuropathy, pancreatitis and hepatitis; 4) doses of 3 of 10 mg/kg/day were well tolerated in the majority of patients with AIDS or AIDS-related complex and were associated with long-term clinical and laboratory improvement. Based primarily on the results of this study (with supportive evidence from 2 other Phase I studies), 3 Phase II/III trials of ddI, sponsored by the NIAID and Bristol-Myers Squibb Company were launched in October of 1989 in medical centers around the country. In addition, the FDA enabled patients who could not tolerate AZT or had failed AZT to receive ddI under the mechanisms of a Treatment IND or open label protocol, respectively. At present, more than 10,000 patients have received ddI throughout the United States under these protocols. We are continuing to follow our Phase I patients receiving ddI. We have learned that survival can be excellent with this drug - 80% of AIDS patients entered on the study are alive at 20 months. In addition, we have observed that patients with AIDS dementia can have improvement on ddI. Finally, we have observed that patients with extensive prior AZT use have limited CD4 rises on ddI, whereas they do respond with decreases in HIV p24 antigen. We are now exploring the combination of ddI and DHPG (a drug used for retinitis) and ddI used with interferon.

PHS 6040 (Rev. 1/84) GPO 914-918

INTRODUCTION

The purine analogue 2',3'-dideoxyinosine (ddI) was found to have activity against HIV *in vitro*. Upon being activated to dideoxyadenosine-5'-triphosphate, it is believed to act at the level of reverse transcriptase. Several properties of ddI made it suitable for clinical testing: it had a comparatively low toxicity for T cells and bone marrow progenitor cells; it had potent anti-HIV activity in monocytes; and once activated to the active triphosphate moiety, it could remain in cells for a long time (half life over 12 hr). With this background, we instituted a Phase I study of ddI in July 1988 in patients with AIDS or ARC who had less than 4 months of prior therapy with AZT. ddI was found to be well absorbed when given orally with antacids and was well-tolerated for short-term therapy. In addition, most patients receiving doses of 3.2 mg/kg/day or greater had short-term clinical, immunologic and virologic improvement. However, therapy of patients with AIDS or earlier HIV infections will probably have to be administered for months or years. We have thus extended this Phase I trial to evaluate the long term activity and toxicity of ddI in patients with AIDS or ARC. We have also examined its effect on patients with prior long-term AZT therapy or who had HIV-induced cognitive impairment.

MATERIALS AND METHODS

Patients

Three groups of patients with AIDS or ARC were studied: (1) 37 patients who entered the original dose-escalating Phase I study of ddI starting in July 1988. All but 2 had 4 or less months of prior AZT therapy, (2) 5 patients originally treated with intravenous 2',3'-dideoxyadenosine (ddA), a pro-drug of ddI, starting in March 1988, and switched to oral ddI in August 1988, (3) 16 additional patients entered on an extension of the Phase I study starting in July of 1989. Preference was given in this third group to patients who had had more than 4 months of AZT therapy or who had HIV-induced cognitive dysfunction.

In all, 22 patients had AIDS and 36 had ARC. All had anti-HIV antibodies. The AIDS patients included 12 with a history of *Pneumocystis carinii* pneumonia (1 each also had *Mycobacterium avium-intracellulare* infection, cytomegalovirus retinitis, and dementia), 3 with esophageal candidiasis, 5 with Kaposi's sarcoma, and 2 with wasting. Thirty of the ARC patients had oral candidiasis, 5 had weight loss, and 1 had mild cognitive impairment. Three patients were female. Four patients became infected with HIV by heterosexual contact, 1 by occupational parenteral exposure, 1 by receiving Factor VIII concentrate, 1 by using intravenous drugs, and the rest by homosexual contact. The median number of $CD4^+$ cells at entry was $47/mm^3$ (range 4 to 267). All patients had a hemoglobin of $\geq$8.5 g/dl and $\geq$600 neutrophils/mm^3. All but 2 were free of active opportunistic infections at entry. All but 4 of the patients received no antiretroviral therapy during the 4 weeks prior to entry.

Administration of ddI

The protocol was approved by the Institutional Review Board of the National Cancer Institute, and all patients gave informed consent. ddI was provided by the Developmental Therapeutics Program of the National Cancer Institute and Bristol-Myers Squibb Company. After a 2-week period of intravenous dosing, the 37 patients who comprised the original dose-escalating Phase I trial received ddI at oral doses ranging from 0.8 to 51.2 mg/kg/day, divided into 2 or 3 daily doses. Since ddI is acid labile, oral drug was administered to fasting patients either with antacids or in a citrate/phosphate/sucrose ddI formulation. The pharmacokinetic profile of this latter formulation was similar to that of ddI given with antacids. As the trial proceeded, patients initially entered on the lower dose groups were escalated up to a maximum of 9.6 mg/kg/day (except patients who started at 6.4 mg/kg/day were maintained at that dose). All patients on higher doses had their dose reduced to 19.2 and later to 9.6 mg/kg/day when it became apparent that higher doses were associated with a high incidence of toxicity. Patients generally received suppressive therapy for *P. carinii* pneumonia when appropriate.

Monitoring of patients

Patients were followed for up to 21 months on ddI (median 8.9 months); 28 of the patients have received ddI for over 1 year. Those who originally received ddA have received ddA or ddI for over 2 years. The patients were closely monitored for clinical and laboratory changes. Most had more than one determination of lymphocyte subsets and HIV p24 antigen during the 3 weeks prior to therapy; in such cases, mean values were used as the time zero determination. To assess aspects of neuropsychological function known to be affected by HIV, we used the Wechsler Memory Scales to assess memory, the Trail Making Test to evaluate psychomotor speed and attention, and, in one impaired patient, the Mattis Dementia Rating Scale. Mood was assessed with the Beck Depression Inventory.

Statistics

The significance of changes was assessed by the two-sided Wilcoxon signed rank test for paired observations, $p<0.05$ being regarded as significant. The influence of prior AZT therapy on the changes in CD4 cells was assessed using the two-sided Wilcoxon rank sum test, and the influence of initial CD4 cells and the influence of ddI dose on this effect was analyzed using linear multivariate analysis. The method of Kaplan and Meier was used to analyze survival and the relationship of ddI toxicity to time on drug.

RESULTS

Anti-HIV Activity of ddI

In the initial dose-escalating Phase I study, we found that patients receiving 3.2 to 51.2 mg/kg/day of ddI by the oral route had an increase in the total lymphocytes; enhanced delayed type hypersensitivity; and where evaluable, a decrease in HIV p24 antigen during the first 10 weeks of

therapy. As will be discussed below, side effects were most frequent in patients receiving more than 9.6 mg/kg/day of ddI. Thus, doses of 3.2 to 9.6 mg/kg/day appeared to be tolerated and have short-term activity.

As experience was gained with higher doses of ddI, patients originally entered on low doses were escalated up to a maximum of 6.4 or 9.6 mg/kg/day. Thus, the 13 patients who entered at 3.2 to 9.6 mg/kg/day can provide data on the long-term activity of ddI at doses which may be clinically feasible. These 13 patients have received a median of 17 months of ddI (range 8 to 20 months), and all but three are still on drug. The changes in $CD4^+$ lymphocytes and total lymphocytes in these patients is shown in Table 1. They had a mean entry CD4 count of 157 $\pm$ 26 (mean $\pm$ SEM). Upon receiving ddI, they had a maximum mean increase in $CD4^+$ lymphocytes of 78 $\pm$ 31 entry. Statistically significant increases persisted for at least 9 months. Of the 13 patients, 6 had detectable serum p24 antigenemia at entry (mean 220 pg/ml). Four of these 6 patients were still receiving ddI 15 months after entry, and 3 of these 4 patients had undetectable p24 antigen at that time. None became p24 antigenemic on ddI. Thus, the immunologic and virologic improvements in patients receiving ddI can persist for at least 9 months, and in some patients at least 15 months.

In the initial escalating dose study, we preferentially entered patients who had received AZT for 4 or else months. We subsequently entered 16 additional patients at doses of 6.4, 9.6 and 19.2 mg/kg/day, in part to evaluate the effect of prior AZT therapy. Pooling these with the patients from the original Phase I study who started at those doses, the 24 evaluable patients at these doses overall had an increase in $CD4^+$ lymphocytes during the first 10 weeks of study from 115 $\pm$ 20 cells/mm^3 (mean $\pm$ SEM) to 165 $\pm$ 29 cells/mm^3 (P=0.0096). However, the patients who had previously received AZT for less than 4 months had a significantly greater percentage increase in CD4 cells than those with >4 months prior AZT therapy (P=0.00270). This effect of prior AZT therapy was still discernable if the absolute increase was examined or if we corrected for the effect of initial CD4 cell number and the dose of ddI using multivariate analysis. In the patients who had antigenemia, the serum p24 antigen fell from 154 x/: SEM) at entry to 52 x/: 1.27 pg/ml at week 10 (P=0.0049). Substantial decreases in HIV p24 antigen were observed both in patients who had little prior AZT therapy and in those with prior long-term therapy.

In three patients where this was examined, the mean CSF:plasma ratio of ddI concentration 1 hr after an intravenous dose was 0.21. With this background, we examined the effect of ddI on 4 patients who had cognitive dysfunction compared to what one would expect based on their educational and professional backgrounds. Each of these patients, who generally received 9.6 or 19.2 mg/kg/day of ddI, had improvement during 6 to 12 weeks on ddI. The mean Memory Quotient increased from 97 (range 80 - 113) at entry to 110 (range 94 - 121) after 6 to 12 weeks of therapy. Also, mean performance on the Trailmaking B test, a measure of attention and psychomotor speed, improved from 106 seconds (range 152 - 78) at entry to 90 seconds (range 117 - 68). A fifth patient, who was too impaired to

utilize these tests, had improved performance on the Mattis dementia scale from -4.83 SD below the mean of a normal elderly population to -1.64 below the mean after 6 weeks on ddI. These improvements could not be attributed to changes in mood.

Toxicities and Other Clinical Observations

At high daily doses of ddI, painful peripheral neuropathy, sporadic pancreatitis, and sporadic hepatitis were dose-limiting toxicities. In all, 12 patients developed peripheral neuropathy, 3 developed frank pancreatitis, 2 developed mild pancreatitis, and 2 (both with hepatitis B infection) developed hepatitis. In addition, 3 patients seized; however, each was found to have an underlying cerebral disorder, and the relationship to ddI therapy is unclear. No patient died of toxicity. Patients receiving more than 9.6 mg/kg/day had a relatively high probability of developing neuropathy, pancreatitis, or hepatitis during the first 6 months of therapy (Figure 1). By contrast, only 3 of 35 patients developed toxicity on doses of 3.2 to 9.6 mg/kg/day of ddI for up to 21 months of therapy (median 10 months) ($P<0.00001$ for dose effect). This differential toxicity rate is even more striking if one considers that the 35 patients in the latter group received 6.4 or 9.6 mg/kg/day most of the time.

Ten of the 12 patients who developed ddI-associated neuropathy had received high doses of ddI (at least 12.8 mg/kg/day) and had a cumulative total dose of at least 1.5 gm/kg of ddI. By contrast, only 2 of 35 patients receiving lower daily doses developed neuropathy in spite of receiving up to 4.4 gm/kg. Thus, dose intensity is a crucial determinant of ddI neuropathy. The neuropathy generally appeared as a painful or burning sensation in the feet, intermittent at first but then becoming more constant. We found that if ddI was withdrawn when the pain became mild to moderate in intensity and lasted for several hours, the neuropathy generally subsided within several weeks. In 6 patients, ddI was restarted without recurrence.

Frank pancreatitis was observed in 3 patients: 2 receiving 19.2 mg/kg/day, and 1 on 25.6 mg/kg/day. One patient required admission to the intensive care unit. In each case, the pancreatitis resolved rapidly without sequelae. Two of the patients had histories of ethanol abuse. In one, pancreatitis was preceded by marked triglyceride elevation (> 800 mg/dl) which may have been a harbinger of the pancreatic damage. Two patients were subsequently restarted on 6.4 mg/kg/day of ddI without pancreatitis recurring. Two additional patients taking 9.6 mg/kg/day developed what may have been mild pancreatitis. Six patients developed transient asymptomatic amylase evaluations (>1.5 X upper limit of normal); drug was transiently held in two of these patients. In addition, 3 patients had more persistent amylase elevations; in two cases this was associated with xerostomia. Fractionation revealed predominantly salivary isoamylase. The significance of this finding is unclear at this point. Of the 14 patients with pancreatitis or amylase elevations, 8 were receiving aerosolized pentamidine, 2 had recent exposure to trimethoprim/sulfamethoxazole, and 1 had received ranitidine.

Several milder reactions were observed. Five patients reported anxiety, 29 reported some insomnia, 13 reported increased irritability, and 11 reported headaches; none of these effects required drug discontinuation. Two patients became confused after taking triazolam (Halcion) with ddI; this cleared upon temporarily stopping both drugs and did not recur upon readministration of ddI alone. Two patients developed mild erythematous macular eruptions. Most patients receiving more than 9.6 mg/kg/day had asymptomatic increases of 0.5 to 5 mg/dl of uric acid, almost certainly from ddI catabolism. Fifteen patients reported transient abdominal pain or vomiting not associated with hyperamylasemia and not requiring drug discontinuation. Several patients developed diarrhea which appeared to be from the buffer formulation (magnesium-based antacids or citrate/phosphate/sucrose vehicle) rather than from the ddI itself, and patients on average had a slight decrease in serum potassium (mean 0.11 meq/L) upon being switched to the citrate/phosphate/sucrose ddI formulation. Finally, about a third of the patients had elevations of their serum triglycerides above 500 mg/dl at some point.

More than half the patients reported increased energy, reduced sleep or nap requirements, and improved appetite on ddI. On average, they gained 1.4 kg when started on therapy. During the course of therapy, the following opportunistic infections were diagnosed: P. carinii pneumonia (8 pts.); symptomatic cytomegalovirus infection (5 pts); M. avium-intracellulare infection (6 pts.); herpes zoster (3 pts.); cryptococcal meningitis and tosoplasmosis (2 pts. each); and pulmonary tuberculosis (1 pt.). Four of the infections developed during the first 6 weeks of therapy and most likely predated ddI. One patient developed squamous cell carcinoma of the maxillary sinus. No patients developed lymphoma.

In all, 6 patients have died. For those who originally entered with AIDS, the 21 month survival was 80%, and for those with ARC, the 21 month survival was 80%, and for those with ARC, the 21 month survival was 93% (Figure 2). These survival values may not be comparable with historical controls as most patients were stable at entry, many received chemoprophylaxis for P. carinii pneumonia, and 10 patients received AZT at some point while their ddI was held. However, given that the overall median number of CD4 cells at entry was 47/mm^3, controlled trials to formally determine the effect of ddI on survival would appear warranted.

Earlier work with ddI suggested that ddI can improve immunologic and virologic parameters during short-term therapy of patients with AIDS or ARC. We now extend those results and show that whereas high doses can be associated with serious toxicities, many patients with AIDS or ARC can tolerate up to 9.6 mg/kg/day for at least 12 to 21 months without developing such toxicity. In addition, the data suggest that doses of 3.2 to 9.6 mg/kg/day of ddI are associated with virologic and immunologic improvements which can be sustained for at least 9 months. However, while patients with extensive prior AZT therapy may have a decreased viral load on ddI, these patients may have only a slight or absent rise in CD4 cells. Finally, the results demonstrate that ddI can improve cognitive dysfunction in certain patients.

The relationship of ddI toxicity to dose intensity is noteworthy. While toxicity often developed within 6 months in patients receiving more than 9.6 mg/kg/day, lower doses could be tolerated for long periods of time in most patients. Even at the highest doses tested, bone marrow suppression was not prominent, and in fact, hematologic parameters have been observed to improve during ddI administration. It is worth noting that we do not understand the mechanism(s) for ddI toxicities, and one cannot necessarily extrapolate from other drugs causing similar reactions. Azathioprine (Imuran) or 6-mercaptopurine, for example, causes acute pancreatitis in 5% of patients in certain settings. Once pancreatitis occurs with these drugs, rechallenge with lower doses will nearly always induce a recurrence. By contrast, the two patients with ddI-induced pancreatitis who were rechallenged did not have a recrudescence of the pancreatitis. Clinicians should bear in mind that the principal toxicities of ddI (neuropathy, pancreatitis, or hepatitis) may be found in AIDS patients as a result of underlying HIV infection, secondary complications or other medications. (Indeed, ddI has been reported to reverse HIV-induced neuropathy in certain patients.) In particular, cytomegalovirus infection can cause both painful peripheral neuropathy and pancreatitis, and other drugs commonly used in AIDS (such as pentamidine, even given by aerosol, or trimethoprim/sulfamethoxazole) can cause pancreatitis.

It will be important to learn how to best prevent and manage ddI toxicities. Pancreatitis in particular can be a life-threatening complication; as of March, 1990, 7 of approximately 8300 patients with AIDS or severe ARC receiving ddI in clinical trials or in an "expanded access" program died of pancreatitis. Many of these patients were AZT-intolerant and had advanced AIDS, and more needs to be learned about the factors contributing to pancreatitis in this setting. It is clear from the data in this study, however, that attention to dose intensity is a crucial parameter in reducing the risk of toxicities from ddI. Also, until more is know, patients on ddI should avoid alcohol and other drugs associated with pancreatitis or neuropathy. For example, ddI should be temporarily stopped if patients receive intravenous or intramuscular pentamidine. (However, it would be impractical to avoid using ddI along with aerosolized pentamidine, particularly as little drug is absorbed from the lungs.) We now measure amylase and triglyceride levels and temporarily hold ddI when the amylase rises to 1.5x the upper limit of normal or when the triglyceride levels rise above 700 ug/dl in patients starting with normal triglycerides; ddI is then reinstituted when the levels approach normal. Transient asymptomatic elevations of triglycerides or amylase are often seen in HIV-infected patients, but do not constitute clinical pancreatitis. As noted, two patients on ddI had xerostomia and elevated salivary isoamylase, and this finding will bear further study. In regard to neuropathy, we now stop ddI when patients develop foot pain of mild to moderate severity and several hours duration. Finally, until more data emerge, we would avoid the use of cimetidine or ranitidine (which could enhance ddI absorption or cause pancreatitis in their own right) and triazolam (which may have caused confusion in two patients). The specific formulation is also important. As noted, the buffers used to effect absorption of this acid-labile drug can cause symptoms independent from ddI. The citrate/phosphate/sucrose vehicle may cause substantial diarrhea in certain patients (and even

decreased potassium) which is probably unrelated to the drug itself. Additional formulations are now being examined.

Overall, the results of this trial suggest that certain doses of ddI (3.2 to 9.6 mg/kg/day) may be tolerated in most patients for long-term therapy and are associated with evidence of anti-HIV activity. However, the optimal dose of ddI will not be known without further study. Indeed, the optimal dose of AZT is still under discussion 3 years after its approval as an anti-retroviral drug. Finally, while relatively few patients died on this study, it should be stressed that this extended Phase I study cannot conclusively address the questions of whether ddI reduces disease progression or mortality in patients with HIV infection, or whether it is superior, equal, or inferior to AZT as an anti-AIDS drug. The resolution of these questions will require carefully controlled clinical trials.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 07210-01 CO

PERIOD COVERED

June 1, 1989 through May 31, 1990

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

Study of non-Hodgkin's lymphoma in the setting of severe HIV infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Lymphoma is a well known complication of HIV infection. Previous studies by this laboratory have identified several mechanisms of B cell activation in patients with HIV infection which may lead to lymphoma: 1) increased numbers of Epstein-Barr virus infected B cells; 2) T-cell dependent polyclonal B cell activation induced by HIV; and 3) antigen-specific B cell activation by HIV. We have recently observed that eight of 55 patients (14.5%) with AIDS or severe ARC entered onto three long-term phase I trials of azidothymidine (AZT) or AZT-containing regimens at the NCI between 1985 and 1987 developed a high-grade non-Hodgkin's lymphoma (NHL), B cell type after initiating antiretroviral treatment. The lymphomas developed a median of 23.8 months after the initiation of therapy. The estimated probability of developing lymphoma by 36 months was 46.4%. The patients who developed NHL had <100 T4 cells/mm^3 for a median of 17.8 months prior to that diagnosis. All patients presented with NHL in extranodal sites. Patients with symptomatic HIV infection who survive for up to three years on antiretroviral therapy may have a relatively high probability of developing lymphoma. As improved therapies for the treatment of HIV and its complications result in prolonged survival, NHL may become an increasingly significant problem. We are now exploring means of improving therapy in this disorder. In collaboration with Dwight Kaufman, M.D. in the Radiation Oncology Branch, we are conducting a trial of combination chemotherapy with AZT and GM-CSF. Preliminary results suggest that some patients may respond to this regimen but that toxicity can be a problem.

INTRODUCTION

In 1981, acquired immune deficiency syndrome (AIDS), a disorder now known to be caused by infection with human immunodeficiency virus (HIV), was first recognized as a new illness occurring in individuals in certain risk groups. Soon after the recognition of AIDS as a new disease, the clustering of high-grade non-Hodgkin B cell lymphomas in individuals in these same risk groups and infected with HIV was noted. These lymphomas frequently occurred in extranodal sites, particularly the central nervous system. In 1985, the definition of AIDS was expanded by the Centers for Disease Control (CDC) to include high grade non-Hodgkin lymphoma AIDS as an AIDS-defining illness in certain clinical settings. At present approximately 3% of new AIDS cases have non-Hodgkin lymphoma as their AIDS-defining illness. The incidence of HIV-associated non-Hodgkin lymphoma continues to rise.

The occurrence of non-Hodgkin lymphoma in other settings of immunosuppression has been recognized for years. Indeed, the inter-relationship between immunodeficiency and cancer has been a major focus of research for several decades. In 9873, the Immunodeficiency Cancer Registry was established to maintain a central registry of malignancies that develop in patients with genetically determined immunodeficiency diseases. As of 2987, nearly 500 such cases have been reported, of which 50.7% are non-Hodgkin lymphoma. In addition, an increased incidence of neoplasms has been documented in patients iatrogenically immunosuppressed following organ transplantation. Thirty-six percent of such neoplasms are non-Hodgkin lymphoma. Many of these tumors have unusual sites of presentation, including the central nervous system. Thus, it would appear that non-Hodgkin lymphoma occurs with substantially increased frequency in the setting of immunosuppression, particularly in patients with defects in T cell function.

The course of HIV infection is changing as a result of therapeutic advances. In particular, the life expectancy of patients with HIV infection is presently increasing as a result of improved therapies both for the treatment of HIV-associated infectious complications and of HIV infection itself. Relatively little information now exists on the incidence of lymphoma in AIDS patients who are followed longitudinally, particularly since the advent of antiretroviral therapy.

The National Cancer Institute (NCI) has a cohort of patients that has received anti-HIV therapy for an extended period of time. These patients represent some of the earliest recipients of zidovudine (azidothymidine, AZT) and zidovudine-containing regimens, and as such my provide a database of what may be observed with the widespread use of such therapies. We have observed the development of non-Hodgkin lymphoma in an unexpectedly high number of these patients, particularly those who were long-term survivors with decreased T4 lymphocytes. It is possible that an increased cumulative incidence of such lymphomas may be an ironic by-product of prolongation of survival by effective antiretroviral therapy.

MATERIALS AND METHODS

Patients: We examined the charts of 55 HIV-seropositive patients receiving long-term dideoxynucleoside antiretroviral therapy entered into three Phase I studies in the Clinical Oncology Program (COP) of the NCI from 1985 to 1987. The studies were: (1) the Phase I study of zidovudine alone (29 patients); (2) a pilot study of zidovudine with simultaneously administered acyclovir (8 patients); and (3) a pilot study of zidovudine alternating with 2',3'-dideoxycytidine (18 patients). These were the only studies initiated during that period in which patients administered antiretroviral therapy were followed for more than six months. All of the patients in these three trials had either AIDS or severe AIDS-related complex, the latter having either oral candidiasis or greater than 10% weight loss. All patients had less than 350 T4 cells/mm^3 at the time of entry. Patients with a Karnofsky Performance score less than 70, active opportunistic infections, or an expected survival of less than three months were not included. In general, these were patients with AIDS or poor-prognosis AIDS-related complex who were clinically stable at the time of entry.

Of these 55 patients, 8 developed non-Hodgkin lymphoma. In seven of the patients, the diagnosis was made antemortem, while one patient was diagnosed postmortem. Each of those patients diagnosed antemortem underwent staging evaluation and treatment at the NCI with combination chemotherapy, radiation therapy, or a combination of these modalities.

Patient evaluations: All patients on these studies were followed at the Warren G. Magnuson Clinical Center of the National Institutes of Health (NIH), Bethesda, Maryland. Clinical and laboratory evaluation were performed every two to four weeks, and patients were admitted for evaluations and treatment when medically indicated. In all patients, lymphocyte subsets reacting to Leu 3 (CD4+, T4+, helper-inducer T cells) or to Leu 2 (CD8+, T8+, suppressor-cytotoxic T cells) were periodically analyzed by flow cytometry. The 1987 revised CDC criteria for AIDS was used in defining the onset of each patient's AIDS-defining illness.

Immunophenotyping: The immunologic phenotype of the lymphoma cells was determined by immunostaining with monoclonal antibodies to antigens expressed on B cells, T cells and mononuclear phagocytes. The six cases (patients 2, 4-8) immunostaining was performed on paraffin sections using the avidin-biotin-complex immunoperoxidase technique as previously described. Cells were stained for the B cell associated antigen, L-26, the T cell associated antigen, UCHL-1, and lysozyme. In patients 1 and 3, immunostaining was performed on air-dried cytocentrifuge preparations from the lung aspirate and peritoneal fluid respectively. In patient 3, cells derived from the pleural fluid were stained with monoclonal antibodies and subjected to phenotypic analysis using a flow cytometer and the avidin-biotin-complex immunoperoxidase technique applied to cytocentrifuged preparations. An extensive battery of monoclonal antibodies directed against T cells, B cells, mononuclear phagocytes, and myeloid cells was employed. Both a skin biopsy of recurrent large cell immunoblastic lymphoma and a brain biopsy showing small non-cleaved cell lymphoma from

patient 3 were also subjected to immunoperoxidase staining on paraffin sections.

Statistical Analysis: The method of Kaplan and Meier was used to estimate the probability of lymphoma developing in this group of patients as a function of time on antiretroviral therapy. Follow-up was available to the time of death or to the present in 52 of the 55 patients; three patients lost to follow-up were censored at the most recent point at which data was available. Twenty-nine patients who died without developing NHL were censored at their times of death, and 15 other patients who are alive and have not developed NHL were censored at the time last known alive. Confidence intervals for the Kaplan-Meier analysis were determined using the method of Rothman.

The 95% confidence intervals for the overall proportion of patients developing lymphoma were calculated by an exact method. For each of the eight patients who developed non-Hodgkin lymphoma, the time elapsed from the initial fall of their T4 cells below 100 or 50 cells/mm^3 to the development of lymphoma was calculated. In this analysis, two consecutive determinations of T4 values below 100 or 50 cells/mm^3 were required, and the time was calculated from the first of these determinations.

Survival of patients with non-Hodgkin lymphoma was determined from the time the diagnostic biopsy was performed until the time of death. Median values, with the appropriate ranges, were then determined for each time period.

Non-Hodgkin lymphoma developed in 8 of the 55 patients (14.5%, 95% confidence interval of 6.5% to 26.7%). As analyzed by the method of Kaplan and Meier, the estimated probability of developing lymphoma in the patients within 30 months of starting antiretroviral therapy is 28.6% (95% confidence interval of 13.7% to 50.3%) (Figure 1). After 36 months of therapy, the estimated probability of lymphoma developing increases to 46.4% (95% confidence interval of 19.6% to 75.5%); this later number is clearly based on a very small number of patients remaining at risk for its development. Since 15 of the original 55 patients are still alive and without non-Hodgkin lymphoma, this number may increase with time. One patient (#3) developed a second distinct lymphoma 16 months after his initial occurrence of non-Hodgkin lymphoma; however, only the first occurrence of lymphoma in this patient was considered in the above calculations.

The median T4 cell counts at the initiation of antiretroviral therapy for all 55 patients was 74 cells/mm^3 (range 0 to 953 cells/mm^3). Median survival for the 55 patients overall was 22 months (data not shown).

Patients who developed lymphoma had received antiretroviral therapy for a median of 23.8 months (range 13 to 34.5 months) before the onset of lymphoma (Table 1). The median time from the diagnosis fo their AIDS-defining illness to the development of non-hodgkin lymphoma was 22.5 months (range 9 to 77 months). Median T4 cell counts at initiation of antiretroviral therapy in these patients and at the occurrence of

non-Hodgkin lymphoma was 26 cells/mm^3 (range 8 to 135 cells/mm^3) and 6 cells/mm^3 (range to 21 cell/mm^3) respectively (data not shown). Patients who developed non-Hodgkin lymphoma had had less than 100 T4 cells/mm^3 for a median time of 17.8 months (range 7 to 35 months). In addition, these same patients had had less than 50 T4 cells/mm^3 for a median of 15.3 months (range 5.5 to 35 months) prior to the occurrence of lymphoma.

All of the lymphomas reported here histologically were of high-grade, four being large cell immunoblastic lymphoma and four small non-cleaved cell lymphoma (Table 2). Seven of ten lymphomas were B cell type and one was null cell type. Each of the patients had serological evidence of infection with Epstein-Barr virus. All occurred in extranodal sites. Five patients had primary involvement of the central nervous system: four had mass lesions within the brain and one had leptomeningeal disease only. The other three patients presented with visceral involvement of the lung (patient 1), esophagus, liver, and spleen (patient 2), and ascites and pleural fluid (patient 3).

Median survival for the four patients with primary central nervous system involvement diagnosed antemortem was 1.8 months (range 0.6 to 3.2 months) (Table 1). The three patients who presented with visceral involvement had survivals of 0.4, 7 and 18 months.

DISCUSSION

The occurrence of non-Hodgkin lymphoma in the setting of HIV infection is well-established, and high-grade non-Hodgkin lymphomas account for approximately 3% of the initial AIDS-defining illnesses in reported adult and adolescent cases of AIDS. However, there is presently little available literature on the temporal development of NHL in cohorts of patients with AIDS or severe AIDS-related complex, particularly since the development of effective antiretroviral therapy. We selected these three protocols for analysis because they represent the first three protocols of our group in which patients were followed for long periods of time on antiretroviral therapy. Included in this cohort are the first patients to have ever received zidovudine. It is worth noting, however, that we have also observed the development of lymphoma in HIV-infected patients on other antiretroviral protocols. In particular, one patient developed stage IE primary small non-cleaved cell lymphoma of the liver while studied on a Phase I trial of dideoxyadenosine, while a second patient initially entered on a study of recombinant soluble CD4 developed stage IVB large cell immunoblastic lymphoma. A third patient initially entered on the Phase I study of 2′,3′-dideoxycytidine subsequently developed stage IVB Hodgkin disease.

The patients reported here were followed for up to 38 months while receiving continuous antiretroviral therapy with zidovudine or zidovudine-containing regimens. It is possible that because of teh screening process (e.g., patients had to be free of active opportunistic infections), the study patients may not be representative of AIDS or AIDS-related complex patients in the general population. Nevertheless, the estimated probability of lymphoma developing in 46.4% of patients by 36 months after

starting antiretroviral therapy is a striking finding, although the 95% confidence interval of 19.6% to 75.5% reflects the variability of the estimate at 36 months. Further evaluations of larger populations will be needed to more accurately define the probability of patients with severe HIV infection on antiretroviral therapy developing a non-Hodgkin lymphoma. The data presented here indicate that non-Hodgkin lymphoma may well become a limiting factor in the survival of patients with HIV infection as improved antiretroviral therapies are developed. It will be important to learn how to prevent this complication. For example, earlier intervention with antiretroviral therapy may delay the decline of the T4 cells below $100/mm^3$, and this may result in a lower incidence of lymphoma. This possibility will require further study.

Lymphomas can be difficult to diagnose in patients with HIV infection, and this may result in an underreporting of non-Hodgkin lymphoma in these patients. For example, two of the cases of lymphoma in this study (patients 5 and 7) were diagnosed in patients being treated for cerebral toxoplasmosis who developed new or enlarging brain lesions. Also, none of the eight cases presented here had the diagnosis established by biopsy of a lymph node. Patients with lymphoma in the setting of AIDS pose substantial therapeutic challenges. In spite of the interventions used, the overall survival in the patients described here following the development of lymphoma was poor (Table 1). Interestingly, one patient developed a dramatic flare of his Kaposi sarcoma when administered a chemotherapeutic regimen for the lymphoma which contained steroids, consistent with previous reports. Improved therapeutic strategies for these conditions are needed, and this will be an important area for future research.

Although the mechanism of AIDS-related non-Hodgkin lymphoma development is not known, many inter-related factors have been postulated to be involved. It has been found that patients with AIDS or AIDS-related complex have a polyclonal B cell proliferative lymph node expansion. Several factors may contribute to this process. Polyclonal B cell activation can be a direct response to HIV infection, either through mitogenic or antigenic stimulation. HIV-infected patients have increased numbers of circulating Epstein-Barr virus infected cells, which may in part be due to their profound defect in T cell immunity. However, direct Epstein-Barr virus involvement of tumor has not been documented in the majority of patients with HIV-associated lymphoma, and this issue bears further study. Whatever the mechanism, polyclonal B cell proliferation may provide a milieu for the development of transforming events to occur. While these transforming events have not yet been delineated, there is evidence that in many cases of AIDS-related Burkitt lymphoma, a c-myc oncogene rearrangement similar to that seen in endemic and sporadic Burkitt lymphoma occurs. Unregulated oncogenic expression could then become the proximal cause of the transformed state in patients. Alternately, it is possible that the replication of an as yet unidentified oncogenic virus may be enhanced in patients infected with HIV.

Although the cellular events involved in the pathogenesis of AIDS-related non-Hodgkin lymphoma have not been elucidated, it is likely that immunosuppression plays a substantial role. The occurrence of high-grade, B cell lymphomas (particularly at extranodal sites) developing in patients with other forms of immunodeficiency, either primary (e.g., Wiskott-Aldrich syndrome, ataxia telangiectasia) or from immunosuppressive therapy, has been well-documented. Prolongation of survival in patients with primary immunodeficiency has been felt to increase the cumulative risk for the development of a non-Hodgkin lymphoma. In patients with Wiskott-Aldrich syndrome, for example, the overall risk of developing a malignancy has been calculated to be 126 times that of the general population, with a cumulative risk of 2% per year for the first 25 years of life. The majority of these malignancies are lymphoreticular in origin (74.5%), and high-grade, B cell lymphomas, frequently occurring in extranodal sites, particularly the central nervous system, predominate. Thus, with enhanced control of infection and other therapeutic advances, the cumulative probability of such patients developing a non-Hodgkin lymphoma has increased along with the prolongation of life expectancy.

Recent evidence suggests that as a result of clinical advances in the therapy of HIV infection, patients with this disease are experiencing an improvement in survival. For example, the median survival of patients diagnosed with AIDS reported to the San Francisco Department of Public Health has increased from 10.8 months for those diagnosed in 1985 to 15.6 months for those diagnosed in 1987. This improvement has been particularly striking for those who present with Pneumocystis carinii pneumonia as their AIDS-defining illness; in these patients, the median survival during the same period has increased from 10.5 to 17.9 months. While earlier diagnosis and improved methods of treatment and prophylaxis of P. carinii pneumonia may have contributed to this phenomenon, there is evidence that the use of zidovudine has resulted in prolonged survival over and above any affect of PCP prophylaxis. The median survival of 22 months in this study population lends further support to this impression. Therefore, patients with profound immunodeficiency are living longer, theoretically allowing more time for the development of non-Hodgkin lymphoma or other malignancies.

It is noteworthy that patients in our series had had AIDS for a median period of 22.5 months (range 9 to 77 months) and had less than 50 T4 lymphocytes /mm^3 for a median of 15.3 months (range 5.5 to 35 months) prior to the development of lymphoma. Prior to the development of antiretroviral therapy, it would have been relatively unusual to have such prolonged survival after the development of AIDS or profound T4 depletion. Lymphomas may develop in HIV-infected patients at any point in the course of their illness. However, while the numbers are small, the findings here suggest that patients who survive for long periods with profound immunodeficiency manifested by less than 50 T4 cells/mm^3 may have a particularly high likelihood of developing high-grade lymphomas. Indeed, assuming a 10 year incubation period from the initial infection with HIV to the development of AIDS, and that non-Hodgkin lymphoma represents 3% of AIDS-defining illnesses, then the yearly incidence of non-Hodgkin lymphoma developing in

early stages of HIV infection is approximately 0.3%. If we were to assume that the development of non-Hodgkin lymphoma is constant over the period of follow-up, then the estimated incidence of non-Hodgkin lymphoma in our cohort of patients with AIDS and severe AIDS-related complex is nearly 9% per year of follow-up. This wide disparity again indicates that non-Hodgkin lymphoma is much more likely to develop in the setting of severe immunodeficiency and thus may be considered an "opportunistic" tumor.

Finally, one must wonder if zidovudine or other antiretroviral drugs might directly contribute to the development of lymphoma in these patients. In this regard, zidovudine can act as a mutagen, and vaginal malignancies have been reported to develop with increased frequently in mice and rats receiving lifelong high dose zidovudine. Further research will be required to determine if the same effects occur in humans. It is worth noting that the lymphomas in our patients are of the same type as those which typically develop in the setting of HIV infection (Table 2), suggesting that zidovudine therapy was less likely to be a direct cause of these tumors. There is no question that zidovudine has improved rates of teh morbidity and mortality of HIV infection. Nevertheless, teh direct oncogenic potential of zidovudine and related drugs cannot be discounted. This serves as an incentive to find the lowest effective doses for such agents. It will be important to sort out the relative contribution of immunosuppression, prolonged survival, a possible effect of antiretroviral therapy, and perhaps other unrecognized factors in the development of lymphomas in order to learn how to minimize the occurrence of this condition.

The AIDS epidemic is already changing the demographics of lymphoma in this country, and such effects are likely to be amplified in the near future. In 1989, there were 34,598 cases of AIDS in adults and adolescents reported to the CDC, of which 948 (3%) initially presented with high grade non-Hodgkin lymphoma as their AIDS-defining illness. The number of cases of AIDS is expected to rise over the next decade. As stated previously, there was a 14.5% incidence of non-Hodgkin lymphoma in AIDS or severe AIDS-related complex patients on long-term antiretroviral therapy in our patient cohort. If this sample is roughly representative, and presuming that none of the remaining 15 patients who are alive without non-Hodgkin lymphoma develop this malignancy, then of the population initially diagnosed with AIDS in 1989, nearly 4900 (95% confidence interval of 2187 to 8985) of those patients with an AIDS-defining illness other than lymphoma may be estimated to develop an "opportunistic" non-Hodgkin lymphoma at some time during their illness. While this sample selected for Phase I studies may not be representative, and many other factors may influence this extrapolation, the results do suggest that the incidence of non-Hodgkin lymphoma in the setting of AIDS is likely to significantly increase with time.

In the last 17 years, the Surveillance, Epidemiology and End Results (SEER) Program database of the NCI has shown a steady increase in the incidence of non-Hodgkin lymphoma. From 1973 to 1987 (the last year in which final figures are available), the incidence of non-Hodgkin lymphoma has increased

by more than 50%. The factors contributing to this rise are not known. The rise was observed before the epidemic of HIV infection, and available information indicates that lymphomas associated with AIDS account for only a very small percentage of this increase. For example, in 1990 there will be an estimated 36,000 cases of non-Hodgkin lymphoma, and approximately 18,000 non-Hodgkin lymphoma-related deaths. Most of these estimated cases will be outside the setting of HIV infection. The addition of an increasing number of AIDS-related non-Hodgkin lymphoma to the HIV-independent group will represent a new burden and pose crucial challenges to physicians and the health care delivery system of this country. For this reason, it is important that we learn more about the prevention and optimal treatment of this formidable complication of HIV infection.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07211-01 CO

PERIOD COVERED

October 1, 1989 through September 30, 1990

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

Inhibition and modulation of HIV infection in monocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

We have investigated the infection of human peripheral blood monocyte/macrophages by HIV and ways of inhibiting this process. 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 do novo HIV infection in monocyte/macrophages. In regard to AZT, this was surprising, as monocytes have very low levels of thymidine kinase (responsible for catalyzing the first step of AZT phosphorylation) and there were very low levels of AZT-5'-triphosphate in monocytes exposed to AZT. We further found that monocytes have very low levels of thymidine-5'-triphosphate. Thus, the ration of AZT-triphosphate to thymidine-triphosphate is actually higher in monocytes than in T cells, and this can account for its activity. In further experiments, we found that granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) both enhanced the replication of HIV in monocytes. GM-CSF, however, also stimulates the acti-HIV activity of AZT and other thymidine analogues such as 2',3'-didehydro-2',3'-dideoxythymidine (D4T). In the case of AZT, the increased activity appears to occur because of a combination of increased entry and increased phosphorylation. GM-CSF does not enhance the anti-HIV activity of other dideoxynucleosides such as ddC and ddI. Interestingly, M-CSF does not appear to enhance the anti-HIV activity of AZT or the other dideoxynucleosides. We further explored whether CD4 binding was a necessary component of the entry of HIV into monocytes. Infection of monocytes was inhibited by agents which block gp120 binding to CD4 such 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 Lau 3 or soluble CD4.

Introduction

It is well established that CD4 acts as the principal receptor for the binding of human immunodeficiency virus (HIV) to T cells . However, as the number of identified cellular targets for HIV infection has expanded, it has been hypothesized that other receptors for HIV may exist. There is recent evidence, for example, that some muscle or glioma cell lines, which lack detectable surface CD4, may be infected by HIV. Moreover, it has been observed that infection of human lymphocytes or the monocytoid cell line U937 by HIV can be enhanced by low titers of anti-HIV antibodies, and it has been hypothesized that Fc receptors or complement receptors may act as an entry site for HIV under these conditions.

Human peripheral blood monocyte/macrophages (M/M) can be easily infected by certain strains of HIV, and there is increasing evidence that infection of such cells plays a crucial role in the pathogenesis and progression of acquired immunodeficiency syndrome (AIDS) and related disorders. M/M are phagocytic cells, and it seemed possible that this could provide a route for viral entry. In addition, M/M bear Fc and complement receptors and in the case of certain other viruses (e.g. flaviviruses) are prime targets for enhancement of infection by anti-viral antibodies. It therefore seemed conceivable that a CD4-independent pathway for infection may exist for M/M, either in the presence or absence of low titers of anti-HIV antibodies. This question is potentially important from a therapeutic viewpoint as strategies to inhibit HIV-CD4 binding are actively being investigated as therapies for HIV infection . The present set of experiments were designed to study whether infection of M/M could be enhanced by low titers of anti-HIV antibodies, and in addition, whether a second entry mechanism for HIV infection of monocyte/macrophages might be identified, particularly in the presence of such antibodies.

Materials and Methods

Cells. Monocyte-enriched peripheral blood mononuclear cells (PBMC) were obtained from healthy, HIV-seronegative donors using a cell separator (Fenwall C3000; Travenol Inc., Deerfield, IL). Fresh elutriated M/M were subsequently purified from this population by elutriation as described by Gerrard et al.. In certain experiments, the fresh elutriated M/M were cultivated for 5 days in the presence or absence of 100 chronic myelogenous leukemia (CML) U/ml of human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF)(Sandoz Research Institute, East Hanover, NJ) as described (1). Also, in certain experiments, mature adherent M/M populations (called 5-day adherent M/M) were obtained by incubating 10^6 PBMC/well in 48-well plates (Costar, Cambridge, MA) for 5 days followed by extensive washing to remove non-adherent cells(2). Using this method, the yield after removal of the non-adherent cells was approximately 10^5 M/M per well. The viability of cells obtained with each of these methods was consistently >95%. M/M obtained with each of these methods were <1% E-rosette positive, >95% non-specific esterase positive (Technicon Instruments, Tarrytown, NY), and >95% morphologically resembled monocytes when examined after Giemsa staining. Other characteristic of these cells have been previously described. In certain experiments, U937, a $CD4^+$

monocytoid cell line, or ATH8, an HTLV-1-infected human T cell line (gift of Dr. H. Mitsuya, National Cancer Institute, Bethesda, MD) were used.

The expression of surface antigens on the cells was assessed by flow cytometry using the following fluorescein-conjugated monoclonal antibodies: CD4, Leu-3a (Becton Dickinson, Mountain View, CA); Cd11b, OKM1 (Ortho Diagnostics, Raritan, NJ); CD36, OKM5 (Ortho); HLA-DR, OKIa (Ortho). Paired isotype-specific control antibodies were run with each sample. Dead cells were excluded from analysis using propidium iodide, and the percentage of antigen-positive cells was calculated by straight channel integration, with the integration channel set so that less than 1% of the isotype control cells appeared positive. The density of CD4 on the surface of the cells was compared by staining cells with fluorescein-labeled Leu-3a monoclonal antibody (Becton Dickinson), followed by analysis on a profile flow cytometer (Coulter Electronics). Fluorescence was then compared with fluorescein-labeled isotype controls using logarithmic-to-linear conversion tables supplied by Coulter Electronics.

Virus. A monocytotropic strain of HIV-1, HTLV-III_{Ba-L} (gift of Drs. S. Gartner, R.C. Gallo, and M. Popovic, National Cancer Institute, Bethesda, MD), and a lymphocytotropic strain, HIV-1, HTLV-III_B (Electro-Nucleonics Laboratory, Inc., Silver Spring, MD), were used as previously described. These will be referred to as HIV-1_{Ba-L} and HTLV-IIIB respectively. Supernanants from infected cultures of fresh M/M were used as the source of HIV_{Ba-L}; these were filtered and stored in liquid nitrogen prior to use. Titration to determine infectivity was performed in a primary M/M system; the minimum infectious dose (MID) was defined as the minimum amount of virus that induced viral replication in at least two of four M/M cultures. Pelleted supernates of infected H9 cells which were concentrated by centrifugation were used as the source of HTLV-IIIB. The MID of this strain was assessed in the AHT8 cell line.

Anti-HIV antibodies. Preparations of human antibodies to HIV (called HIV-Ab1 and HIV-Ab2) were the gifts of Dr. Larry Cummins, Abbott Laboratories, North Chicago, IL. These antibodies were prepared from the plasma of asymptomatic, HIV-infected individuals who lacked detectable serum HIV p24 antigen. HIV-Ab1 was prepared from donors who had high titers of antibody to p24 and gp120 but low titers of antibody to gp41. HIV-Ab2 was prepared from a donor who had high titers of antibody to gp120 and gp41, but no detectable anti-p24 antibody. Plasma from these donors was inactivated by treatment with 1% tri-N-butyl-phosphate and 1% Tween 80 for four hr at 30° C and fractionated by the Cohn-Oncley cold ethanol procedure. Additional purification was obtained using QAE Sephadex gel. The final preparation contained more than 95% monomeric IgG as determined by high performance liquid chromatography (HPLC). These preparations were then diluted at 50 mg/ml in saline, sterile filtered, and stored at 4° C until used. A control preparation of human IgG, obtained from HIV-seronegative donors, was purified in the same manner.

Preliminary experiments showed that HIV-Ab1, but not HIV-Ab2, interfered with the radioimmune assay used to measure HIV p24 antigen (Dupont Co., Wilmington, DE). However, even at the highest concentration used (5000

μg/ml), the washing procedure performed 48 hr after viral challenge (see below) reduced the concentration of antibody by approximately 10^{-8} so that it no longer had any detectable effect on the assay. Neither antibody preparation interfered with the enzyme-linked immunosorbent assay (ELISA) used to measure HIV p24 antigen (Dupont), even at the highest concentrations utilized.

Anti-HIV agents and controls. Recombinant soluble CD4 (sCD4) containing the four extracellular domains was provided by Dr. Dan Capon of Genentech, Inc. (So. San Francisco, CA)(3). OKT4A, an IgG_{2a} murine monoclonal antibody against CD4 (Ortho); OKT4C (Ortho), another IgG_{2a} anti-CD4 murine monoclonal antibody which binds to a different determinant; and D3-2H2-9-21 anti-dengue antibody (ATCC, Rockville, MD), a IgG_{2a} murine monoclonal antibody used as an isotype control; were diluted in phosphate buffered saline (PBS), extensive dialyzed against PBS, and passed through a 0.4 micron filter prior to use. The final protein concentration in these preparations was evaluated by spectrophotometric measurement. All reagents were stored at 4° C until used.

Infection and culture of M/M populations. 10^5 M/M prepared by the various techniques described above (fresh elutriated cells, elutriated cells cultured 5 days with or without GM-CSF, or 5-day adherent M/M) were suspended in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 20% heat-inactivated low-endotoxin fetal calf serum (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, 50 U/ml penicillin, and 50 g/ml streptomycin (Gibco Laboratories) (complete medium). The cells were exposed to various concentrations of sCD4, OKT4A, OKT4C, or D3-2H2-9-21 for 30 minutes to 2 hr at 37°, challenged with 100 to 500 MID/well of HIV-1_{Ba-L}, and incubated at 37° C in a humidified atmosphere supplemented with 5% CO_2. In some experiments, the sCD4 or antibodies were added to the cultures 1 or 6 days after the viral challenge. Two days after viral exposure, the M/M were extensively washed to remove excess virus and then cultured under the same conditions and drug concentrations as before. The supernatant was partially replaced with fresh complete medium every 5 days. Viral production into the supernatant was evaluated every 4 to 5 days starting from day 6 by radioimmunoassay or ELISA for HIV p24 antigen (Dupont). In some experiments, inhibition of syncytia formation by these agents was evaluated by visual inspection; this was only done in cultures of mature or GM-CSF-exposed M/M as fresh M/M usually do not form syncytia in our hands.

Evaluation of the effects of anti-HIV antibody. To evaluate the enhancement of HIV infection by low concentrations of anti-HIV antibody, 3 MID/well of HIV-1_{Ba-L} were mixed with serial 10-fold dilutions of HIV-AB1 or HIV-Ab2 in 200 l of complete medium and incubated at 4° C for 2 hr. The virus-antibody mixtures were then added to fresh or 5-day adherent M/M which were previously incubated for 30 min at 4° C with sCD4, OKT4A, D3-2H2-9-21, or control medium in 48-well plates. The cells were cultured for 2 days at 37° in a humidified atmosphere supplemented with 5% CO_2 as described above. The M/M were then washed extensively to remove excess virus, sCD4, or antibodies, resuspended in 1 ml of complete medium (without either anti-HIV antibody or other agents), and continued in culture. They

were fed every 5 days as above, and viral production into the supernatants periodically assessed as described above.

Toxicity assessments. The toxic effects of antibodies or sCD4 were assessed by the trypan blue dye-exclusion method, by evaluation of phagocytosis of 0.8 μ latex beads, and by measure of ^{3}H-thymidine incorporation in the U937 cell line. For the latter, $2x10^{4}$ U937 cells/well were suspended in 0.2 ml of complete medium in wells of a 96-well plate (Costar) and then cultured for 5 days in the presence or absence of different concentrations of sCD4, OKT4A, D3-2H2-9-21 antibody, HIV-Ab1, or HIV-Ab2. Cells were then pulsed for 6 hr with 1 Cu of ^{3}H-thymidine, harvested, and counted in a liquid scintillation spectrometer (Beckman).

Statistics. The statistical significance of the effects of OKT4A and CD4 was assessed with the two-sided Wilcoxon signed rank test for paired values.

Results

Most of the M/M expressed CD11 and HLA-DR. Fresh elutriated M/M also expressed CD36; however, consistent with previous results, we found that expression of this antigen declined somewhat in mature, cultured M/M. The expression of CD4 was quite variable in the M/M populations, with the mean expression ranging between 39% in M/M cultured with GM-CSF to 56% on elutriated M/M cultured without GM-CSF. In each instance, however, the percentage of M/M expressing CD4 was less than either helper T cell clones (such as ATH8) or the U937 monocytoid cell line. In addition, those M/M which expressed CD4 generally had a low density of antigen as compared to ATH8 T cells (13.8 fold less) or U937 cells (5.3 fold less). These results indicated that while some M/M expressed CD4, overall the expression of this antigen was less in this population than in prototype $CD4^{+}$ T cells.

With this background, we asked whether OKT4A or sCD4, two agents which block the binding of HIV env gp120 to CD4, inhibited HIV-1 infection of the various M/M populations. As seen in Figure 2a, fresh M/M exposed to 100 to 500 MID/well of HIV_{Ba-L} produced substantial amounts of HIV p24 antigen starting after 2 weeks and continuing at least up to day 35. However, HIV replication was inhibited throughout this period of observation by the addition of 1 μg/ml of OKT4A or 5 μg/ml of sCD4; these concentrations are equal to or less than the concentrations of these agents required to inhibit T cells. Similar results were observed with 5-day adherent M/M and with elutriated M/M precultured for 5 days with or without GM-CSF. It is worth stressing that while GM-CSF-stimulated M/M had a relatively low expression of surface CD4 and in the absence of inhibitors produced substantially more HIV p24 antigen than the other populations, the level of inhibition on this population induced by OKT4A or sCD4 was comparable with that of the other M/M populations.

In contrast to the above results, HIV infection of the M/M was not inhibited by comparable concentrations of OKT4C, a murine monoclonal antibody which binds to a different domain on CD4 than does OKT4A and which

does not either inhibit gp120-CD-binding or block HIV infection of T cells. Also, the IgG_{2a} control monoclonal antibody, D3-2H2-9-21, had no inhibitory activity. Thus, agents which inhibit CD4-gp120 binding, but not control antibodies, consistently blocked productive HIV infection in the various M/M populations.

We next asked whether these agents might be effective if added to the cultures after the time of initial viral challenge. As seen in Table II, partial inhibition was still observed when OKT4A or sCD4 were added 24 hr after the exposure of fresh M/M to HIV_{Ba-L}. This may be the result of these agents blocking secondary spread of HIV. Alternatively, the partial activity of OKT4A added at 24 hrs may have resulted from an effect on a post-binding step of viral replication (e.g. fusion or entry). In contrast, if either OKT4A or sCD4 were added at day 6 and the supernatants harvested at day 10, no inhibitory effect was observed. As expected, no inhibitory effect was observed if the IgG_{2a} control antibody D3-2H2-9-21 was added either at 24 hr or at day 6. Taken together, these results suggest that in the M/M populations studied, none of the substances tested act at late stages of HIV replication (e.g. viral budding). In addition, they provide evidence that neither substance was toxic for M/M per se.

More direct evidence of a lack of toxicity of these substances was provided by the fact that neither caused cell death, as evidence by trypan blue exclusion, and that neither affected the phagocytosis of latex beads up to the highest concentrations tested (10 g/ml OKT4A or D3-2H2-9-21 and 50 μg/ml sCD4). To further assess the possible toxicity of these agents, we examined their effects on the proliferation of the U937 monocytoid cell line. As seen in Table III, the above agents had no appreciable toxic effects.

We next asked whether infection of the M/M populations could be enhanced by anti-HIV antibodies and if so, whether this might occur by a CD4-independent pathway. To assess this, we used purified IgG anti-HIV preparations as described above. In preliminary experiments, we found that HIV-Ab1 had neutralizing activity against HIV infection of ATH8 cells even at a high multiplicity of infection of virus (1000 MID/well): 50% inhibition of the cytopathic effect of HTLV-IIIB was achieved with 500 μg/ml HIV-Ab1, while >95% inhibition was achieved with 5000 μg/ml HIV-Ab1. This preparation did not cause appreciable toxicity at those concentrations. In contrast, HIV-Ab2 had no discernable anti-HIV activity at equivalent concentrations in this T cell system. Also, a control preparation of IgG prepared from HIV-seronegative donors had no activity at up 5000 μg/ml.

HIV-Ab1 likewise had substantial anti-HIV activity in M/M exposed to HIV_{Ba-L} (3 MID/well). Interestingly, HIV-Ab2 also had activity against HIV_{Ba-L} in M/M, in spite of its not being active against HTLV-IIIB in ATH8 cells. This differential activity may have been due in part to the lower multiplicity of infection used in the M/M cultures. However, 50 μg/ml of either HIV-Ab1 or HIV-Ab2 inhibited infection of both fresh and mature M/M exposed to 300 MID/well of HIV_{Ba-L} (data not shown), suggesting that these antibodies were indeed more potent inhibitors of HIV in the M/M than in T

cells. It is possible that differences between the virus preparations used, or alternatively intrinsic differences between the target cells, may have contributed to these effects.

It has previously been reported that low levels of anti-HIV antibodies can paradoxically enhance HIV infection of lymphocytes or the U937 monocytoid cell line. We next sought to determine whether HIV-Ab1 or HIV-Ab2 had enhancing activity and if so, whether this was inhibited by OKT4A or sCD4. In cultures of M/M utilizing a high multiplicity of infection (300 MID/well of HIV_{Ba-L}), we found no or inconsistent evidence of enhancement. However, when we used a lower multiplicity of infection (3 - 10 MID/well), an increase in HIV infection (enhancement) was observed in mature (5-day adherent) M/M with extremely low concentrations of HIV-Ab1 or HIV-Ab2 ($5x10^{-2}$ to $5x10^{-4}$ g/ml) in four out of five experiments. The degree of enhancement averaged four to five fold. Also, in five additional experiments using 3 MID/well of HIV_{Ba-L}, productive infection of fresh or 5-day adherent M/M was attained only in the presence of $5x10^{-4}$ to $5x10^{-6}$ μg/ml of HIV-Ab1 or HIV-Ab2. In no case was enhancement observed using a control IgG preparation. The addition of complement had no consistent effect on enhancement in this system. In this regard, it should be remembered that these antibody preparations contained >95% monomeric IgG, and these results do not exclude the possibility that complement may affect the results seen with other antibody preparations. Enhancement of infection in M/M was not restricted to HIV_{Ba-L}; in the one experiment where this was examined, 100 MID/well of HTLV-IIIB (a lymphocytotropic strain) failed to infect fresh M/M in the absence of antibody, while productive infection was observed in the presence of $5x10^{-3}$ g/ml of HIV-Ab1 (data not shown). Overall, with HIV_{Ba-L}, there was variation in the optimal concentration of antibodies yielding enhancement in M/M populations from various donors (range from $5x10^{-2}$ to $5x10^{-6}$ μg/ml). In addition, there was variation in the degree of enhancement. However, although the effect was sometimes moderate and was observed only with a low viral inoculum and very low concentrations of antibody, this was a reproducible phenomenon under these experimental conditions.

It has been hypothesized that antibody-mediated enhancement of HIV infection might involve viral binding and entry via a CD4-independent mechanism. To explore this possibility, we studied the effect of OKT4A and sCD4 on M/M infection which was enhanced by anti-HIV antibodies. Infection of either fresh or mature M/M under conditions of enhancement (and with a low MID/well of HIV_{Ba-L}) was consistently inhibited by more than 95% by 1 to 5 μg/ml of OKT4A or by 5 μg/ml of sCD4. Inhibition was observed even when these agents were removed from the cultures two days after viral exposure. Inhibition was seen both in experiments where there was infection of the M/M in the absence of anti-HIV antibody and in experiments where infection was only observed in the presence of anti-HIV antibody. Overall, inhibition by OKT4A and by sCD4 was seen in each of six experiments where this was examined ($P<0.05$).

We also examined the activity of OKT4A in U937 cells exposed to HTLV-IIIB in the presence of low concentrations of anti-HIV antibodies. We found that viral production was increased in the presence of $5x10^{-1}$ to $5x10^{-4}$

μg/ml of HIV-Ab1 or HIV-Ab2. At the same time, complete inhibition of infection of these monocytoid cells was observed with 5 g/ml of OKT4A, even under conditions of enhancement. It is worth stressing that our system utilized a measurement of HIV p24 antigen produced directly by the M/M or the U937 cells and did not involve a second "indicator cell" population. Also, M/M were subjected to extensive washing to remove excess virus after viral challenge as previously described (2), and additional controls were established in each experiment to verify this point. Finally, neither the anti-HIV antibodies, nor the OKT4A or sCD4 at the concentrations in the supernatants, had any appreciable effect on the assays used to measure HIV p24 antigen.

One concern in these experiments was that by binding to HIV, sCD4 might somehow interfere with subsequent viral fusion or entry, and that inhibition might occur even if HIV entered by a CD4-independent pathway. However, this concern would not apply for OKT4A. On the other hand, the effect of OKT4A could conceivably have resulted from competitive inhibition of OKT4A with the anti-HIV antibodies for binding to the Fc receptor on the M/M. However, 5 μg/ml of D3-2H2-9-21 (an irrelevant murine monoclonal antibody of the same isotype as OKT4A) failed to inhibit HIV infection of 5-day adherent M/M which was enhanced by low concentrations of HIV-Ab1. Thus, the overall evidence suggests that binding of gp120 to CD4 is an essential step in the infection of these M/M populations by HIV_{Ba-L}, even in the presence of enhancing antibodies.

Discussion

The results of this study demonstrate that agents which block the binding of env gp120 to CD4 inhibit the infection of both fresh and precultured (mature) human peripheral blood M/M. In addition, they demonstrate that infection of HIV in M/M can be enhanced by very low concentrations of anti-HIV antibodies, but that even under such conditions, infection is still blocked by inhibitors of gp120-CD4 binding. Overall, these results suggest that even under conditions of "enhancement", CD4 binding is an essential component of infection of M/M by HIV.

The ability of low concentrations of antiviral antibodies to enhance viral infection in vitro is a well described phenomenon for a number of viruses (especially flaviviruses) and may in certain cases be clinically important. Dengue, for example, may be more severe in individuals with low levels of anti-dengue antibodies than in seronegative individuals. There is evidence that for flaviviruses two mechanisms may contribute to this phenomenon. First, antibodies may serve to attach the virus to either Fc-receptors or (indirectly) to complement receptors; the viruses then enter by receptor-mediated endocytosis. Secondly, the antibodies may increase the specific infectivity of bound virus; flaviviruses enter the cytoplasm by an acid-dependent uncoating process and antibodies appear to make this process more efficient by altering the pH of virus-containing endosomes. In contrast to the flaviviruses, HIV can enter cells both via acid-dependent and acid-independent pathways, and the latter mechanism may not apply. Also while a cellular receptor for flaviviruses has not been clearly identified (in the absence of antibodies), CD4 is a defined

receptor for HIV. Indeed, in the present experiments, CD4 appears to act as an essential receptor for HIV even in the presence of enhancing antibodies. It is possible that anti-HIV antibodies anchor HIV to the cell surface (via binding of the antibody to the Fc receptor), and thus increase the likelihood that the virus will come into contact with CD4. It is conceivable that this mechanism might exert a greater effect in cells (such as M/M) where there is a relatively low expression of surface CD4. Alternatively, it is possible that anti-HIV antibodies may enhance viral fusion or some other step which follows binding to CD4. Finally, binding of antibody-virus complexes might increase expression of surface CD4 on target cells. Whatever the mechanism(s), the available evidence suggests that under the conditions studied, antibody-mediated enhancement of M/M still requires viral binding via CD4 as an essential step.

One must consider whether the present results might have been caused by toxicity of sCD4 or OKT4A. This is unlikely to be the case, however, as late addition of these agents had no effect on HIV production, and there was no other evidence of toxicity. The lack of suppression of HIV replication from a late addition of sCD4 also argues against the possibility that this agent might have an effect on virus budding or other late stages in replication in M/M, as has been proposed in other cell systems. However, as noted above, it is still possible that upon binding to HIV, sCD4 might interfere with cell fusion or viral entry (as opposed to viral binding). Such a mechanism, however, could not account for the suppression observed with OKT4A, and taken in toto, the results suggest that CD4 binding is an essential step for HIV infection of M/M in the presence of enhancing antibodies.

It should be noted that the results presented here are somewhat in contrast to these of Homsy et al. who reported that antibody-induced enhancement of HIV infection was not always blocked by inhibitors of gp120-CD4 binding. Variations in the M/M populations, the source or strain of HIV, or anti-HIV antibodies might account for the differing results, and these issues will have to be explored further. Also, the experiments reported here do not involve coculture of M/M with indicator cells which might simply be infected by HIV absorbed onto the surface of M/M (by antibodies) and thus not appear to be inhibited by anti-CD4 antibodies or by sCD4. Additionally, certain M/M preparations may release proteases which may destroy proteins which inhibit gp120-CD4 binding.

It has been proposed that antibody-induced enhancement of HIV infection might have clinical implications in disease pathogenesis, the development of a vaccine against HIV, or certain therapeutic modalities. However, we could elicit enhancement only under very stringent conditions (low multiplicity of infection and very low concentrations of antibodies), and even then, the effect seen was only moderate. It would thus appear that clinically significant effects would be observed only under very limited conditions if at all. During a narrow window of time early in the course of HIV infection, for example, enhancing antibodies might cause a burst of HIV infection. Subsequently, however, antibody titers would be higher than those associated with enhancement. In regard to therapeutic modalities, enhancement has been proposed to be a theoretical concern in the

administration of hyperimmune anti-HIV gamma globulin. However, the titers attained in patients with HIV-Ab1 would be at least 10,000 fold higher than those associated with enhancing activity. Lack of activity against M/M or enhancement may also be a theoretical concern in the use of CD4-immunoglobulin fusion proteins . However, such proteins are effective inhibitors of HIV infection of M/M, and in preliminary experiments, we have not been able to detect enhancement with this agent. Additional studies, however, will be needed to further assess the potential clinical implication of these phenomena.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07212-01 CO

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In vitro inibition of HIV by combination of multiple anti-HIV agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH
Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

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☐ (a1) Minors
☐ (a2) Interviews

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

We tested the in vitro inhibitory activity of three 2',3'-dideoxynucleosides (ddN) and two viral binding inhibitors in combinations against the infectivity and cytopathic effect of human immunodeficiency virus type 1 (HIV-1). We found that either 3'-azido-2',3'-dideoxythymidine (AZT), 2'3'-dideoxyinosine (ddI), or 2',3'-dideoxycytidine (ddC) combined with soluble recombinant CD4 (sCD4) brings about a synergistic antiretroviral activity without toxicity at clinically achievable concentrations. Combinations of ddNs plus dextran sulfate also exerted similar synergistic antiviral effects without concomitant increases in toxicities. When sCD4 and dextran sulfate were combined, an apparent antagonism was observed. We confirmed that none of the combinations of sCD4 plus AZT, ddI, or ddC exerted significant increase in inhibitory effect on colony formation of human myeloid/monocytic bone marrow cells in vitro at concentrations used in this study. These data might have a clinical relevance to the treatment of patients with HIV infection.

PHS 6040 (Rev. 1/84) GPO 914-918

INTRODUCTION

In the past few years, efforts aimed at prevention and control of replication of human immunodeficiency virus (HIV) have gained increasing importance,and potentially useful strategies for therapy against acquired immunodeficiency syndrome (AIDS) have arisen from the accumulated knowledge of the life cycleof HIV. Notably, there have been certain successes at a clinical level inusing reverse transcriptase inhibitors. For example, 3′-azido-2′,3′dideoxythymidine (also termed 3′-azido-3′-deoxythymidine or AZT), one member of a broad family of 2′,3′-dideoxynucleosides, can improve the clinical course and prolong the survival of some patients with advanced AIDS and its related disorders. However, one of the major toxicities of AZT is significant bone marrow suppression, which has been a dose-limiting toxicity in many patients. A closely related drug, 2′,3′-dideoxycytidine (ddC), which has been shown to exert a potent antiviral effect against HIV-1 and HIV-2 in cultured T-cells, and an in vivo virustatic effect in patients with AIDS and AIDS-related complex, has a different dose-dependent toxicity in the form of peripheral neuropathy, which limits its use in some patients. Finally, in the treatment of HIV infection, the emergence of drug-resistant virus variants should always be considered possible, and indeed, Larder and his coworkers have recently reported that AZT-insensitive HIV-1 variants were isolated from patients who received AZT therapy. There are now a number of agents with potential usefulness in the treatment of HIV-1 infection. Logical extension of current therapeutic approaches would be the use of combinations of such drugs which have different antiretroviral mechanism(s). Combination therapy might enhance the efficacy and minimize the toxicity of each drug, and also could minimize or retard the emergence of drug-resistant variants. It is also worth noting that the judicious application of combination chemotherapies made it possible to successfully treat a variety of microbial and neoplastic diseases, which were not treatable with single agents. In the current work, we attempted to evaluate antiretroviral effect of combinations of three 2′,3′-dideoxynucleosides (ddN) and two virus binding inhibitors, all of which have been given to patients with HIV infection.

Materials and Methods

Viruses and Cells

HIV-1 was pelleted by ultracentrifugation from the culture supernatant of HIV-1/III_B-producing H9 cells and prepared to contain $1.2x^{11}$ virus particles per ml. The 50% tissue culture infectious dose ($TCID_{50}$) per ml of the stock cell-free virus preparation was determined by an endpoint titration method using ATH8 cells (see below). The titration was performed in 10 replicate cultures, and $TCID_{50}$ values were calculated by the method previously described elsewhere. An HTLV-1 transformed $CD4^+$ T-cell clone (ATH8) and a normal tetanus toxoid-specific helper/inducer T-cell clone (TM11) were used as target cells for infection by HIV-1. Characteristics of clones ATH8 and TM11 have been described elsewhere. Cell cultures were not synchronized as to cell cycle.

Reagents

2',3'-Dideoxyinosine (ddI), and ddC were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. AZT was kindly provided by the Wellcome Research Laboratories (Research Triangle Park, North Carolina). Soluble recombinant CD4 (sCD4) and dextran sulfate (molecular weight approximately 8,000) were kindly provided by Genentech Inc. (South San Francisco, California), and Ueno Fine Chemical Co. (Itami, Japan), respectively.

Assay for Inhibition of the HIV-1 Cytopathic Effect

Inhibition of the HIV-1 cytopathic effect was assessed as previously described. Clone TM11 cells were stimulated by 2 limiting flocculation units of tetanus toxoid (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain, MA) per ml plus irradiated autologous peripheral blood mononuclear cells (PBM) 5 days prior to the assay. The stimulated TM11 cells were cultured in complete medium [RPMI 1640 supplemented with 4 mM of L-glutamine, 15% (vol/vol) undialyzed and heat-inactivated fetal calf serum (FCS), and 50 units penicillin and 50 ug streptomycin per ml] containing 15% (vol/vol) interleukin 2 (IL-2, lectin-depleted: Advanced Biotechnologies, Silver Spring, MD) and 25 units of recombinant IL-2 (Amgen Biological, Thousand Oaks, CA) per ml until the HIV cytopathic effect inhibition assay. ATH8 cells were used without the antigen stimulation. In the assay, the target ATH8 cells and TM11 cells ($2x10^5$) were exposed to 14,300 $TCID_50$ (1,000 viral particles per cell) and 28,600 $TCID_{50}$ of HIV-1 per cell (2,000 viral particles per cell)rspectively for 1 hr, resuspended in 2 ml of fresh complete medium with IL-, and incubated at 37C in 5% CO_2-containing humidified air. Drugs were added to the culture after resuspension of target cells following exposure to the virus. Control cells were similarly treated but were not exposed to the virus. At various time points, viable cells were counted in a hemocytometer by the trypan blue-exclusion method. Variability in cell number determinations is ±10% of the value shown.

Calculation of Antiretroviral Effect and Cytotoxicity

Percent antiretroviral effect of drugs on the survival and growth of target T-cells exposed to the virus was determined by the following formula: 100 x [(number of viable cells exposed to HIV-1 and cultured in the presence of the compound) - (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]/[(number of viable cells cultured alone) - (number of viable cells exposed HIV-1 and cultured in the absence of the compound)]. By this formula, when the number of viable cells exposed to the virus and the compound is equal to or greater than the number of virus- unexposed cells cultured alone, 100% is given. Calculated percentages ≤0 are expressed as 0%. In quantitative analysis of combination effects, 100% and 0% inhibitions were assumed to be 99.0% and 0.01% inhibitions to facilitate the calculation. Percent cytotoxicity of drugs on the growth of ATH8 cells was determined by the following formula:

100 x [1 - (number of total viable cells cultured in the presence of the compound)/(number of total viable cells cultured alone)]. Calculated percentages $\leq$ 0 are expressed as 0%.

Synergy Calculation

The effects of combined drugs were determined according to the method of Chou and Talalay. Synergism or antagonism of combined effects are quantitatively represented by the combination index (CI), where CI<1, =1, and >1 indicate synergism, summation (additivism), and antagonism, respectively.

Assay for Bone Marrow Toxicity of Drugs

Bone marrow cells were obtained from normal volunteers. The bone marrow cells were washed and diluted with sterile phosphate-buffered saline at 4C and the mononuclear cell population was further separated by Ficoll-Hypaque gradient centrifugation as previously described. Briefly, the cells were suspended in McCoy's 5A media (M.A. Bioproducts, Walkersville, Md.) supplemented with 20% FCS, 2 mM glutamine and 15% (vol/vol) colony stimulating factor/granulocytes-monocytes (CSF/GM)-derived from an HIV-negative leukemic cell culture (P38 cell line)(Kindly provided by Z. Salahuddin). The cells were plated by the soft agar method at a final concentration of 0.3% agar, 20% FCS, 15% CSF-media with $2x10^5$ mononuclear cells per well in a total volume of 1 ml in triplicate with or without various drugs that did not exceed 50 microliters per well. The agar was allowed to gel for 15 min at 30C and the suspension was incubated for 10-12 days at 37C in a humidified atmosphere of 5% CO_2 in air. Colonies greater than 40 cells were counted by an inverted microscope. Sample wells were stained by the method of Kubota for myeloid morphological examination. Approximately 100-200 colonies formed per control well and each drug, alone and in combination, was tested for inhibitory effect on the colony formation. All the experiments were performed in triplicate.

RESULTS

Combinations of ddNs and sCD4 Exert Synergistic Antiretroviral Effect on ATH8 Cells Exposed to HIV-1

We first tested the anti-viral activity of combinations of the various ddNs plus sCD4. We have previously shown that AZT, ddI, and ddC, as single agents, can exert a potent inhibitory effect against the infectivity and replication of HIV-1 at doses that do not affect the growth of target cells in the HIV cytopathic effect inhibition assay, and can completely suppress the virus at concentrations of $\geq$1 uM, $\geq$10 uM, and $\geq$0.5 uM, respectively. It has recently been shown that sCD4 can also suppress the infectivity of HIV-1 *in vitro* at concentrations of 1 to 5 ug/ml. In the current HIV cytopathic effect inhibition assay in which we used susceptible ATH8 cells and a high multiplicity of infection, 8 or 9 combinations of sCD4 with several concentrations of each dideoxynucleoside were tested for antiretroviral activity against HIV-1. In this assay, sCD4 alone could give only a partial protection in the range of 0.2 to 1 ug/ml, and each low

concentration of dideoxynucleoside (AZT at 0.5 uM, ddI at 5 uM, and ddC at 0.05 uM) also exerted a partial inhibition against the cytopathic effect of the virus. However, when combined, sCD4 plus either of ddNs provided synergistic protection. 0.5 uM AZT plus 1 ug/ml of sCD4 gave an almost complete protective effect on virus-exposed ATH8 cells without damaging their survival and growth rate with a CI value of 0.53. The antiviral activity of combination of ddI and sCD4 was further tested under the same conditions. Although 5 uM ddI alone and 1 ug/ml sCD4 alone exerted partial protective effects, when combined, both drugs together exhibited a substantial synergistic antiviral effect with a CI value of 0.13. The growth of the target ATH8 cells was not significantly affected at any concentrations of combined drugs employe. We also tested the effect of combination of ddC plus sCD4 under the same conditions. Combination of 0.05 uM ddC with 0.5 and 1 ug/ml sCD4 exerted a strong synergistic antiretroviral effect and gave a virtually complete protection with CI values of 0.17 and 0.21, respectively.

Anti-Retroviral Effect of a Combination of ddI Plus sCD4 on Normal CD4+ TM11 Cells Exposed to HIV-1

The antiretroviral effect of ddI combined to sCD4 was further tested in normal helper/inducer T-cell clone, TM11, following exposure to HIV-1. In the absence of the drugs, HIV-1 exerted a substantial cytopathic effect on TM11 population by day 15 of culture, resulting in an approximately 60% decrease in the number of viable cells. However, 1 uM ddI combined to 0.2, 0.5, and 1 ug/ml sCD4 exerted an enhanced antiretroviral effect. The CI values obtained exhibited substantial synergistic antiretroviral effect in 8 out of 9 points of combinations.

Combinations of ddNs and Dextran Sulfate Can Inhibit the Infectivity and Replication of HIV-1 Synergistically

We then tested the antiviral effect of ddI combined with dextran sulfate, an anionic polysaccharide which inhibits the binding of HIV-1 virions against CD4+ T-cells in vitro. Both $\leq$5 uM ddI and $\leq$1.25 uM dextran sulfate exerted only a partial protective effect on ATH8 cells exposed to the virus. When combined, however, both drugs also exhibited a substantial enhanced antiretroviral effect. In 8 out of 9 points of combinations, the synergistic protective effect appears to have taken place as assessed by CI. When ddC and dextran sulfate were combined, substantial synergistic antiviral activity was observed in 7 out of 8 points.

Effect of Combinations of Dextran Sulfate and sCD4 on the Replication of HIV-1.

Dextran sulfate can block the binding of HIV-1 virions to various target cells, inhibit syncytia formation, and exert a potent inhibitory effect against the replication of HIV-1 and HIV-2 in vitro. We and others have recently shown that dextran sulfate is capable of inhibiting virion attachment and/or fusion-dependent events which depend on the interaction between cellular CD4 molecules and viral gp120. Soluble CD4 has a high

binding affinity to gp120, which is comparable to cellular CD4, and can competitively inhibit the binding of HIV-1 to CD4+ target cells. It istherefore possible that dextran sulfate blocks the interaction of sCD4 and gp120, which may result in the reduction of antiviral activity of sCD4. We then asked if dextran sulfate could affect the antiviral activity of sCD4 in ATH8 cells exposed to the virus. However, there was an apparent antagonism observed at about equipotent combinations (0.5-1 ug/ml sCD4 plus 0.625 uM dextran sulfate), and a weak synergistic effect was observed.

Bone Marrow Toxicity of Combinations of ddN plus sCD4

We tested on the toxicity of the drugs in combinations to human myeloid/ monocytic bone marrow cells in vitro (CFU-GM). Testing given drugs for the possible toxicity against bone marrow cells in vitro can predict bone marrow suppression in the patients under therapy with the given drugs. Indeed, AZT, whose major toxicity is bone marrow suppression in patients with AIDS and AIDS-related complex, has been shown to be substantially toxic to bone marrow cells in vitro. We then asked if combinations of ddN and viral binding inhibitors could be toxic to bone marrow cells in vitro. When normal bone marrow cells were cultured in the presence of colony-stimulating factor (CSF/GM) and various concentrations of the drugs in combinations, we did not detect any significant increase in toxicity to bone marrow cells in any combination tested.

DISCUSSION

Combination chemotherapies have been widely explored as curative approaches to many other diseases including cancers and infectious diseases, notably in tuberculosis, and leukemias/lymphomas. In this study we demonstrate that combinations of ddN and two different viral binding inhibitors, sCD4 and dextran sulfate, can exert at least an additive effect in vitro when combined, and at certain concentrations, can exhibit a synergistic antiviral effect without any significant additive or synergistic toxicity to the target cells or bone marrow cells in vitro.

It is worth noting that in this study cautions should be taken against overinterpretation of the magnitude of the synergistic and antagonistic effects. The method of Chou and Talalay used in the present study is based on the median-effect principle of the mass-action law and thus emphasizes the importance of IC_{50}. One major feature of this method is to fit the data to the mass-action principle instead of drawing the empirical curve to fit the data. The applicability of data to this principle is routinely checked with the linear correlation coefficient of the median-effect plot without exception. In the present studies, statistical reliability of data was thus tested at the beginning of analysis rather than at the end of analysis after many mathematical transformations have occurred. It should also be stressed that experimental and biological variations will also be reflected in the CI values we used in this study. However, we would like to emphasize that the CI values for each set of data in the present studies consistently indicated synergism (or antagonism). In fact, the distribution of CI

values in Tables 1-4 provided an indication of the general trend of drug interaction of each set of combinations.

Soluble CD4 and dextran sulfate have been shown to be potent inhibitors against the infectivity and replication of HIV-1 in vitro. Soluble CD4 and dextran sulfate may act on an early phase(s) of HIV-1 replication, perhaps on binding of the virus to the target cell or its entry, while dideoxynucleosides, following phosphorylation in the cytoplasm, appear to serve as substrates for viral reverse transcriptase and function as DNA-chain terminators, although this may not be the only mechanism. Thus, combinations of these drugs, which target different stages in the HIV-1 replicative life cycle, may synergize each antiviral activity and may also decrease side effects in patients treated with the combination therapy.

Interestingly, when two viral binding inhibitors, sCD4 and dextran sulfate, were combined, there was an apparent antagonism in five of the nine combinations, although at higher concentrations a weak synergy was observed. This suggests that one of the two agents may inhibit the interaction of the other agent with the virus. With this regard, it is noteworthy that although 1 uM dextran sulfate inhibits the binding of radiolabelled HIV-1 virions to $CD4^+$ cells, the binding of radiolabelled recombinant gp120 to soluble CD4 is not inhibited by even 10 uM dextran sulfate (H. Mitsuya et al, unpublished). These data might suggest that dextran sulfate may not affect the CD4 molecule-viral gp120 interaction itself but affect other events of the cell-virion interaction. The antiviral mechanism(s) of dextran sulfate and sCD4 requires further research.

The current data indicate that 2′,3′-dideoxyinosine combined with sCD4 brings about substantial synergism without concomitant increases in toxicities in vitro. This could be noteworthy since ddI has been shown to suppress HIV-1 replication in patients with AIDS and ARC and thus far, this agent appears to be the least toxic of the nucleoside analogues we have tested as antiretroviral drugs. If the optimal effectiveness of soluble CD4 for clinical application is determined, such combination could represent a potent antiviral therapy regimen against HIV-1 infection. Combination chemotherapy utilizing ddI and other drugs could also be useful, and in particular, could be most suitable for long-term administration for the treatment of individuals with HIV infection.

Taken together, the data in this report may have theoretical and clinical implications in the development of antiretroviral therapies against AIDS and its related diseases.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07213-01 CO

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Lipophilic dideoxynucleoside derivatives active against HIV in vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

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

Four 2-amino-6-halo- and four 6-halo-2′,3′-dideoxypurine ribofuranosides (ddP) were synthesized and tested for in vitro activity to suppress the infectivity, cytopathic effect, gag protein expression, and DNA synthesis of HIV. The comparative order of in vitro anti-HIV activity of the eight 6-halo-ddPs was : 2-amino-6-fluoro, 2-amino-6-chloro, 6-fluoro > 2-amino-6-bromo > 2-amino-6-iodo, 6-chloro > 6-bromo > 6-iodo. 2-Amino-6-fluoro-, 2-amino-6-chloro- and 6-fluoro-ddPs showed a potent activity against HIV comparable to that of 2′,3′-dideoxyinosine (ddI) or 2′,3′-dideoxyguanosine (ddG), and completely blocked the infectivity of HIV without affecting the growth of target cells. The lipophilicity order was : 2-amino-6-iodo > 2-amino-6-bromo > 2-amino-6-chloro > 2-amino-6-fluoro >> ddG > ddI. All eight 6-halo-ddPs were substrates for adenosine deaminase (ADA). The relative rate of hydrolysis by ADA was : ddA, 2-amino-6-fluoro >> 2-amino-6-chloro, 2-amino-6-bromo > 2-amino-6-iodo. In the presence of an ADA-inhibitor, 2′-deoxycoformycin, all 2-amino-6-halo- and 6-halo-ddPs failed to exert their in vitro antiretroviral effect. Taken together, these compounds may represent a new class of lipophilic prodrugs for ddI and ddG, and may also provide a new strategy for endowing therapeutic purine nucleosides with desirable lipophilicity.

INTRODUCTION

Several drugs active against human immunodeficiency virus (HIV) have now been transferred from laboratory to clinical settings to produce therapeutic benefits in patients with HIV infection. One such drug, 3′-azido-2′,3′-dideoxythymidine (AZT or zidovudine) has been formally proven to reduce the morbidity and mortality of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). Other members of the dideoxynucleoside family, including 2′,3′-dideoxycytidine (ddC), 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T) (Browne, M., G. Curt, and Brown University Phase I working group; unpublished data), and 2′,3′-dideoxyinosine (ddI), have recently been shown to be active against HIV in some patients with AIDS and ARC in short-term phase I clinical trials. However, the lipophilicity of 2′,3′-dideoxypurine ribofuranosides (ddPs) including ddI is generally low and perhaps in part this limits their penetration into the central nervous system. One of the devastating features of AIDS and its related disorders is HIV-induced neurological abnormalities. It is of note that HIV-associated neurological disorders in both adults and children with AIDS or ARC have been substantially improved during therapy with AZT. Thus, development of lipophilic antiretroviral drugs might have a direct clinical relevance in the therapy of HIV infection.

MATERIALS AND METHODS

Viruses and Cells

HIV-1 was pelleted by ultracentrifugation from the culture supernatants of HIV-1/III $_B$-producing H9 cells and was prepared to contain 5.54×10^{10} virus particles per ml. The 50%-tissue culture infective dose ($TCID_{50}$) per ml of the cell-free HIV-1 preparation was determined by an endpoint titration method using $CD4^+$ T-cells (ATH8).

The supernatant of monocyte/macrophage (M/M) culture following exposure to HIV-1_{Ba-L} (11) was collected and used as a source of infectious monocytotropic virus.

Three $CD4^+$ T-cell lines, ATH8, MT2, and H9, were used as target cells for infection by HIV-1 in this study.

Reagents

2-Amino-6-chloropurine and 2-amino-6-iodopurine were purchased from Sigma Chemical Co. 2-Amino-6-fluoropurine, and 2-amino-6-bromopurine, and 2′,3′-dideoxyuridine were synthesized by published methods. Four 2-amino-6-halo-ddPs and four 6-halo-ddPs were synthesized by using a method newly developed by Murakami, K. et al (in preparation) employing pelleted live Escherichia coli JA-300 cells as a source of pyrimidine nucleoside phosphorylase and purine nucleoside phosphorylase.

Briefly, each 2-amino-6-halopurine or 6-halopurine was mixed with 2′,3′-dideoxyuridine and pelleted JA-300 cells, and incubated for 3 hr in 50 mM

potassium phosphate buffer (pH 6.5) at 50°C, followed by centrifugation at 8000 rpm for 20 min at 10°C. The supernatant was then chromatographed on a DIANION HP-20 column (Mitsubishi Kasei Co., Tokyo, Japan) and eluted with water followed by methanol. Following methanol evaporation, resulting compounds were purified by crystalization. Compounds tested in this study are listed in Table 1 with their in vitro antiviral activity against HIV. Compounds 6, 11 and 12 in Table 1 have been described elsewhere, while compounds 1-5, 7-10, 13-15 were newly synthesized and will be described in detail elsewhere. All synthesized ddPs were > 99% pure (except 2,6-diamino-ddP whose purity was ~95%) as assessed by nuclear magnetic resonance spectra and high performance liquid chromatography (HPLC). Satisfactory elementary analyses have been obtained for all the new compounds described in this paper. 2′,3′-Dideoxyadenosine (ddA), 2′,3′-dideoxyinosine (ddI), and 2′,3′-dideoxyguanosine (ddG) were provided by the Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, while 3′-azido-2′,3′-dideoxythymidine (AZT) was purchased from Sigma Chemical Co. Adenosine deaminase from calf spleen was purchased from Boehringer-Mannheim (Indianapolis, IN).

HIV Cytopathic Effect Inhibition Assay

The HIV cytopathic effect inhibition assay was performed as previously described. Briefly, target $CD4^+$ T cell lines, ATH8 or MT2 cells, were exposed to HIV-1, resuspended in 2 ml of fresh complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/ml of penicillin, and 50 mg/ml of streptomycin) containing 15% (vol/vol) interleukin 2 (lectin-depleted; Advanced Biotechnologies Inc., Silver Spring, MD) and 50 U/ml of recombinant interleukin 2 (Amgen, Tousand Oaks, CA), and the cells were incubated at 37°C in 5% CO_2-containing humidified air.

Determination of HIV-1 gag Protein Expression

Determination of HIV-1 gag protein expression was performed. Briefly, H9 cells ($2x10^5$) were pretreated for 2 hrs with various drugs, exposed to a dose of 4.3×10^3 $TCID_{50}$ HIV-1 per cell, and cultured at 37°C in 5% CO_2-containing humidified air. On days 5 and 7, the percentage of the H9 cells expressing p24 gag protein was determined by an indirect immunofluorescence assay using a murine monoclonal antibody reactive against HIV-1 p24 gag protein (M26).

Southern Blot Hybridization

Southern blot hybridization was performed as previously described by Southern with minor modifications. Briefly, high molecular weight DNA was extracted from cultured cells, and 40mg of such DNA was digested with Kpn I (Boehringer, Mannheim), subjected to electrophoresis, transferred to nitrocellulose, hybridized with a radiolabelled DNA [env region of BH10 containing a 1.3 kilobase Bgl II fragment], and the viral DNA was detected by autoradiography. Relative levels of the detected viral DNA were compared by densitometry (X-Rite, 301; X-Rite Inc. Grand Rapids, MI). The percent reduction of HIV proviral DNA content was determined by the

following formula: 100 x [1-(O.D. for a sample DNA - the background O.D.)/(O.D. for no drug control DNA - the background O.D.)], where O.D. represents optical density reading and the background represents the lowest density site within each lane.

Determination of HIV-1 gag protein expression in Monocytes/Macrophages

Briefly, target M/M (10^6) were preincubated with drugs for 20 min, exposed to 100 l of HIV-1_{Ba-L} preparation (one l of the supernatant represented the minimum infectious dose, and cultured in 1 ml of complete medium in the presence or absence of the drug. On day 6 in culture, the cells were extensively washed and further cultured in 1 ml of fresh medium. On day 12 and beyond, the amount of p24 gag protein in the supernatant was assessed by radioimmunoassay (Du Pont, NEN Reseach Products, Boston, Mass.).

Partition Coefficient Determination

n-Octanol/water partition coefficients (P) were determined by a micro shake-flask procedure. A 20 l aliquot of a 0.5 mg/ml DMSO solution of a given compound was dissovled in 1 ml octanol-saturated, pH 7.0, 0.01 M potassium phosphate buffer, and thoroughly mixed with 1.0 ml buffer-saturated n-octanol in a 2-ml Mixxor apparatus (Lidex Technologies, Bedford, MA) at 24-26°C. The phases were separated, centrifuged individually, and the relative concentration of sample in a 50 l aliquot of each phase was determined by HPLC analysis. The partition coefficient was calculated by dividing the absolute area of the appropriate integrator peak from the octanol phase by that of the buffer phase. The values were expressed as log P's.

Studies of Enzymatic Hydrolysis by Adenosine Deaminase

Relative rates of hydrolysis of ddPs by ADA and characterization of their products were determined.

RESULTS

In Vitro Antiretroviral Activity of 2-Amino-6-halo- and 6-Halo-2',3'-dideoxypurine ribofuranoside (ddP) against HIV.

Four 2-amino-6-halo-ddPs, (compounds 1-4 in Table 1) and three 6-halo-ddPs (compounds 5-7) exerted a potent anti-HIV-1 activity in vitro. In the HIV cytopathic effect inhibition assay, almost all ATH8 cells were destroyed by the virus by day 7 after exposure to HIV-1 in the absence of drugs (Figure 2). However, when the cells were cultured in the presence of 2 and 5 M of each compound, a partial protective effect on ATH8 cells was obtained. At 20 and 50 mM, the cells were virtually completely protected against HIV-1 and grew comparably to virus-uninfected cells (Table 1). These compounds exhibited comparable antiviral activity when MT2 cells were employed as the target cells (Table 1). At concentrations 100 M, however, the drugs appeared to be somewhat more suppressive to cell growth as compared to the reference compounds ddI and ddG (compounds 17 and 18 in Table 1, respectively). Among ddPs tested, compounds substituted with a bromine or

an iodine were the most toxic for cell growth. Other structurally related ddP congeners (compounds 9, 10, 12-15) were not active against HIV under the conditions described above. It was of note that, contrary to a previous report, 2-chloro-6-amino-ddP (compound 12 in Table 1) was toxic and failed to exert significant protective effect against HIV-1 in both ATH8 and MT2 cells.

In this initial screening assay, 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs were most potent and least toxic among the ddPs tested. Based on such antiviral and toxicity profiles, 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs were further investigated for their antiviral activity against HIV-1.

2-Amino-6-fluoro- and 2-Amino-6-chloro-ddPs Inhibit HIV-1 gag Protein Expression In Vitro

When $CD4^+$ H9 cells were exposed to HIV-1, by day 5 in culture, approximately 40% of the H9 cells expressed p24 gag protein as assessed by an indirect immunofluorescence technique, and on day 7, ~60% of the cells became positive for gag protein. However, 10 mM 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs suppressed the gag expression by 75-100% and at 20 mM both compounds virtually completely blocked the expression of HIV-1 throughout the 7 day period of time in culture. At concentrations used in this experiment, the viability of H9 cells was always 90~100%.

Inhibition of HIV-1 DNA Synthesis by 2-Amino-6-halo-ddP In Vitro

To further characterize the antiviral activity of 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs, the amount of proviral DNA synthesized in PHA-stimulated peripheral blood mononuclear cells (PBM) following the exposure to HIV-1 was assessed using the Southern blot hybridization technique. In the absence of the drugs, proviral DNA was readily detectable on day 2. However, in the presence of 80 M 2-amino-6-fluoro- or 2-amino-6-chloro-ddP, the synthesis of proviral DNA was virtually completely suppressed. Densitometric analysis of the exposed film revealed that 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs reduced the HIV-1 DNA synthesis by 98% and 94%, respectively, as compared to the amount of viral DNA detected in the cells cultured in the absence of drugs.

In Vitro Inhibition of Monocytotropic HIV-1 Replication in Monocytes/macrophages by 2-Amino-6-halo-ddP

We asked if 2-amino-6-fluoro- and 2-amino-6-chloro-ddP could also block replication of a monocytotropic HIV-1 strain, $HIV\text{-}1_{Bal}$, in M/M in vitro. In the absence of drugs, by day 12 in culture, M/M following the exposure to $HIV\text{-}1_{Bal}$ began to produce a detectable amount of HIV-1; and by day 21, they produced as much as 25 ng/ml p24 gag protein as assessed by radioimmunoassay. However, when M/M were cultured in the presence of 2 mM 2-amino-6-fluoro- or 2-amino-6-chloro-ddP, replication of the virus was virtually completely inhibited.

Lipophilicity of 2-Amino-6-halo- and 6-Halo-ddP

Antiretroviral drugs with high degree of lipophilicity may theoretically possess an enhanced ability to penetrate the blood-brain-barrier and may therefore inhibit infectivity and replication of HIV-1 in the CNS. We, therefore, asked whether 2-amino-6-halo- and 6-halo-ddPs had a high level of lipophilicity (Table 2). It was found that all eight 2-amino-6-halo-ddPs had substantially higher octanol partition coefficients than the reference compounds, ddA, ddI, and ddG. The partition coefficients of 2-amino-6-fluoro-ddP and 6-fluoro-ddP were close to that of AZT; however other 2-amino-6-halo- and 6-halo-ddPs had higher coefficients than AZT.

The 2-Amino-6-halo- and 6-Halo-ddPs are Substrates for Adenosine Deaminase

Since 2-amino-6-halo- and 6-halo-purine ribo-nucleosides are readily hydrolyzed by ADA, we asked whether the corresponding dideoxynucleosides were the substrate for this enzyme. This turned out to be the case shown in Table 3, which depicts the rate constants of selected 2-amino-6-halo- and 6-halo-ddPs as substrates of ADA. When the kinetic experiments were performed in culture media containing 15% fetal calf serum, the 2-amino-6-halo-ddPs were still hydrolyzed to ddG, but at a rate that was approximately 60 times slower than the rate in the presence of excess enzyme. Thus, both ddA and 2-amino-6-fluoro-ddP had an approximate 2 hr half-life in RPMI 1640 media.

We then asked if 2-amino-6-halo-ddP could have antiviral activity against HIV-1 in ATH8 cells in the presence of 2′-deoxycoformycin (2′-dCF), a potent inhibitor of adenosine deaminase. We found that the antiviral activity of all these compounds at a concentration of 50 M was completely abrogated in the presence of 5 M 2′-dCF and essentially all the target ATH8 cells were destroyed by the virus (data not shown).

DISCUSSION

Human immunodeficiency virus (HIV) not only causes pathologic effects in immunocompetent cells but also often causes a variety of neurological disorders including acute and chronic meningitis, inflammatory and sensory neuropathy and myelopathy, and encephalopathy. HIV in the central nervous system (CNS) may replicate more actively than in other tissues and the CNS may serve as a principal reservoir of the virus in the whole body. Thus, the capacity of antiviral agents against HIV to penetrate into the CNS may constitute an important feature of therapeutics against HIV. We describe here that the substitution with a halogen atom at the 6 position of the base can confer a substantial lipophilicity on 2′,3′-dideoxypurine nucleosides without reducing their in vitro antiretroviral activity. It should be noted, however, that the principal determinants of entry of any drugs into the CNS include lipophilicity, protein binding, and carrier systems; and the lipophilicty of a given congener per se may not necessarily determine its penetration potential into the CNS. It is also possible that a drug with a high lipophilicty may not have an improved therapeutic index and may even exert increased toxicity. Only in vivo studies could resolve these issues.

The 6-halo versions of 2′,3′-dideoxypurine ribofuranosides are of interest in view of their activity/structure relationships. 2-Amino-6-halo- and 6-halo-ddPs appear to exert antiviral activity only upon conversion to ddG and ddI, respectively (data not shown). In this regard, replacement of a phenolic oxygen (e.g. the oxygen in the position 6 of purine) has been shown to increase the lipophilicity under certain circumstances and 2-amino-6-halo- or 6-halo-purine ribofuranoside, has been identified as a substrate for ADA by Chassy and Suhadolnik. However, it should be noted that there is still no reliable algorithm for predicting which congeners will exert more antiretroviral activity or more cellular toxicity. For example, replacement of a phenolic oxygen of ddI, by a hydrogen, generating 2′,3′-dideoxypurine ribofuranoside, negates the potent antiretroviral activity of ddI. The same replacement of ddG, generating 2-amino-2′,3′-dideoxypurine ribofuranoside, also abolishes the antiretroviral activity of ddG. Indeed, in the present study, we also found that neither of two 6-mercapto-ddPs (compounds 9, 10 in Table 1) exerted antiretroviral activity in vitro. This may be due to the fact that these 6-mercapto-ddPs are not good substrates for ADA (unpublished data) and may not convert to ddG or ddI.

We now have data that each of 6-halo-ddPs discussed in this study is as sensitive as ddI and ddA to solvolysis in acid reactions and decomposes to a purine base and a dideoxyribose, thus losing its antiretroviral activity (unpublished data). However, it has been shown that ddI is orally bioavailabile when administered with antacids, and plasma concentrations higher than those that exert potent antiviral activity in vitro can be achieved.

Taken together, these newly synthesized 6-halo-ddPs may represent a new class of lipophilic antiretroviral drugs against HIV-1. Our current observations may also provide a new strategy to develop lipophilic purine nucleoside derivatives for different clinical applications.

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Antiviral Activity of 2',3'-Dideoxynucleosides Tested

Compound	Target[a]	Concentration (μM)	Protective Effect (%)[b]	Cytotoxicity (%)[c]
2-Amino-6-Halo-2',3'-Dideoxypurines (ddP)				
(1) 2-amino-6-fluoro-ddP	ATH8	2, 5, 20, 50, 100, 200	48, 62, 100, 100, 77, 70	0, 0, 0, 0, 26, 32
	MT2	2, 20, 50, 100, 200	0, 100, 89, 57, 54	0, 0, 0, 33, 42
(2) 2-amino-6-chloro-ddP	ATH8	2, 5, 20, 50, 100, 200	38, 48, 100, 100, 84, 78	0, 0, 0, 0, 19, 25
	MT2	2, 20, 50, 100, 200	0, 100, 100, 62, 38	1, 0, 2, 30, 63
(3) 2-amino-6-bromo-ddP	ATH8	2, 5, 20, 50, 100, 200	35, 35, 100, 100, 78, 64	0, 0, 0, 0, 20, 33
	MT2	2, 20, 50, 100, 200	0, 100, 66, 62, 27	0, 0, 32, 35, 71
(4) 2-amino-6-iodo-ddP	ATH8	2, 5, 20, 50, 100, 200	21, 21, 100, 93, 73, 47	0, 0, 0, 9, 26, 47
	MT2	2, 20, 50, 100, 200	0, 97, 70, 34, 7	2, 5, 25, 63, 96
6-Halo-2',3'-Dideoxy-purines (ddP)				
(5) 6-fluoro-ddP	ATH8	2, 20, 50, 100, 200	64, 100, 87, 61, 43	0, 5, 10, 32, 52
(6) 6-chloro-ddP	ATH8	2, 20, 50, 100, 200	28, 100, 69, 56, 49	8, 1, 20, 41, 55
(7) 6-bromo-ddP	ATH8	2, 20, 50, 100, 200	25, 80, 63, 48, 36	7, 17, 17, 52, 61
(8) 6-iodo-ddP	ATH8	2, 20, 50, 100, 200	17, 41, 39, 30, 20	4, 39, 37, 55, 81
6-Mercapto-2',3'-Dideoxy-purines (ddP)				
(9) 2-amino-6-mercapto-ddP	ATH8	2, 20, 200	6, 1, 2	29, 83, 78
(10) 6-mercapto-ddP	ATH8	2, 20, 200	0, 2, 8	59, 46, 49

Table 1. In Vitro Antiretroviral Activity of 2',3'-Dideoxynucleosides Tested (Continued)

Compound	Target[a]	Concentration (μM)	Protective Effect (%)[b]	Cytotoxicity (%)[c]
Other 2',3'-dideoxypurine (ddP) anlogues				
(11) 2,6-diamino-ddP	ATH8	2, 20, 200	17, 90, 100	0, 0, 0
	MT2	2, 20, 200	0, 3, 77	2, 13, 27
(12) 2-chloro-6-amino-ddP	ATH8	2, 20, 200	1, 1, 0	0, 77, 95
	MT2	2, 20, 50, 200	2, 0, 0, 0	0, 99, 100, 100
(13) 2,6-dichloro-ddP	ATH8	2, 20, 200	0, 0, 0	0, 45, 100
(14) 2',3'-dideoxyxanthosine	ATH8	2, 20, 200	1, 0, 0	3, 2, 11
(15) 2',3'-dideoxypurine	ATH8	2, 20, 200	0, 0, 3	0, 0, 5
(16) 2',3'-dideoxyadenosine (ddA)	ATH8	5, 20, 100, 200, 500	10, 100, 100, 100, 66	0, 0, 0, 0, 0, 28
(17) 2',3'-dideoxyinosine (ddI)	ATH8	1, 10, 100, 200, 1000	7, 97, 100, 100, 46	9, 6, 0, 0, 40
	MT2	2, 5, 10, 20, 50	25, 81, 95, 92, 100	0, 0, 0, 0, 0
(18) 2',3'-dideoxyguanosine (ddG)	ATH8	5, 10, 100, 200, 500	34, 90, 100, 91, 66	0, 0, 0, 1, 41
	MT2	5, 10, 50, 100, 500	0, 5, 40, 76, 38	4, 8, 12, 12, 59
(19) 3'-azido-2',3'-dideoxy-thymidine (AZT)[d]	ATH8	0.5, 1, 5, 10, 50	56, 100, 93, 93, 48	0, 0, 4, 4, 51
	MT2	1, 2, 5, 10, 20	28, 100, 95, 100, 81	0, 0, 5, 0, 12

Legend to Table 1

[a] ATH8 or MT2 cells (2×10^5) were exposed to 4.3×10^3 50% tissue culture infectious doses of HIV-1/III_B per cell (1,000 viral particles per cell) for 1 hr and cultured in the presence of various concentrations of each compound. On days 5 to 7, the total viable cells were counted. Data obtained by using ATH8 or MT2 cells are so indicated in the Table. Orders of numbers in the column for concentrations correspond to the orders of numbers in other columns.

[b] The percentage of protective effect of each compound on the survival and growth of target cells exposed to the virus was determined by the following formula: 100 x [(number of viable cells exposed to HIV-1 and cultured in the presence of the compound - number of viable cells exposed to HIV-1 and cultured in the absence of the compound)/(number of viable cells cultured alone - number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]. By this formula, when the number of viable cells exposed to the virus and the compound is the same as or more than the number of viable cells cultured alone, 100% is given. Calculated percentages equal to or less than zero are expressed as 0%.

[c] The percentage of toxicity of each compound on target cells was determined by the following formula: 100 x [1-(number of total viable cells cultured in the presence of the compound/number of total viable cells cultured alone)]. Calculated percentages equal to or less than zero are expressed as 0%.

[d] 3'-Azido-2',3'-dideoxythymidine (AZT) was listed as a reference compound.

Table 2. Octanol-water partition coefficient

Compound	Log P
2-amino-6-halo-2',3'-dideoxypurines (ddP)	
(1) 2-amino-6-fluoro-ddP	-0.050 ± 0.007
(2) 2-amino-6-chloro-ddP	0.211 ± 0.011
(3) 2-amino-6-bromo-ddP	0.338 ± 0.007
(4) 2-amino-6-iodo-ddP	0.523 ± 0.008
6-halo-2',3'-dideoxypurines (ddP)	
(5) 6-fluoro-ddP	-0.002 ± 0.005
(6) 6-chloro-ddP	0.237 ± 0.007
(7) 6-bromo-ddP	0.354 ± 0.006
(8) 6-iodo-ddP	0.526 ± 0.012
2',3'-dideoxypurines	
(16) 2',3'-dideoxyadenosine	-0.287 ± 0.005
(17) 2',3'-dideoxyinosine	-1.242 ± 0.028
(18) 2',3'-dideoxyguanosine	-1.091 ± 0.006
(19) 3'-azido-2',3'-dideoxythymidine (AZT)†	0.052 ± 0.009

† 3'-azido-2',3'-dideoxythymidine (AZT) was listed as a reference compound.

Table 3. Rate Constants of 2-Amino-6-halo- and 6-Halo-ddPs as Substrates of Adenosine Deaminase

Substrate	K_m (Molar)	Relative V_{max}
adenosine	6.9×10^{-5}	100
2-amino-6-fluoro-ddP (1)	1.3×10^{-3}	127
2-amino-6-chloro-ddP (2)	6.2×10^{-3}	50
6-fluoro-ddP (5)	1.0×10^{-3}	76
2',3'-dideoxyadenosine (16)	1.6×10^{-4}	65

Adenosine deaminase activity was assessed as described in Materials and Methods. For comparison, the relative V_{max} of adenosine, the reference compound, was defined to be 100.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07214-01 CO

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Quantitative analysis of HIV-1 proviral DNA in samples from patients with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Hiroaki Mitsuya, M.D., Visiting Scientist, COP, NCI
Shizuko, Aoki, Visiting Associate, PB, COP, NCI
Mary O'Brien, Biologist, COP, NCI
Samuel Broder, Director, NCI

COOPERATING UNITS *(if any)*
DTP, DCT, NCI: Dr. Harry Ford, Jr., Dr. James A. Kelley, Dr. David A. Cooney
CHB; NHLBI: Dr. Hiroyuki Fujii

LAB/BRANCH
Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

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

HepG2-derived hepatoblastoma cells (2.2.15) which actively produce hepatitis B virus (HBV) were cultured in the presence or absence of the antiretroviral agents 2′,3′-dideoxyguanosine (ddG), 2′,3′-dideoxyinosine (ddI), or 3′-azido-2′,3′-dideoxythymidine (AZT). All of these agents diminished the viral replication in 2.2.15 cells as assessed by the amount of extrachromosomal HBV DNA without affecting cellular growth. Among the three dideoxynucleosides, ddG was the most potent agent, diminishing viral replication by as much as 97%. Northern blot analysis revealed no apparent difference between treated and untreated cells in the pregenomic RNA profile, suggesting that dideoxynucleosides suppress HBV replication at the stage of reverse transcription. The effect of varying the time of drug exposure showed that these agents can suppress HBV replication even when added late in culture, suggesting that if de novo DNA synthesis mediated by reverse transcriptase is continuously suppressed, the extrachromosomal DNA molecules are unstable and disappear after a certain period of time. HPLC analyses using ^{3}H-ddG showed that anabolic phosphorylation of ddG to an assumed active metabolite, ddGTP, occurs in 2.2.15 cells.

These data suggest that 2′,3′-dideoxynucleosides may be potential experimental drugs for the treatment of HBV infection by targeting the reverse transcription step, although the data do not address the in vivo toxicity profile or therapeutic index.

INTRODUCTION

During the last decade or so, a great deal has been learned about the replicative cycle of Hepatitis B virus (HBV), the causative agent of acute and chronic hepatitis, certain types of liver cirrhosis, and hepatocellular carcinoma. The latter complication causes over one million deaths worldwide every year. HBV infection is an increasingly serious problem among gay men and intravenous drug abusers, many of whom are co-infected with human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS) and its related diseases. Although there are a number of distinct differences between HBV and HIV, there is at least one notable resemblance between these two human pathogenic viruses: their replicative cycle involves an RNA intermediate and a process of reverse transcription step.

In 1985, we found that a broad family of 2′,3′-dideoxynucleosides can suppress replication of HIV-1 in cultured cells through inhibition of the retroviral reverse transcriptase. One such drug, 3′-azido-2′,3′-dideoxythymidine (AZT or zidovudine), has now been formally proven to confer prolonged survival and improved quality of life in patients with advanced HIV infection. Certain clinical benefits also occur in asymptomatic individuals. Two other didexoynucleosides, 2′,3′-dideoxycytidine (ddC) and 2′,3′-dideoxyinosine (ddI) have recently been shown to be active against HIV-1 in patients with AIDS and AIDS-related complex (ARC) in short term phase I clinical trials. These two drugs are now under phase II cliniclal trials nationwide. We have postulated that therapy for HBV infection by targeting its reverse transcriptase using 2′,3′-dideoxynucleosides was worth persuing. While caution in extrapolating from this model to human disease is necesaary, several members of the 2′,3′-dideoxynucleoside family have turned out to be active in suppressing replication of duck hepatitis B virus (DHBV) in chronically DHBV-infected Pekin ducks in vivo.

In the current study, we asked if dideoxynucleosides could be active against replication of HBV in an actively HBV-producing human hepatoblastoma cell line, 2.2.15.

MATERIALS AND METHODS

Cells

A human hepatoblastoma (HepG2)-derived cell line, 2.2.15, transfected with a plasmid containing HBV DNA, was employed in this study. The 2.2.15 cells carry four 5′-3′ tandem copies of the HBV genome positioned such that two dimers of the genomic DNA are 3′-3′ with respect to each other as chromosomally integrated sequences. These cells episomally express relaxed circular, covalently closed, and incomplete copies of the HBV genome.

Nucleoside analogues

2′,3′-dideoxyinosine (ddI) was provided by the Division of Cancer Treatment, the National Cancer Institute, while 2′,3′-dideoxyguanosine (ddG) and 3′-azido-2′,3′-dideoxythymidine (AZT) were purchased from Sigma Chemicals and

Pharmacia, respectively. [^{3}H]-2′,3′-dideoxyguanosine (specific activity, 44 Ci/mmol) was purchased from Moravek Biochemicals, Inc.

Cell culture and drug treatment

The 2.2.15 cells were trypsinized, resuspended, and seeded in 25 cm^2/tissue culture flasks (Corning) and cultured in complete media [RPMI 1640 supplemented with 15 % heat-inactivated fetal calf serum (Gibco), 4 mM L-glutamine, 50 U/ml penicillin and 50 mg/ml streptomycin] at 37°C in 5 % CO_2 containing air. The culture medium was replaced with fresh complete medium every 2 to 3 days. Each drug was added to the culture at various time points following cell seeding and the concentration was kept constant throughout the drug treatment period.

Isolation and analysis of DNA

Total DNA was isolated from 2.2.15 cells. Briefly, cells were washed in phosphate buffered saline, and lysed in proteinase K-containing solution. Following incubation at 37°C for 4 hours, total DNA was extracted in phenol/chloroform/isoamyl alcohol followed by two extractions with chloroform/isoamyl alcohol. The DNA was precipitated in ethanol and stored at -20°C overnight.

Undigested samples of DNA were electrophoresed on a 1% agarose gel and transferred to nitrocellulose. Hybridization was performed overnight at 42°C with a full length genomic DNA probe (AM12: kindly provided by Dr. John L. Gerin) and radiolabelled by [^{32}P]-nick translation. HBV DNA species were visualized by autoradiography. Relative levels of the detected viral DNA were compared by densitometry (X-Rite 301; X-Rite Inc. Grand Rapids, MI). The percent reduction of HBV DNA content in a sample was determined by the following formula: 100 x [1-(O.D. for a sample DNA - the background O.D.)/(O.D. for no drug control DNA - the background O.D.)], where O.D. represents optical density reading and the background represents the lowest densitiy site within each lane.

Isolation and analysis of HBV-specific RNA

Total RNA was isolated from 2.2.15 cells by the guanidinium thiocyanate procedure and purified on a 5.7 M CsCl cushion. The purified RNA was electrophoresed on a 1 % agarose gel containing 1.1M formaldehyde, transferred overnight to nitrocellulose, and hybridized overnight at 42°C with the radiolabelled AM12 DNA probe. HBV RNA species were visualized by autoradiography.

Metabolism

Anabolic phosphorylation of ddG was analyzed using a minor modification of previously published procedures. Briefly, 2.2.15 cells in 10 ml of RPMI 1640 complete media were incubated with [^{3}H]ddG (5 mCi/ml) in the presence of 5 uM unlabelled ddG for 6 hours just before cells reached confluency following seeding in 75 cm^2 cell culture flasks (Costar 3075). Cells were then trypsinized, harvested, and extracted with 600 ml of 60% (vol/vol)

methanol. The methanol extract was heated at 95°C for 1.5 minutes, clarified by centrifugation, and a 200 ml aliquot was loaded onto a radial compression column of Partisil SAX equilibrated with 20 mM ammonium phosphate. The nucleotides were eluted isocratically for 5 minutes then developed with a highly convex (10 min) followed by a slightly convex (15 min) gradient to 25% and 100% of 700 mM ammonium phosphate with 10% methanol, respectively, and held at the latter for an additional 10 minutes (flow rate 2 ml/minute). Elution was measured by scintillation counting.

RESULTS

Reduction of Extrachromosomal HBV DNA by Dideoxynucleosides In Vitro

We first asked if three dideoxynucleosides, AZT, ddG, and ddI, could affect the replication of HBV in 2.2.15 cells by comparing the amount of extrachromosomal viral DNA produced by untreated or drug treated cells (Table 1-A). The cells were cultured with or without each drug throughout the entire 7 weeks. Total DNA was then isolated from a half of cultured cells, and undigested DNA samples were analyzed by the Southern blot hybridization technique. Total RNA was extracted from another half of cultured cells (vide infra). In the absence of drugs,a dominant band with a mobility of approximately 3.6 kb was readily identified, which corresponds to the relaxed circular form of extrachromosomal HBV DNA. However, when the cells were cultured with 20 mM AZT, 100 and 200 mM ddG, and 100 mM ddI, we observed a substantial reduction in the amounts of extrachromosomal HBV DNA by 77 %, 92%, 97%, and 94%, respectively. At the drug concentrations used, toxicity of these compounds did not appear to be significant as assessed by microscopic morphology, pellet sizes of the cells after trypsinization and centrifugation, and the yield of total DNA from each cell population (cell counting is inaccurate in 2.2.15 cells which do not readily form a single-cell suspension).

We then asked whether the decrease in extrachromosomal DNA was dose-related. The cells were cultured with various concentrations of AZT, ddG, and ddI throughout the 3 week period. In this relatively shorter period of drug exposure, ddG demonstrated a substantial dose-related decrease in viral DNA of 60%, 74%, and 92% at 5uM, 20uM, and 50uM, respectively, and of 24%, 60%, and 64% at 5 mM, 20 mM, and 50 mM, respectively. 100 mM ddG was as effective or nearly as effective as 200 mM ddG. Similar dose-related inhibition of DNA synthesis was observed in cultures with either AZT or ddI; however, these drugs appeared to be less potent than ddG.

Antiviral Effect of Varying Time of Drug Exposure

We asked if drugs could inhibit HBV replication when they were added at various time points to the culture. It has been shown that 2.2.15 cells begin to produce HBV efficiently at near confluency. In our assay, 2.2.15. cells reached confluency ~7 days after seeding. Consequently, drugs were added to 2.2.15 cells on days 7, 17, and 28 of culture, and the cells were further cultured for ~25 days. No significant difference in the magnitude of the decrease in extrachromosomal HBV DNA was seen when the drug exposure was delayed compared to the entire 25 days of drug exposure. Among the three

dideoxynucleosides, ddG was again the most potent agent, and produced substantial levels of HBV DNA reduction by 88% at 50 mM, 85%, 87%, and 95% at 50, 100 and 200 mM, and 75% at 50 mM.

Dideoxynucleosdies Do Not Alter The 2.2.15 HBV RNA Profiles

Dideoxynucleosides are thought to block the infectivity and replication of retroviruses by inhibition of reverse transcriptase. If AZT, ddG, and ddI inhibit HBV replication in 2.2.15 cells at the step of reverse transcription, the HBV RNA pregenome profile, in theory, would not be significantly altered. To address this question, total RNA was extracted from untreated 2.2.15 cells and cells cultured with 20 mM AZT, 100 and 200 mM ddG, and 100 mM ddI, and was analyzed by Northern blot hybridization. In this particular experiment, these three compounds had shown a substantial level of inhibition of HBV DNA synthesis. When total RNA extracted from each cell population was hybridized with a full-length genomic HBV DNA probe, no apparent reduction in the amount of 2.5 kb and 3.5 kb HBV RNA species was observed.

Active Metabolite, ddG-triphosphate is Formed in 2.2.15 Cells

Dideoxynucleoside analogues are succesively phosphorylated in the cytoplasm of human cells ultimately yielding their corresponding dideoxynucleoside-5′-triphosphates, the active inhibitory metabolites of reverse transcriptase. The pathways for anabolic phosphorylation are different for the various dideoxynucleosides. In the case of ddG, anabolic phosphorylation may depend on 2′-deoxycytidine kinase or 5′-nucleotidase (or both).

The 5′-triphosphate forms of several dideoxynucleosides have been extensively studied and shown to have higher affinities for reverse transcriptase than for DNA polymerase a, a key enzyme for cellular DNA synthesis and DNA repair during cell growth), whereas cellular DNA polymerases b and g may be comparatively sensitive to the 5′-triphosphate form. We then asked if the triphosphate of ddG was formed in 2.2.15 cells. To evaluate the metabolism of ddG, 2.2.15 cells were incubated with [^{3}H]-ddG for 6 hours, and the cell lysate was analyzed for the amount of phosphorylated ddG using the reverse phase HPLC. Detected in the eluate were the parent nucleoside, ddG, and its mono-, di-, and triphosphates. The eluted radioactivity of mono-, di-, and triphosphates were 33 picomoles per one nanomoles of ATP, 2.5 picomoles per one nanomoles of ATP, and 1.6 picomoles per one nanomoles ATP, respectively.

DISCUSSION

In the present study, we found that all three dideoxynucleosides tested, AZT, ddG, and ddI, could suppress replication of HBV in the HepG2-derived human hepatoblastoma cell line, 2.2.15. Among these compounds, ddG was the most potent agent against HBV, and suppressed the episomal DNA synthesis by up to 97%. When we studied the metabolism of ddG in 2.2.15 cells, a relatively low, but distinct level of ddGTP formation was identified. Although the life cycle of hepadnaviruses has been shown to involve a reverse transcription step, a functional reverse transcriptase of hepadnaviruses has not been isolated. Furthermore, the precise intracellular metabolic pathway of ddG is not, as yet, completely understood. However, taking into consideration

that (i) ddG is a recognized potent inhibitor of reverse transcriptase, (ii) anabolic phosphorylation of ddG occured in the cytoplasm of 2.2.15 cells, and (iii) the HBV RNA profiles were not affected by the addition of dideoxynucleosides, it seems likely that the suppression of HBV replication by dideoxynucleosides takes place at the step of reverse transcription in the life cycle of HBV. Future research will be needed to elucidate the mechanism(s) involved.

In the current study, higher concentrations of dideoxynucleosides were required for suppression of HBV replication in 2.2.15 cells than are needed to suppress the infectivity and replication of HIV-1 and HIV-2 in vitro. In this regard, substantial differences in efficiency of nucleoside phosphorylation among various cell populations have been reported. The 2.2.15 cell line might have an inherently low level of the kinases required for phosphorylation of dideoxynucleosides tested here, or its uptake of these compounds might be relatively low.

The 2.2.15 cells begin to synthesize extrachromosomal HBV DNA over 7 to 10 days after seeding as assessed by Southern blot hybridization technique. In the current assay, the inhibition of viral DNA synthesis by dideoxynucleosides was most significant when the cells were cultured in the presence of drugs for 47 days). However, when the drug exposure was started 7 to 28 days after seeding and the cells were cultured for an additional 3 weeks, substantial levels of suppression were observed. These findings strongly suggest that if de novo HBV DNA synthesis mediated by reverse transcriptase is continuously suppressed by drugs, extrachromosomal viral DNA which was present at the time of drug addition may degrade and disappear after a certain period of time. This observation may be notable since it implies that extrachromosomal DNA may be eradicated by treatment of infected cells with these drugs for a sufficient length of time.

Cellularly integrated viral sequences are not detected during replication of duck hepatitis B virus (DHBV) except under rare circumstances. Therefore, an integration step of the hepadnavirus genome is thought to be unnecessary for hepadnavirus replication and, indeed, the presence of only free viral DNA without integrated sequences is observed in the chronic carrier stage. However, viral integration has been linked to the development of hepatocellular carcinoma (HCC) in humans and woodchucks. Integrated viral sequences have nearly always been detected in both human HCC-derived cell lines and human tumor samples. Nevertheless, both virus replication and virus expression are generally absent in the poorly differentiated hepatocytes of advanced tumors. Taken together, an effective treatment that suppresses the de novo HBV DNA synthesis in actively HBV-producing cells may theoretically lead to termination (or a "cure ") of the chronic carrier state. In this regard, we have seen that several dideoxynucleosides, including ddC, ddG, and ddI, can produce substantial levels of DHBV DNA synthesis inhibition in chronically infected Pekin ducks after as short as 5 to 6 days' administration.

To date, various attempts have been made to treat HBV infection; however, it is still a formidable challenge to provide chronic HBV carriers with a safe and effective therapy. Based on the short-term experience that ddI can

bring about in vivo activity against HIV, a pilot short-term phase I trial of ddI on patients with chronic hepatitis B has just begun in the National Institute of Diabetes, Digestive and Kidney Diseases.

Recently, several groups have shown that various nucleoside analogues can suppress HBV DNA synthesis in actively HBV-producing hepatoma cell lines in vitro (41,42); however, antiviral activity of such compounds against HBV varies to some extent from one cell line to another. This might be due to: (i) different HBV integration and expression patterns, (ii) different cellular regulation of HBV production, (iii) different metabolic pathways of nucleosides, (iv) different experimental procedures, and other unknown factors. Nevertheless, it appears that at least some of the nucleoside analogues including dideoxynucleosides are active against the replication of hepadnaviruses.

The current data suggest that 2′,3′-dideoxynucleosides, including ddG, may be worth pursuing as experimental drugs for the treatment of HBV infection, although one should use caution in ranking the activity and toxicity of individuals agents based on these in vitro studies. We would also now like to postulate that if we can suppress HBV replication in patients with chronic hepatitis B and provide a considerable level of viral load reduction, it may be possible to decrease the probability of HBV integration into hepatocytes and thereby reduce the incidence of hepatocellular carcinoma. However, more extensive research on agents active against HBV is required.

PUBLICATIONS

1. Kassianides, C., J.H. Hoofnagle, R.H. Miller, E. Doo, H. Ford, S. Broder, and H. Mitsuya. (1989) Inhibition of duck hepatitis B virus replication by 2′,3′-dideoxycytidine. Gastroenterology. 97: 1275-1280.

2. Martin, P., C. Kassianides, J. Korenman, J.H. Hoofnagle, H. Ford, S. Broder, and H. Mitsuya. (1989) 2′, 3′-Dideoxyinosine (DDI) and dieoxyguanosine (DDG) are potent inhibitors of hepadnaviruses in vivo. Proceedings of American Association for The Study of Liver Diseases. A-3:11 (Abstr.)

3. Mitusya, H. and Broder, S. (1989) Antiretroviral chemotherapy against human immunodeficiency virus (HIV) infection: Perspective for therapy of hepatitis B virus infection. Cancer Detection and Prevention, 14: 299-308.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07215-01 CO

PERIOD COVERED

October 1, 1989 through September 30, 1990

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

In vitro inhibition of hepatitis B virus by 2',3'-dideoxynucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Hiroaki Mitsuya, M.D., Visiting Scientist, COP, NCI
Shizuko Aoki, Visiting Associate, PB, COP, NCI
Robert Yarchoan, Senior Investigator, COP, NCI
Rose V. Thomas, Medicine Branch, NCI
Kathy Marczyk, Medicine Branch, NCI
Samuel Broder, Director, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

A rapid and simple method for determining the proviral DNA content in peripheral blood mononuclear cells (PBM) from patients with human immunodeficiency virus type 1 (HIV-1) infection was established by using the polymerase chain reaction (PCR) technique. We found that the majority of HIV proviral DNA copies detectable in unfractionated PBM resided in T cells, while B cells/monocytes contained lesser amounts of HIV DNA ($93.9\pm3.5\%$ for T cells versus $6.1\pm3.5\%$ for B cells/monocytes: $p<0.05$). When we compared the amount of HIV proviral DNA in PBM from 13 patients with AIDS or ARC before and during antiretroviral therapy with 2',3'-dideoxyinosine (ddI) which was given as an escalating dose in a phase I clinical study, a significant decrease was observed in 9 out of 12 evaluable patients receiving the drug for 8 to 14 weeks ($p<0.02$). The decrease appeared more pronounced in patients receiving relatively high doses of the drug.

These data suggest that the quantitation of HIV viral DNA in PBM by PCR is feasible and may theoretically contribute to an overall monitoring of patients receiving experimental therapy. However, larger studies will be required to determine the sensitivity and specificity of this assay and further longitudinal studies will be essential.

HS 6040 (Rev. 1/84) GPO 914-918

INTRODUCTION

It has been shown that the replication of human immuno-deficiency virus type 1 (HIV-1) can be suppressed in patients by several 2′,3′-dideoxynucleosides. One such drug, 3′-azido-2′,3′-dideoxythymidine (AZT or zidovudine), a member of the 2′,3′-dideoxynucleoside family, has been demonstrated to confer prolonged survival and improved quality of life in patients with advanced HIV infection. 2′,3′-Dideoxyinosine (ddI) is another dideoxynucleoside which has a potent antiretroviral activity against HIV in vitro presumably by inhibiting the reverse transcriptase after undergoing conversion to ddATP inside of the cytoplasm of target cells. This drug has recently been given to patients with AIDS or severe ARC in phase I clinical trials. The results of this pilot study demonstrated that ddI could induce an increase in circulating $CD4^+$ T cells and total lymphocytes, a decrease in serum p24 antigen, and improvement in immunologic function. This drug is now being studied in several controlled clinical trials and expanded access protocols.

It is believed that the clinical effects of antiretroviral drugs reflect both their activity in inhibiting viral propagation as well as the capacity of the host to repair and deploy the immune system in the presence of drugs. It is, therefore, likely that antiretroviral chemotherapy would ultimately reduce total body viral load, provided that many of infected cells die out in a finite time span or disappear by anti-viral host defenses. In theory, a population of uninfected immunocompetent cells, protected by the drug, could then be recruited and participate in a regeneration of the immune system, or at least, prevent further immunologic deterioration. Indeed, the decrease of serum p24 antigen levels concomitant with the rise in circulating $CD4^+$ T cells, which has been observed in some patients during therapy with AZT, ddC, or ddI, provides evidence for such an antiviral effect. However, the current assay system for measuring p24 antigen levels (either radioimmunoassay or enzyme linked immunosorbent assay) has substantial limitations as a means of assessing the viral load : it is affected by the level of serum anti-p24 antibody (which can itself change), and in fact it is undetectable in many patients with AIDS or ARC.

In this study, we have attempted to directly evaluate the effects of antiviral therapy with ddI on viral load. To this end, we established a rapid and simple assay for determining the HIV proviral DNA content in peripheral blood mononuclear cells (PBM) from patients with advanced HIV infection by using polymerase chain reaction (PCR), and measured the HIV DNA content before and after initiating treatment with ddI.

MATERAILS AND METHODS

Patients

Patients were selected for this study during the escalating dose phase I clinical trial with ddI, conducted at the National Cancer Institute starting in July 1988.[6-7] The protocol was approved by the Institutional Review Board of the National Cancer Institute, and each patient gave an

informed consent prior to entry. We established our assay system during this phase I study, and therefore, we assessed patients at an oral ddI dose of 3.2mg/kg every 12 hours (dose E), 3.2mg/kg every 8 hours (dose F), 6.4mg/kg every 12 hours (dose G), 12.8mg/kg every 12 hours (dose I) or 12.8mg/kg every 8 hours (dose J). These dose ranges were previously described in detail. A total of thirteen patients, randomly selected, was studied. Of these 13 patients, there were 2 patients with AIDS and 11 patients with ARC according to the CDC criteria. Clinical profiles of patients are summarized in Table 1.

Cells

Peripheral blood mononuclear cells (PBM) were obtained by Ficoll-Hypaque gradient centrifugation from the heparinized venous blood of patients at entry and at different time points during therapy. When applicable, PBM were further separated into T lymphocytes (T cells) and B lymphocytes/monocytes (B/M) by one cycle of sheep red blood cell rosetting as previously described.[12] T cells obtained by this method contained more than 99 % $CD3^+$ cells and the B/M population contained less than 5 % $CD3^+$ cells as assessed by fluorescence-activated cell sorter (FACS) analysis. Immediately after cells were separated, they were washed twice in phosphate buffered saline and then lysed in lysis buffer (50 ml per $3.75x10^5$ cells), containing 50 mM KCL, 10 mM Tris-HCL, 10 mM $MgCl_2$, 0.45 % NP40 and 0.06 mg/ml proteinase K, at 50 c for 1 hr, followed by further incubation at 95^oC for 12 min. The cell lysates were stored at -70 C until assay.

ACH-2 cells, which have been shown to contain one HIV proviral DNA copy per cell[13,14] were employed as calibration standards. To produce a standard curve of HIV DNA, 10 to 10^4 ACH-2 cells were combined with 10^6 PBM obtained from an HIV-1 negative normal volunteer. The cell lysate was prepared from each combination of ACH-2 cells plus PBM and subjected to PCR assay. This standardization was performed in each PCR assay.

Polymerase Chain Reaction

PCR was performed as described by Saiki & colleagues or others[15-18] with some modifications. Briefly, 50 ml cell lysate was mixed with 50 ml PCR working buffer, containing 50 mM KCL, 10 mM Tris HCL, 10 mM $MgCl_2$, 0.02% gelatin, 50 pmoles of either of HIV primer pairs, SK38/39[17] or SK68/69, 50 pmoles of an HLA primer pair (GH26/27), 200 mM dATP, dGTP, dTTP, dCTP and 5 units of Thermus aquaticus (Taq) polymerase, and subjected to 30 cycles of enzymatic DNA amplification using DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) as follows. The samples were heated at 94 C over 1 minute (to denature DNA), cooled to 55 C over 2 minutes (to anneal the primers), and heated at 72 C for 3 minutes (to activate the polymerase and to extend the annealed primers). The amplified DNA was then extracted by 100 ml chloroform and hybridized in solution with a ^{32}P-labeled probe, SK19, SK70 or GH64 for SK38/39, SK68/69 or GH26/27 respectively. The location of the HIV primer pairs and probes are illustrated in Figure 1. Hybridized products were subjected to electrophoresis on 8 % polyacrylamide gels and visualized by the autoradiography, exposing the gels to KODAK X-OMAT™ film at a room temperature for 1 to 4 hours.

The density of bands on the X-ray film exposed to a gel was measured by a densitometer (X-Rite 301; X-Rite Inc. Grand Rapids, MI). The radioactivity of an area encompassing these bands on the same gel was also counted by a radioisotope scanner (Ambis Mark II Radioanalytic Imaging System; AMBIS System Inc. San Diego, CA). The mobilities of hybridized products were determined by using ^{32}P-labeled Hae III digest of fX174.

Definition of Change in HIV Viral DNA Content in PBM

The percent change of HIV proviral DNA content was determined by the following formula: 100 X [(HIV DNA copy number at a given time/baseline HIV DNA copy number at entry)-1]. We defined a change in proviral DNA copies of +20% and above compared with the entry baseline value as an increase, a change between +20% and -20% as no change and a change of -20% and below as a decrease. The 20 % used as a cut-off represented two times the standard deviation of 10 replicate determinations of DNA copy in a representative test, yielding 95 % confidence level.

Statistical Analysis

Wilcoxon's signed-rank test was employed for non-parametric statistical comparison. Spearman rank correlation coefficient was also determined for analyzing an association between HIV proviral DNA copy numbers and $CD4^+$ T cell counts or serum p24 antigen levels. Also, Fisher's exact test (two-tailed) was used for assessing the association between change in HIV proviral DNA and different doses of ddI.

RESULTS

Quantitation of the Amount of Proviral DNA in PBM

We first attempted to quantitate the amount of proviral DNA in PBM from patients by using the PCR technique. For standardization, various numbers of ACH-2 cells were combined with 10^6 PBM from a normal volunteer and subjected to PCR using a gag primer pair, SK38/39, in the presence of HLA primer pair, GH26/27. The HLA primer pair was added in the reaction mixture of standard PCR to ensure that desirable conditions for amplification were used (vide infra). The amplified products were hybridized with the radiolabeled probes in liquid phase. The liquid hybridization yields several radioactive bands on the gels, indicating the existence of different forms of hybridized products such as heteroduplex, heterotriplex and heteroquadriplex. For example, the HIV gag primer pair, SK38/39, produces positive stranded and negative DNA with 114 bases; and upon hybridization with SK 19, a 41-mer positive stranded DNA probe, the resulting heteroduplex may have a mobility of 77 base pairs [(114+41)/2] by electrophoresis. A heterotriplex can also be formed which represents both positive and negative stranded amplified products plus the radioactive probe. This heterotriplex may have a mobility of 135 base pairs [(114+114+41)/2]. The density of two bands at sizes of 191 [representing heteroquadriplex: (114+114+114+41)/2] and 77 base pairs (Figure 2, inset) was measured by densitometry. The radioactivity of an area encompassing these two bands on the same gel was also directly counted by radioisotope

scanning for each sample. Both results were plotted against numbers of ACH-2 cells. Two standard curves thus obtained yielded a virtually identical pattern. This PCR standardization was also performed using an env primer pair, SK68/69, which produced a standard curve comparable with that obtained by using the gag primer pair(data not shown). In the subsequent studies, standardization using the gag primer pair was employed and the numbers of proviral DNA copies in a series of the lysate samples obtained from a given patient at different time points were determined in one assay using the linear portion of the standard curve produced in the same experiment.

T-cells Harbor the Majority of Proviral DNA Detected in PBM

We then asked which cell population(s) in PBM contained HIV proviral DNA. Of 13 patients studied at entry, sufficient PBM were obtained from 6 patients to separate into T cells and B/M to yield more than 3.75×10^5 of each population for PCR assay. Figure 3 shows three representative profiles of the PCR products derived from unfractionated PBM, T cells, and B/M. An HLA primer pair (GH26/27) was included as an internal control in all reaction mixtures and the HLA PCR products were visualized following hybridization with the radiolabeled HLA probe (GH64). Only when the amounts of HLA products were comparable in all lanes, did we assume that the reaction proceeded under evaluable conditions. The HIV PCR data were then further analyzed. We found that in all of 6 patients tested, the majority of proviral DNA detected in PBM resided in T cell fractions ($93.9 \pm 3.5\%$), while B/M fractions contained lesser amounts ($6.1 \pm 3.5\%$) of HIV-1 DNA ($p<0.05$). The numbers of proviral DNA copy in PBM, T cells, and B/M in all patients at entry are summarized in Table 1.

The HIV proviral DNA content in each population was also determined in 10 patients during the therapy with ddI. Again, we found that in all 10 patients tested, T cells but not B/M harbored the majority of the viral DNA throughout the study. ($p<0.01$)

Changes in the Proviral DNA Content in PBM from Patients with AIDS or ARC during ddI Treatment

In this short-term phase I clinical trial of ddI, patients receiving oral doses of ddI 6.4mg/kg/day and above had statistically significant increases in their circulating $CD4^+$ T cells and CD4/CD8 ratios, while at the same time exhibiting significant decreases in serum HIV p24 antigen levels. We then asked if the decrease in viral load (as measured in terms of HIV DNA) could be detected by PCR in unfractionated PBM from these patients following the treatment with ddI. We collected PBM lysates from patients at entry and at various time points during the treatment, determined the numbers of proviral copies in a series of PBM samples in one PCR assay, and compared those with the baseline value of proviral DNA in PBM at entry. The assay was performed at least twice for each series of PBM samples, and in all cases comparable data were obtained showing virtually the same magnitude of changes. For example, panel A shows a substantial decrease in the amount of viral DNA after treatment with ddI. (In this patient, ddI was stopped at week 12 because of an episode of pancreatitis.) It should

be noted that this patient had a substantial increase in the number of circulating $CD4^+$ T cells during the treatment, while there was a decrease after ddI was stopped (104, 239, 216, and 84 $CD4^+$ T cells/mm^3, and 1290, 1530, 2240, 1218 PBM [lymphocytes plus monocytes]/mm^3 at entry, week 7, week 10, and week 15 of treatment respectively), indicating that the observed decrease in the amount of viral DNA during the treatment was not simply due to a decrease of circulating $CD4^+$ T lymphocytes or a dilution of HIV-containing cells by an increase of PBM counts. Panel B shows a profile of one patient who did not have a substantial change in the amount of viral DNA throughout the study. Panel C illustrates another representative profile of a patient who showed a substantial increase in he viral DNA amount in PBM during the treatment.

Patients at oral ddI doses of 3.2mg/kg every 12 hours (dose E) and 3.2mg/kg every 8 hours (dose F) in panel A, patients at a dose of 6.4mg/kg every 12 hours (dose G) in panel B, and patients at doses of 12.8mg/kg every 12 hours (dose I) and 12.8mg/kg every 8 hours (dose J) in panel C. In order to analyze these data, we defined a change of +20% and above compared with the baseline value at entry as an increase, a change between +20% and -20% as no change, and a change of -20% and below compared with the baseline value at entry as a decrease. Analysis of the data according to this definition revealed that there was a statistically significant decrease in 9 out of 12 evaluable patients treated with ddI for 8 to 14 weeks ($P < 0.02$) (patient 10 was not included since PBM were not obtained during this period). In addition, there was also a decrease in 5 out of 7 patients receiving ddI for 20 to 31 weeks (not significant).

We further asked if the observed decrease in viral DNA content in PBM had any correlation with doses of ddI each patient received. It was then noted that 8 out of the 9 patients who had a decrease during 8 to 14 week treatment had received 9.6mg/kg/day or more of ddI (doses F, G, I, and J), although this dose effect was not statistically significant.

No Apparent Correlation between the Amount of Proviral DNA in PBM and Clinical Parameters

Finally, we asked whether the observed decrease in proviral DNA in PBM could be correlated with clinical parameters, such as absolute numbers of circulating $CD4^+$ T cells or serum p24 antigen levels during the administration of ddI (Table 2). Of 3 patients whose serum p24 antigen was positive and became undetectable after 8 to 14 weeks of treatment, one (patient 7) showed a proviral DNA decrease (1585 and 776 copies/10^6cells at entry and week 11 respectively), while two (patients 5 and 11) showed virtually no change (patient 5: 550 and 525 copies/10^6cells at entry and week 13, patient 11: 457 and 398 copies/10^6cells at entry and week 11 respectively). Of 8 patients who had no detectable serum p24 antigen through 8 to 14 weeks of ddI treatment, 7 patients had a moderate to substantial decrease in proviral DNA and 1 patient (patient 9) had a substantial increase (955 at entry to 2399 copies/10^6cells at week 12). One patient (patient 1) receiving dose F had an increase in serum p24 antigen level with a moderate proviral DNA decrease in PBM without significant change in $CD4^+$ T cell numbers (294 and 358 pg/ml serum p24

antigen, 2884 and 1549 copies/10^6cells, 128 and 136 /mm^3 $CD4^+$ T cells at entry and week 13 respectively).

We also determined correlation coefficients between the proviral DNA content in PBM and the $CD4^+$ T cell numbers or serum p24 antigen levels at entry or at every 4 week period of treatment (week 1 through week 4, week 5 through week 8, week 9 through week 12, and so on). Spearman's method revealed that there was no significant correlation between the HIV DNA contents in PBM and the $CD4^+$ T cell numbers or serum p24 antigen levels throughout the study.

DISCUSSION

We established the use of a rapid, simple, and reproducible quantitative PCR assay for determination of HIV DNA content. Further we have demonstrated a significant decrease in the amount of HIV viral DNA in PBM from patients with AIDS or ARC following treatment with ddI. The current PCR assay system required only a small number of cells (3.75×10^5 cells per assay). This may be important, as patients with HIV infection are often anemic and lymphopenic. The use of cell lysates in the current assay also made the procedure simple and less labor-intensive. It is noteworthy that a repetition of thawing and freezing the cell lysates up to 7 times over a long period of time (for up to 31 weeks) did not affect the reproducibility of our PCR data. Taken together, our results suggest this method could be incorporated in the long term follow-up of patients' status.

This quantitative PCR assay using ACH-2 cell lysates as calibrating standard could detect as few as 10 proviral DNA copies per 10^6 PBM. We also found that the majority of HIV proviral DNA detected in unfractionated PBM resided in T cells in all patients tested before the therapy. Such T cells contained about 300 to 7000 HIV DNA copies per 10^6 cells. These findings are consistent with the recent observations. Furthermore, it was found in our study that during the ddI treatment, the predominant presence of HIV proviral DNA in T cells was not still changed.

By using this quantitative PCR assay, we asked if the viral load or the amount of HIV viral DNA in PBM was altered by treatment with ddI during the NCI phase I clinical trial. Interestingly, there was a significant decrease in the HIV viral DNA copies in PBM from 9 out of 12 patients ($P < 0.02$) evaluated during the ddI therapy for 8 to 14 weeks. The observed decrease in the amount of HIV proviral DNA in PBM could have theoretically represented a consequence of the progression of disease, which in turn, might decrease the absolute number of $CD4^+$ T cells. Overall, however, in these 9 patients, there was no decrease in percentage of $CD4^+$ T cells (Yarchoan et al., unpublished data), indicating that the decrease in HIV proviral DNA content in PBM was not due to a proportional decrease in the number of HIV harboring cells in PBM (i.e., dilution effect). In addition, there was no apparent correlation between the viral DNA content and $CD4^+$ T cell numbers. In this study, we also asked whether the observed decrease in proviral DNA content in PBM had any correlation with doses of ddI. Although the number of the patients receiving each dose was small and dose

effect had not reached statistical significance, 8 out of 9 patients who showed a decrease in proviral DNA copies during 8 to 14 weeks of the therapy had received ddI at oral doses of 9.6mg/kg/day or more.

The present study was performed during a phase I clinical trial, and we did not examine PBM from patients who had comparable stages of the disease and did not receive antiretroviral therapy. In this regard, there has been an observation that the amount of viral DNA in PBM from asymptomatic HIV-infected individuals receiving no antiretroviral therapy remains steady or increases as a function of time as assessed by PCR assay (Schnittmann et al., personal communication). Thus, it appears that the decrease in the HIV viral DNA copies observed in the current study was likely due to treatment with ddI as the applied variable.

When we asked if the changes in other clinical markers such as serum p24 antigen levels and absolute numbers of circulating $CD4^+$ T cells correlated with the changes in the amount of viral DNA in PBM, no significant correlation was identified. It is possible that the changes in viral DNA content in PBM simply reflect alterations of certain HIV-harboring T cell populations other than circulating $CD4^+$ T cells. In particular, in patients whose HIV proviral DNA copy numbers increased, certain subsets of already infected cells may have contained high copy numbers per unit cell and expanded or acquired even higher copy number during the study despite the treatment with ddI. It is also possible that there is a time lag between changes of viral DNA content in PBM and the changes of $CD4^+$ T cell numbers or serum p24 antigen levels, and their correlation was not detected in this study. Another possibility is that viral DNA in PBM and two other markers reflect different aspects of the HIV infection. For example, the level of serum p24 antigen could represent the degree of active replication of HIV both in the circulation and tissues including the viral replication in the brain, while the HIV viral DNA content in PBM could reflect the viral load in the circulation per se, and the viral load in various compartments may not necessarily be synchronized with one another.

The HIV proviral DNA content in PBM represents the HIV DNA of both the cells actively producing the virus and cells latently infected. The use of PCR in combination with reverse transcriptase to detect HIV RNA expressed in PBM might identify the compartment of such actively HIV-producing cells. In particular, it is conceivable that by an adroit use of such an RNA PCR technology using judiciously selected primers, one could ask whether some antisense-containing agents act by actually inhibiting specific functions of certain HIV genes. This technique may also help investigate the fate of the target HIV mRNA species upon attack by such antisense constructs. Thus, the combination of a quantitative RNA PCR technique with the current DNA PCR might provide more precise analytical capability for assessing the dynamics of viral load in patients with HIV infection. We could also ask, for example, whether there has been a shift in the HIV RNA profile of patients before and after antiretroviral therapy.

The current data show that the quantitation of HIV viral DNA in PBM by PCR is feasible and may theoretically contribute to an overall monitoring of patients receiving experimental therapy. However, larger studies will be

required to determine the sensitivity and specificity of this assay and further longitudinal studies will be essential to determine if HIV DNA content in PBM could serve as a reliable parameter for assessing the efficacy of antiretroviral treatment.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 07251-03 CO

PERIOD COVERED

October 1, 1989 through September 30, 1990

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

Phase I studies of ddC as a single agent or with AZT

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Robert Yarchoan, Senior Investigator, Clinical Oncology Program, NCI
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Kathleen M. Wyvill, R.N., Medicine Branch, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

In previous annual reports, the first development of anti-retroviral drugs belonging to the dideoxynucleoside family was outlined. One such drug (AZT) has now achieved prescription status. Two others, ddC and ddA, have continued in our development effort. The goal is to find drugs which, alone or in combination, have a better therapeutic index than AZT alone. In a Phase I dose-seeking trial, we administered 5 dose regimens of 2',3'-dideoxycytidine (ddC), a cytidine analogue with potent in vitro activity against human immunodeficiency virus (HIV) which had never previously been given to man, to 20 patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC). ddC was administered intravenously for 2 weeks and then orally for 4 or more weeks. ddC was well absored from the gut and crossed the bloodbrain barrier in these patients. Ten of the 15 patients who received 0.03mg/kg to 0.09 mg/kg ddC every 4 hrs had increases in their absolute number of $T4^+$ cells at week 2 ($p<0.05$); in many of the patients, however, these rises were not sustained. Eleven of 13 evaluable patients had a fall in their serum HIV p24 antigen by week 2 of therapy ($p<0.01$); in a few patients, the p24 antigen subsequently rose to baseline while in msot the decline was sustained. Dose-related toxiciteis included a transient symptom complex of cutaneous eruptions, fever, and mouth sores; thrombocytopenia; and neutropenia. A late toxicity appearing after 6 to 14 weeks on ddC was reversible painful peripheral neuropathy. These results suggest that ddC has activity against HIV in vivo and has a different toxicity profile than 3'-azido-2',3'-dideoxythymidine (AZT). Based on these different toxicity profiles, we subsequently administered a regimen of AZT (200 mg orally every 4 hrs for 7 days) alternating with ddC (0.03 mg/kg every 4 hrs for 7 days) as a feasibility study. The regimen appears active and has less toxicity than either agent alone. Some patients have taken this combination for 3 years.

PHS 6040 (Rev. 1/84)

GPO 914-918

INTRODUCTION

The chemotherapy of pathogenic human retroviral infections is rapidly evolving. 2′,3′-dideoxycytidine (ddC), an analogue of 2′-deoxycytidine, belongs to a family of nucleoside analogues that includes 3′-azido-2′,3′ dideoxythymidine (AZT). These agents were shown to have activity against human immunodeficiency virus (HIV) by Mitsuya and Broder in work discussed in previous annual reports, and one agent (AZT) has been proven to induce immunologic, virologic and clinical improvements in patients with fulmanent HIV infections. The use of AZT, however, is associated with substantial toxicity in some patients, with fulmanent HIV infections. The use of AZT, however, is associated with substantial toxicity in some patients, most notably bone marrow suppression. Therefore, improved therapeutic regimens for the treatment of HIV infections are urgently needed.

Dideoxycytidine functions as a chain-terminating pyrimidine. The drug is anabolically phosphorylated (activated) in human T cells by a different pathway than the one involved in phosphorylating AZT. It is efficiently phosphorylated by human T cells and is approximately 10 times more potent than AZT on a molar basis at inhibiting HIV replication in vitro; complete inhibition in T cells is obtained at a concentration of 0.5 uM of ddC under conditions of high multiplicity of infection. When fewer viral particles are used, ddC is effective at even lower concentrations (down to 10nM). The drug, like all dideoxynucleosides is resistant to deamination by cytidine deaminase (a ubiquitous enzyme which degrades many cytidine analogues including arabinosyl cytosine). In this study, we have administered ddC to patients with HIV infection in a Phase I study designed to test the feasibility of using this agent alone or in combination with AZT in patients and to determine the dose-limiting toxicity.

MATERIALS AND METHODS

Twenty male patients with HIV infection, ages 25 to 57, were entered into an initial trial of ddC. Nine of the patients had AIDS with a history of (Pneumocystis carinii pneumonia(PCP). An additional 6 patients with HIV infection, ages 27 to 33, were subsequently entered into a pilo trial of AZT alternating with ddC; 2 of these 6 were AIDS patients who had had PCP, and 4 had ARC. One of the 6 patients had previously participated in the initial ddC trial. Each of the ARC patients in both trials had a history of oral candidiasis.

Overall, 23 of the patients were gay, one had used intravenous drugs, and had both risk factors for AIDS. Each patient had circulating antibodies to HIV, and each had less than $350/mm^3$ helper T cells ($CD4^+$ cells) when assayed prior to entry into the study. The patients were treated at the Warren G. Magnuson Clinical Center of the National Institutes of Health. The protocol was approved by the Institutional Review Board of the National Cancer Institute. Each subject gave informed consent prior to entry.

Initial trial of ddC

ddC was synthesized for this study by the Developmental Therapeutics Program of the National Cancer Institute. Each patient received an intravenous test dose to ddC administered over 1 hr, and some patients received an oral test dose the following day. The patients were then given ddC intravenously for 14 days according to the following regimens: 0.03 mg/kg every 8 hrs (Regimen A); 0.03 mg/kg every 4 hrs (Regimen B); 0.06 mg/kg every 4 hrs (Regimen C); 0.09 mg/kg every 4 hrs (Regimen D); or 0.25 mg/kg every 8 hrs (Regimen E). Each dose was administered over 1 hr. Patients who completed the intravenous therapy then received oral ddC at the same dose schedule for an additional 4 weeks. Patients who tolerated this initial 6 week period of therapy were given the option of continuing on oral ddC for an additional 6 to 8 weeks.

Patients were closely monitored for clinical and laboratory changes. They underwent testing for cutaneous delayed type hypersensitivity at entry and either at the end of 6 weeks or at the end of therapy (whichever came first) with 0.1 ml candida extract, intermediate strength (5 TU) purified protein derivative (PPD), tetanus toxoid, and trichophyton extract. Lymphocyte subsets reacting to Leu 3 ($T4^+$, helper-inducer T cells) or to Leu 2 ($T8^+$, suppressor-cytotoxic T cells) were analyzed by flow cytometry. Patients were also monitored for their ability to mount an in vitro proliferative response to tetanus, diphtheria, and A/Aichi influenza virus antigens as previously described.

Heparinized plasma samples were obtained at various times after doses of ddC for measurement of ddC levels. In some patients, an aliquot of cerebrospinal fluid (CSF) was obtained by lumbar puncture during the second week of in vitro therapy. ddC concentrations in the plasma samples were measured by high-performance liquid chromatography using a modification of a
method previously describe. ddC concentrations in the CSF samples and simultaneously obtained plasma samples were measured by mass spectroscopy as described.

Serum samples obtained at entry and weekly during therapy were assayed for HIV p24 antigen using an enzyme-lined immunosorbent assay (ELISA) developed by Abbott Laboratories (Abbott Park, Illinois). Mitogen-stimulated peripheral blood mononuclear cells were cultured for HIV as previously described at entry, at 2 weeks, and at 6 weeks.

Pilot study of AZT alternating with ddC

The AZT for this study was provided by Burroughs Wellcome Company (Research Triangle, NC). After an initial evaluation, patients received a regimen of 200 mg of AZT orally every 4 hours for 7 day dosing periods, alternating with -.03 mg/kg ddC orally every 4 hrs for 7 day dosing periods; the regimen was continued for 9 or more weeks. The patients were followed on an outpatient basis, and had weekly evaluations of their clinical, immunologic and virologic status as described above except that viral culturing for HIV was not performed.

Statistics

The statistical significance of the rises in T4 counts and the falls in HIV p24 antigen were assessed using the one-sided Wilcoxon signed rank test for paired observations.

RESULTS

Clinical Pharmacology

The peak ddC concentration was roughly proportional to the administered dose, and peak levels of 0.5 uM (a dose providing complete protection against HIV in vitro under conditions of high multiplicity of infection), were attained with one-hr intravenous infusions of -.06 mg/kg or greater. The average half-life of ddC was 1.2 hrs, and the oral bioavailability averaged 70-80%. Most of the drug appears to be eliminated by renal clearance. CSF samples obtained 2 to 3 1/2 hr after the initiation of a intravenous infusion contained an average of 20% (range 9 to 37%) of the concentration in simultaneously obtained plasma samples. Thus, ddC at least partially penetrated across the blood-brain barrier in these patients.

Immunologic and Virologic Changes

There was no clear trend in the absolute number of T4 cells in the 4 patients receiving the lowest dose schedule of ddC. However, 10 of the 15 patients receiving the next three doses (0.03 mg/kg to 0.09 mg/kg every 4 hrs) had rises in their absolute number of $T4^+$ (helper-inducer) T cells by week 2; in these 15 patients, the mean number of $T4^+$ cells rose from 85/mm^3 at entry to 117/mm^3 at 2 weeks ($p<0.05$). In addition, 12 of these 15 patients had increases in their ratio of helper-inducer/suppressor-cytotoxic T cells during the first 2 weeks ($p<0.01$). After week 2, however, there were decreases in the $T4^+$ cells in some of the patients, and by week 6 (or end of therapy if that came earlier), the mean absolute number of T4 cells (90/mm^3) was not significantly different from that at entry ($p>0.05$). Thus, patients receiving 0.03 to 0.09 mg/kg ddC every 4 hrs appeared to have transient increases in their T4 cells during the initial weeks of therapy.

In terms of immunologic function, 3 of the 17 patients who were anergic at entry developed positive skin tests to at least one antigen while on therapy. However, two patients (both on the 0.09 mg/kg every 4 hrs dose) who had weakly positive tests at entry lost their reactivity by week 6. The proliferative responses of peripheral blood lymphocytes to three recall antigens (influenza virus, diphtheria toxoid, and tetanus toxoid) was monitored in 15 of the patients. None had a substantial decline in their responses, and 6 had substantial improvement. In one patient (#19), there was a marked increase in the response to tetanus toxoid (3030 cpm of ^3H-thymidine incorporation at entry versus 24,990 cpm at week and the increased proliferation may in part have been from immunization as a result of skin testing at entry. However, each of the other 5 patients with

improved proliferation (#5, 9, 13, 16 and #18) responded to diphtheria toxoid (4 pts) or influenza virus (2 pts), and it is unlikely that this was the result of antigenic exposure during treatment.

There was no clear trend in the ability to isolate HIV in mitogen-stimulated cultures of peripheral blood mononuclear cells from the patients during therapy (data not shown). However, administration of ddC resulted in a decrease in serum HIV p24 antigen; of the 13 evaluable patients (i.e., those who had detectable HIV p24 antigen at some point during the study), 11 had declines during the first 2 weeks of therapy. In these 13 evaluable patients, the mean p24 antigen fell from 361 at entry to 135 pg/ml at week 2 ($p<0.01$). These decreases could not be accounted for by a change in the patients' serum antibody to p24 antigen. It is noteworthy that the falls in p24 antigen even occurred in the patients receiving the lowest dose tested. In 4 of the patients, there was a alter rise in p24 antigen after these initial 2 weeks; however, at the end of the 6 weeks (or time when patients were taken off drug if earlier), the mean p24 antigen (173 pg/ml) was still less than baseline ($p<0.05$). Thus, although the patient number was small, ddC appeared to induce at least a transient fall in detectable serum p24 antigen in a significant subset of patients.

Clinical Evaluation

In 9 patients, the initial administration of ddC was stopped before the completion of the first 6 weeks. In 2 patients (#6 and #15), this was because of pneumocystis pneumonia (diagnosed during the first 2 weeks); in 6 patients, because of drug toxicity (see below); and in one patient (#1), because of high fevers (the etiology of these was unclear). The most prominent toxicity during this initial therapy was a transient symptom complex of cutaneous eruptions, malaise, fever, aphthous mouth ulcers, and to a lesser extent arthralgias, ankle edema, and/or diarrhea which were present to some degree in 14 patients, particularly those on the higher doses. Some patients had a fall in serum albumin (averaging 0.5 gm/100ml) during this period, and one patient (#14) developed lip swelling. There was considerable variation among the patients in the severity and expression of this symptom complex. It generally appeared after two weeks of therapy (range 8 days to 4 weeks), and in each of the 7 cases in which the ddC was continued after the development of the complex, the symptoms subsided after 1/2 to 3 additional weeks on ddC.

Some patients receiving the lower doses of ddC (up to 0.06 mg/kg every 4 hrs), developed mild transient thrombocytopenia which subsided even with continued drug administration. Three of the patients on the highest two doses developed more substantial thrombocytopenia and/or neutropenia which were dose-limiting toxicities. In contrast to patients receiving AZT in whom this is an early sign of marrow toxicity, the red blood cell mean corpuscular volume generally did not rise. Bone marrow examinations in 3 patients revealed erythroblastic vacuolization (2 patients) or no abnormalities (1 patient); megaloblastic changes were not prominent. Other toxicities, including hepatic, renal, or cardiac toxicity attributable to the drug, were not observed during this 6 week period.

A different toxicity, painful stocking-glove axonal sensorimotor peripheral neuropathy, developed in 10 of the patients who continued on ddC beyond the initial 6 weeks. It appeared to be dependent on the cumulative dose of ddC, generally appeared after 10 weeks except in the patients on the highest doses, and usually presented as a painful dysesthesia of the feet. Later, patients had decreases in light touch, temperature, vibratory, and proprioceptive sensation, and in severe cases numbness, some weakness, and absent ankle jerks. Electrophysiological studies were consistent with axonal degeneration. This neuropathy is similar to the "painful sensory neuropathy" which can develop in patients with severe AIDS, and HIV-induced neuropathy may have contributed to the picture in some patients. THe neuropathy in general worsened for up to 5 weeks after the drug was stopped, but then began to gradually improve both clinically and electromyographically.

Three patients (#2, #6 and #15) developed PCP while on ddC; two of these e cases appeared during the first 2 weeks of therapy. Other serious infections included one case each of cerebral toxoplasmosis (patient #7), progressive multifocal leukoencephalopathy (patient #13), and fatal gram negative pneumonia (organism could not be cultured) (patient #10). This last patient was not neutropenic at the time the pneumonia developed. In total, 5 patients (#2, #3, #7, #10 and #13) have expired at the time of this writing 12 months after the initiation of the protocol; all but 1 had <30 T4 cells/mm^3 at entry.

Evaluation of clinical parameters of improvement in HIV-related symptomatology was somewhat complicated by the toxicities, particularly the fevers and the mouth sores which hampered eating. In spite of this, the patients gained an average of 0.5 kg during the first 6 weeks of the study; weight gain was particularly noticeable in the patients receiving 0.03 mg/kg every 4 hrs (mean increase 2.9 kg) and was not attributable to fluid retention. In addition, 6 of the patients reported increased energy or decreased fatigue while they were on ddC.

Pilot trial of AZT alternating with ddC

The results of this Phase I trial suggested that ddC had activity against HIV and also had a different toxicity profile than AZT. Also, because ddC appeared most active during the first 2 weeks of administration at the doses tested, we hypothesized that it might best be given on an intermittent basis; by administering ddC in an alternating schedule with AZT, we might also take advantage of their different toxicity profiles. To explore this approach, we administered a regimen of AZT (200 mg orally every 4 hours) alternating with ddC (0.09 or 0.18 mg/kg/day) to 18 patients with AIDS or ARC, with each drug being administered for 7 days at a time.

This study was started in June of 1987 and is still ongoing. The preliminary results from this study, however, do suggest that the drugs are better tolerated when administered in this manner than when either is given continuously. The first patient entered on the trial has now completed 3

years of therapy without developing either neuropathy or toxicity from AZT. Indeed, it appears that by administering ddC in an intermittent regimen, as compared to continuous administration, patients can tolerate at least 4 times as much cumulative drug without developing neuropathy. In addition, even in those patients in whom neuropathy developed, it generally subsided within several seeks after stopping the ddC. Hematologic toxicity was less than one would have expected from continuous therapy with AZT.

Overall, the patients had initial increases in their number of T4 cells (average increase 65 T4 cells/mm^3 at week 12 and 52 T4 cell/mm^3 at week 18). In addition, they had increases in their T4/T8 ratios, declines in serum HIV p24 antigen, and a greater than 4 kg average weight gain while on the regimen. Additional studies will be required to determine the role of ddC in this regimen (i.e., whether similar results would be obtained with intermittent AZT therapy and whether this regimen is indeed superior to AZT as a single drug. The results do indicate, however, that reduced toxicity can be attained by alternate drug administration in this manner, and also that this approach is worthy of additional investigation.

DISCUSSION

The results of this study demonstrate that ddC can be administered to patients with AIDS or ARC on a short-term basis; that serum drug levels above 0.5 uM 9an in vitro virustatic dose) can be attained; that the drug is well absorbed when administered orally; that it has straightforward pharmacokinetics; and that it penetrates into the cerebrospinal fluid. The study revealed several toxicities associated with ddC administration: a transient cutaneous eruption symptom complex; hematologic abnormalities (which also resolved in some patients continued on drug); and, after a number weeks, peripheral neuropathy. The results also suggest that immunologic and virologic improvement can be detected in these patients, at least transiently, as a result of ddC and AZT is reasonably well tolerated for up to 3 years and can confer immunologic and virologic improvements in patients with AIDS or ARC.

As already discussed, ddC is a member of a family of nucleoside analogues, dideoxynucleosides, several members of which are potent inhibitors of HIV replication in vitro. Another member of this family, AZT, was shown in initial studies to induce small immunologic improvements in patients with AIDS or ARC. In a subsequent double-blind placebo-controlled trial, AZT was shown to improve the survival of certain patients with AIDS. The T4 cell rises induced by AZT are often transient, however, and it causes substantial bone marrow suppression in many patients. A rise in the red blood cell mean corpuscular volume is an early manifestation of AZT toxicity, and it is thought that this megaloblastic marrow suppression results from AZT-induced depletion of thymidine-triphosphate levels.

Unlike AZT, ddC administration was not found to be associated with megaloblastic changes. However, as noted above, several unexpected toxicities were observed. The mechanisms responsible for these toxicities are unknown. ddC can be more efficiently phosphorylated in certain monocyte populations than is AZT, and it can form a choline adduct in

mammalian cells; it is possible that one of these biochemical characteristics might contribute to the clinical picture. Of particular interest, and encouraging in a practical sense, was that the cutaneous eruption symptom complex cleared even with continued ddC administration. The later development of peripheral neuropathy, however, ultimately limited the time that ddC could be continuously administered as a single agent using these dose schedules. (Persons taking ddC in subsequent trials should probably avoid taking other neuropathic drugs, ave their vibratory sensation monitored, and have the drug stopped, at least temporarily, when early symptoms develop.) It is possible that by understanding the pathogenesis of these reactions, we might be able to prevent their occurrence while preserving the beneficial effects of ddC. The results of this study suggest that periodic drug-free intervals may significantly reduce ddC-associated neuropathy. Some patients have not been treated for more than one year without neurotoxicity.

The results of this study also showed that ddC induced at least transient (and in some patients more long-lasting) improvement in immunologic function and decreases in serum viral p24 antigen. However, ddC administration did not affect the ability to isolate HIV in mitogen stimulated cultures. This latter technique is probably not a useful indicator of an anti-HIV effect: it involves activating latently-infected cells and, unlike the serum p24 antigen, has not been shown to be affected by doses of AZT which are clinically beneficial. The decreases in p24 antigen seen in the patients were often abrupt and were observed even at the lowest dose of the drug that we tested. ddC appears to be an exceedingly potent agent, and our study provides a rationale for studying even lower doses of the drug. Indeed, preliminary results from an ongoing Phase I/II trial of ddC suggest that decreases of p24 antigen can be observed even in patients given 0.01 mg/kg every 4 hrs, and lower doses are now being tested. It is unclear why in some patients the level of p24 antigen rises after a nadir at 2 to 4 weeks (perhaps in some patients there is reduced phosphorylation of ddC after several weeks) and further research is needed to investigate this point.

The difference in toxicity profiles of ddC and AZT suggested that an alternating regimen utilizing both drugs might provide a sustained anti-retroviral effect with reduced toxicity as compared to either drug used alone; a secondary benefit of such a regimen might be that it would enable a repetition of the consistent anti-retroviral activity seen during the first weeks of ddC therapy. It appears that the hematological toxicity of AZT is drastically reduced by this approach.

We were concerned that the early ddC cutaneous symptom complex might recur during each week on ddC on such a regimen, but except in one patient, this did not occur. Indeed, the regimen tested was in general well tolerated. Also, it appeared to induce a later sustained increase in $T4^+$ T cells (comparable to that observed with AZT alone and a decline in HIV p24 antigen. It is too early to substantially different than intermittent AZT administration per se (such a regimen has never been tested and might possibly provide an improved therapeutic index as compared to conventional AZT regimens); such questions will have to be addressed in larger

controlled studies in which an alternating ddC/AZT regimen is compared to intermittent dosing with AZT.

In summary, the results of these trials suggest that although ddC is associated with some toxicity, it has activity against HIV in patients with AIDS or ARC even at the lowest dose tested and can be used in an alternating regimen with AZT. Additional studies will be needed to determine its role in the armamentarium against HIV infection.

Combination AZT and GM-CSF

We are also pursuing other forms of combination chemotherapy. During the past year, we have initiated a pilot study to test whether the growth-stimulating hormone GM-CSF, given in an alternating regimen with AZT, can ameliorate the bone marrow toxicity of AZT without compromising the clinical activity of AZT. The starting dose of GM-CSF was 2 mg/kg per day. After a ten-day induction regimen of GM-CSF, AZT was alternated every other week with GM-CSF.

The results of this pilot study suggest that it is feasible to give AZT (200 mg q 4h) in a combination regimen with GM-CSF. About two-thirds of the patients developed a localized rash at the GM-CSF injection site. This was not a serious toxicity. About an equal number had some fever during the course of therapy. The fevers were easily controlled with non-steroidal anti-inflammatory agents. Other toxicities involved myalgias and arthralgias.

While the results are still preliminary, some patients have had increases in their T4 counts and other clinical parameters on this regimen. It is too soon to draw specific conclusions; however, the regimen appears to cause less neutropenia than standard-dose AZT as a single agent. Based ont eh observation by Dr. Perno that GM-CSF can potentiate the activity of AZT in macrophages, we are now exploring simultaneous administration of AZT and GM-CSF.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07202-07 BDMS

PERIOD COVERED

October 1, 1989 through September 30, 1990

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	Acting 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 MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

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. 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 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. The Section works with the Clinical Center Medical Information System team, allowing COP input into decisions which directly impact patient care and protocol management. The Section assists the Deputy Clinical Director to insure adequate monitoring of protocols through the MIS Toxicity screens and other mechanisms.

10 Collaborative Projects Within Clinical Oncology Program

Members of the Biostatistics and Data Management Section provide to the intramural clinical research program both biostatistical and data management expertise. Our efforts in these areas are described in Sections A) and B) below.

A. Members of the Biostatistics and Data Management Section (BDMS) participate in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the Section also serves on the Clinical Research Sub-Panel to review all intramural clinical trials. BDMS staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The Section provides statistical support for the COP as well as advice on the best ways to use available NIH computer systems or microprocessor based professional workstations for clinical and laboratory research. The Section is also presently developing extensive microcomputer based data management systems for several branches in the COP.

A detailed list of COP projects to which members of the Section have provided statistical input follows:

(1) Performed two interim analyses of ALL (leukemia) protocol 77-02, a cooperative study with 181 patients at five institutions.
(2) Performed two interim analyses of two ALL protocols for average and high risk patients; the high risk protocol is a single arm extension of the successful chemotherapy only (no cranial irradiation) arm on the multi-institutional 77-02 protocol, with modifications in Ara-C administration to prevent CNS relapse and to more aggressively treat systemic disease. The average risk protocol is a randomized extension of 77-02, comparing two chemotherapy only arms -- one with high dose methotrexate and one without.
(3) Arranged for randomizations and eligibility checklists for protocols to be conducted through COP Branches.
(4) Served as member on Institutional Review Board.
(5) Performed major update of results on all soft tissue sarcoma protocols, comparing adjuvant chemotherapy to no chemotherapy in patients with extremity tumors, and with head, neck and trunk tumors, comparing limb-sparing surgery to amputation in patients receiving adjuvant chemotherapy, comparing a short course adjuvant chemotherapy regimen (350 mg/m^2 doxorubicin) with standard course (550 mg/m^2); and comparing radiation to no radiation in patients with high grade soft tissue sarcoma of extremities with local surgical resection or with low grade soft tissue sarcoma of head, neck and trunk, or extremities.
(6) Performed analyses of dose intensity and relation to outcome in pediatric patients with lymphoma treated on a single protocol.
(7) Analyzed data relating to effects of changes in staging technology on prognostic factors in small cell lung cancer.
(8) Performed analyses of data from a study of benign vs. malignant sympathoadrenal paragangliomas.
(9) Analyzed data from the randomized early stage breast cancer clinical trial.

(10) Performed analyses to determine the relationship between dose of IL2 or alpha interferon on cardiac toxicity.
(11) Performed analyses of chromosome abnormality data from patients with small cell lung cancer to determine whether there are any statistically significant associations between location and number of break points.
(12) Analyzed data on P24 antigen concentrations in HIV patients receiving ddI.
(13) Determined sample size requirements for experiments testing the effects of suramin on calcium release.
(14) Analyzed data on prognostic factors in truncal sarcoma.
(15) Performed analyses of data on the effects of ddI and ddC on the anti-bacterial activity of various lymphocytes.
(16) Participated in a meeting with collaborating senior investigators in a multi-institutional trial of thiotepa in pediatric CNS tumors, which is being conducted by Pediatric Branch.
(17) Prepared statistical considerations for a randomized trial of two delivery forms of AZT vs ddI in pediatric patients with AIDS.
(18) Reviewed an article submitted to the New England Journal of Medicine at the request of a COP investigator.
(19) Performed analyses of data from a study exploring the effect of treatment on growth in children with ALL.
(20) Analyzed data from a multi-institutional trial of 6-MP in pediatric patients with solid tumors.
(21) Performed analyses of data relating to prognostic implications of markers in patients with neuroblastoma.
(22) Analyzed data from a study exploring the prognostic importance of flow cytometry and morphometry in patients with soft tissue sarcoma.
(23) Performed analysis of data from a study examining anti-cachectic effects of TNF injections on tumor-bearing rats.
(24) Analyzed results of experiments testing the effects of TNF and EGF on calcium release.
(25) Provided advice regarding the design of a study of quality of life in patients as assessed by family members and medical staff.
(26) Performed analysis of data from randomized trial of IL2 vs IL2 + LAK in patients with advanced cancer.
(27) Analyzed data resulting from a study of infections in catheters used in patients with cancer.
(28) Performed analysis of data from experiments on the effects of G-CSF on neutrophil activity.
(29) Analyzed data on calcium resorption in the presence of PTH and suramin.
(30) Provided advice regarding the design of two protocols studying the effects of administration of erythropoietin and GM-CSF to patients undergoing radiotherapy.
(31) Provided advice regarding the design of a pilot study for the treatment of patients with HIV who have non-Hodgkin's Lymphoma.
(32) Performed analyses of data from a study of the relationship between levels of lymph nodes and prognosis in patients with early stage breast cancer.
(33) Analyzed data from the randomized advanced cancer trial of IL2 vs IL2 + LAK to determine whether there are factors which can be associated with lengthy (>18 months) survival of patients on the study.

(34) Provided statistical considerations for a Phase I/II study of mafosfamide.
(35) Provided advice to a senior investigator regarding interpretation of percent recurrence vs disease free survival computed actuarially in connection with a clinical alert on use of 5FU/levamisole in colon cancer.
(36) Performed analysis of data on hypernatremia.
(37) Performed analyses of data from a Phase I trial of ddI in children.
(38) Performed analyses to examine differences in survival between patients with SCCL who have extensive stage zero site disease vs. those with only one site of disease.
(39) Analyzed data regarding various immune system measurements in patients receiving ddI.
(40) Performed analyses of event rates in a comparative study of complications due to indwelling catheters.
(41) Provided advice regarding analysis of data from a database of ovarian cancer patients treated at NCI over a 20 year period.
(42) Provided advice regarding design of a trial involving treatment of sub-clavian vein thrombosis.
(43) Prepared statistical considerations for a pilot protocol to gather information in order to compare MRI to other scanning techniques.
(44) Provided statistical considerations for a pilot study of combination treatment for bladder cancer.
(45) Delivered two seminars to a branch regarding research methods and statistics in clinical oncology.
(46) Analyzed data regarding chemotaxis of neutrophils in response to IL-6.
(47) Performed analyses of long-term follow-up data on Ewing's sarcoma patients.
(48) Performed analyses of data for a study addressing the relationshp between duration of response to IL-2 therapy and treatment, demographic, and laboratory paramaters.
(49) Met with investigators to discuss development of a database for all lymphoma patients treated at the NCI.
(50) Provided consultation regarding correlation of HIV copy, T4, and P24 values in patients on a ddI trial.
(51) Reviewed a manuscript for statistical validity that had been submitted to the Journal of the National Cancer Institute.
(52) Delivered a COP Grand Rounds on statistical considerations in cancer clinical trials.
(53) Performed analysis from a study of effects of radiation therapy on patients being treated for soft tissue sarcoma.
(54) Provided advice regarding statistical methods for the analysis of data from in-vitro assays of cytokine activity.
(55) Performed analyses to identify the significance of the difference in platinum DNA adduct levels in tumors for which the patients' response was blinded.
(56) Provided advice regarding correlation and linear regression analysis to a senior investigator.
(57) Provided advice regarding the design of a study to detect a reduction in HAMA between two treated groups of patients.
(58) Performed analyses of data pertaining to various markers and their association with outcome in patients with lung cancer.

(59) Provided statistical input into the planning of a protocol for the use of IL2/TIL therapy in the treatment of patients with neuroblastoma.
(60) Performed analysis of data on the association of a genetic marker with survival in patients with brain tumors.
(61) Performed analysis of data on activity of ddI in patients with HIV.
(62) Performed analyses of data for a study of survival in patients receiving radioactive implants for prostate cancer.
(63) Analyzed data from experiments concerning the distributions of cell counts for cells transfected with a number of oncogenes.
(64) Performed analyses of measures of in-vitro effects of ddC and ddI on human neutrophils.
(65) Performed analyses of data from the study of rehabilitation in patients treated on the randomized early breast cancer protocol.
(66) Performed updated analyses of data from two protocols for treatment of locally advanced breast cancer.
(67) Analyzed data regarding the prognostic significance of eosinophils in patients with neuroblastomas.
(68) Prepared statistical considerations for a randomized trial comparing colloid vs crystalloid in patients receiving IL-2 based immunotherapy.
(69) Provided statistical advice regarding a randomized study of IL-2 vs IL-2 plus PEG IL-2 for treatment of several advanced cancers.
(70) Provided consultation regarding analysis of the association between neuroblastoma stage and NPY expression.
(71) Led a seminar on statistical issues in clinical trials for staff in one branch.
(72) Performed analyses of data from patients who were autopsied following death from ovarian cancer.
(73) Performed analyses of data concerning a study of radiation-related complications in patients with soft tissue sarcomas of the extremities.
(74) Performed analysis for a study relating in-vitro response of SCLC cell lines to patient survival.
(75) Prepared statistical considerations for a protocol for treatment of pediatric patients with high risk ALL.
(76) Performed analysis of data on dose intensity in children being treated on the high risk ALL protocol.
(77) Performed analysis of data from a study of factors affecting development of aspergillosis in patients recently treated with amphoteracin-B.
(78) Prepared statistical considerations section for a Phase II/III protocol of combination chemotherapy in patients with upper GI malignancy.
(79) Prepared statistical considerations for a Phase III protocol to test combination chemotherapy in a population of pediatric sarcoma patients in India, which will be conducted by a COP investigator.
(80) Performed analyses of data from animal experiments measuring the effects of TNF treatment on the response to high dose oxygen.
(81) Conducted analyses of data on infection rates and levels of immunoglobulin G in HIV(+) patients.
(82) Provided advice regarding a planned study of the effect of indwelling catheters on the incidence of infections in HIV(+) patients.
(83) Prepared statistical considerations for a Phase II study of suramin in untreated metastatic prostate carcinoma.

(84) Performed analyses of a Phase II study of suramin in patients with advanced stage prostate carcinoma.
(85) Performed analyses of data from a study of patients treated with combination chemotherapy for ovarian cancer.
(86) Performed analyses of data from a study of patients treated with AZT and related therapies who subsequently exhibited a higher than expected incidence of NHL.
(87) Provided advice regarding methods of analyzing data from a study of a new drug and its effects on the immune response in mice.
(88) Prepared statistical considerations for a Phase II trial in colon cancer.
(89) Performed analysis of data on 23 patients treated with IL-2 based therapy to determine whether knowledge of ploidy could increase the probability of response.
(90) Prepared statistical considerations for a Phase II study to treat pediatric brain tumors with cyclophosphamide and GM-CSF.
(91) Performed analysis of data to identify factors associated with development of aplastic anemia.
(92) Performed updated analyses of three trials in osteosarcoma.
(93) Provided advice regarding design of a trial of AZT vs AZT/ddC to be used overseas, at the request of an NCI/COP physician.
(94) Performed update of the extensive stage small cell lung cancer protocol.
(95) Analyzed data on mean arterial pressure in control and TNF-treated rats.
(96) Provided advice regarding statistical considerations for a Phase II protocol of high dose carboplatin.

B. Data Management Activities

The Section has continued the development and maintenance of several systems which facilitate the monitoring of protocols:

(1) Completed the design and development of the Clinical Data Registry (CDR) System, a DB2, COBOL and ISPF based system. Additional modifications and testing currently continue. A Systems Users/Operations Manaual as well as a Program Maintenance Manual was developed and updated as changes occured.

(2) Continuously updated the Cancer Patient Research Information (CAPRI) System. The data in this system will serve as the base for the CDR System.

(3) Continued to provide support to the Surgery Branch Surgical Oncology Lymphokine Immunotherapy Data (SOLID) System that is maintained on the IBM 370 mainframe with SAS and WYLBUR.

(4) Continued development and programming on the Protocol Database Management System (PDMS) in 4th Dimension on the Macintosh for the Medicine Branch. System documentation, including a User's Guide, Programmer's Reference Manual and Program Hierarchy Diagrams, was produced and submitted to branch personnel for review. Changes and modifications have been made as requested.

(5) Continued development and programming on the Pediatric Data Management System in dBASE III+ for the Pediatric Branch as directed by branch staff. System documentation including a User's Guide and a Programmer's Maintenance Manual was produced.

(6) Began development on a Database Management System for the Radiation Branch. The system was originally to be written in FoxBASE+/MAC on the Macintosh but a decision was made to develop the system in Paradox using an IBM compatible PC to take advantage of the powerful capabilities of the software. Analysis has been completed, required fields have been selected and screens have been developed. In addition, staff continued to maintain and update three FileMaker databases that were developed by project programmers to serve as the temporary Data Management System for the branch until the major system is completed.

(7) Added additional enhancements and requested modifications to the Metabolism Branch Data Management System that was developed in FoxBASE+ on an IBM compatible PC. The system converted to FoxPro and the graphics capabilities of Quattro Pro were added to the system. Provided complete systems documentation including a User's Guide and Programmer's Maintenance Manual.

(8) Redesigned the Patient Listing System for the Navy Medical Oncology Branch. Development and programming continues, with the system being written in FoxPro on an IBM Compatible PC. The original design of the system is being considered with enhancements being added in the screen design, data format, and data entry procedures, editing features, and reporting and archiving capabilities.

(9) Continued the development of the Pathology Tracking System for the Navy Medical Oncology Branch using dBase III+ on an IBM Compatible PC. Added requested enhancements to the system.

(10) Developed several adhoc systems to meet requirements of COP researchers that could not be met by currently existing mainframe or microcomputer systems.

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 placement 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, and retrieval and reporting. Data Management involves 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) 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, plots and tabulations.

(3) Developed and revised data collection instruments, as required, for all branches of the COP.

(4) Created, modified and updated mainframe and microcomputer databases for branches of the COP.

(5) Provided support to insure that patients receiving chemotherapy, especially investigational drugs, have a valid Clinical Center protocol number for pharmacy records.

(6) Monitored all daily outpatient clinic appointment lists and maintained a master database of the latest clinic visit by branch for each COP patient.

(7) Maintained various computer packages and hardware used by the Section.

(8) Provided computer requirements analysis and evaluation, and equipment and software purchase recommendations for acquiring personal computers.

(9) Provided support of the COP use of personal computers, including assistance and consultation in the selection and installation of hardware, evaluation of software packages, and the design and implementation of scientific PC programs.

(10) Assisted research nurses, principal investigators, and clinical associates in the training for 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.

(11) Maintained major statistical and plotting programs for PCs to allow BDMS statisticians to perform Kaplan-Meier analyses, logrank tests (including stratified versions), logistic regression, and Cox regression.

(12) Maintained and modified numerous SAS programs on the IBM 370 mainframe used for producing scheduled and ad hoc protocols, branch and disease specific reports and listings.

(13) 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.

(14) Reviewed and monitored progress notes printed from the Medical Information System (MIS) for new protocol patients.

(15) 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.

(16) 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 occured in the protocols, and performing registrations of all branch patients.

(17) Began providing registration support for all Navy Medical Oncology Branch patients, including setting up all support materials and registering all eligible patients.

(18) Acted as a coordinating center for three multi-center pediatric leukemia protocols, involving the registration 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 Cost Monitoring Program for the Administrative Office.

2. Projects Outside COP

A. The BDMS also participates in biometric activities outside of the COP. A detailed list of projects outside of COP in which the Section's statisticians have provided statistical input include the following:

(1) Reviewed eligiblity requirements and made appropriate changes to materials for a randomized study for treatment of follicular thyroid cancer conducted by NIDDK.
(2) Performed analyses of data comparing abilities of CT scans, angiography, and tomography to detect brain tumors for a study conducted by NIDDK.
(3) Performed logistic regression analyses to try to explore the effect that Camp Fantastic has on the psychological well-being of children with cancer at the request of an investigator from NIMH.
(4) Reviewed a manuscript pertaining to radiologic diagnosis, at the request of an investigator from CC-Diagnostic Radiology.
(5) Prepared statistical considerations section for a protocol involving spinal cord compression being conducted primarily by an investigator from NINDS.
(6) Analyzed data on aminopeptidase levels in rat brain fractions for an investigator in CC-Pathology.
(7) Provided advice to an investigator in DCBD regarding the analysis of the difference between two survival curves.
(8) Advised an investigator from DCBD regarding the statistical methods used in a paper which he was reviewing.
(9) Delivered Clinical Center Grand Rounds on statistical issues in clinical trials.
(10) Provided advice to a physician from NIDDK regarding construction and interpretation of confidence intervals about observed proportions.
(11) Performed analyses of data comparing lipoprotein lipase levels in mice treated with IL-6 or a control, for an investigator in NIDDK.
(12) Provided advice regarding analysis of a study exploring the relationship between the sources and dose of radiation versus development of radiation

pneumonitis for an investigator in the Immunology Branch of DCBD.
(13) Prepared statistical considerations for a trial to be conducted by an investigator from the BRMP regarding DTH evaluation in patients with renal cell cancer.
(14) Served as statistical site visit reviewer for grant applications of an extramural cooperative group conducting therapeutic trials in a specific disease.
(15) Provided advice regarding analysis of Kaplan-Meier survival curves for animals failing kidney transplants in experiments conducted by an investigator in DCBD.
(16) Served as a reviewer for an article submitted in Communications in Statistics.
(17) Provided advice to an investigator in Dermatology Branch, DCBD, regarding analysis of the effect of etretinate on mitoses.
(18) Prepared statistical considerations section for a protocol to be conducted by the Clinical Center Diagnostic Radiology Department which addresses the ability of MRI to detect pulmonary metastases.
(19) Performed analysis of data from the BRMP regarding patients receiving interferon in the treatment of hairy cell leukemia.
(20) Provided advice to the Scientific Director, NCNR, regarding potential studies in the relationship of immune function to nutrition in patients who are HIV(+).
(21) Performed analysis of data on the relationship between Epstein-Barr virus and HIV for a physician in Spain who was formerly a member of the Clinical Center Pathology Department.
(22) Analyzed graft survival data for an investigator in the Experimental Immunology Branch of DCBD.
(23) Delivered a seminar to the Office of Device Evaluations of the FDA on statistical considerations in clinical trials.
(24) Performed analyses of data from a Phase I trial of d4T for treatment of AIDS, conducted at Brown University.
(25) Reviewed a manuscript submitted for publication in Applied Statistics.

B. In addition to data management support for intramural trials, the BDMS provides data management services outside the COP. Project staff have provided operations and/or statistical center support to a number of multi-institutional extramural trials. This support includes performing randomizations, design of data collection instruments, software design and development, production of regular status reports, and production of ad hoc reports and tabulations as directed by the study statistician. The extramural trials supported include:

(1) 7601/7602, Treatment of Early Stage Ovarian Cancer
(2) CCSG-191P, CCSG Protocol for Acute Lymphoblastic Leukemia
(3) CCSG-134P, CCSG Protocol for Poor Prognosis Acute Lymphoblastic Leukemia
(4) CCSG-144P, CCSG Protocol for Average Prognosis Acute Lymphoblastic Leukemia

3. Biometric Research

Current biostatistical research being conducted includes:

(1) A two stage method for selected interactions between variables to be evaluated for prognostic importance.
(2) Development of data management systems which may serve multiple purposes.
(3) Development of models for testing in-vitro synergy of chemotherapeutic agents.
(4) Development of estimation methods for parametric transformations in survival analyses.
(5) Development of appropriate non-parametric techniques for analyzing paired data with missing values.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06119 21 M

RIOD COVERED

October 1, 1989 to September 30, 1990

'LE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytogenetic Studies

INCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacqueline Whang-Peng	Head, Cytogenetic Oncology Section	MB, COP, DCT, NCI
Others:	Turid Knutsen	Medical Technologist	MB, COP, DCT, NCI
	Elaine Lee	Medical Technologist	MB, COP, DCT, NCI
	Wing-Keung Chau	Guest Researcher	MB, COP, DCT, NCI

)OPERATING UNITS *(if any)*

Environmental Epidemiology Branch, NCI; NCI/Navy Medical Oncology Branch, NCI; Surgery Branch, NCI; Pediatric Oncology Branch, NCI; Laboratory of Tumor Virus Biology, NCI

B/BRANCH

Medicine Branch

CTION

Cytogenetic Oncology Section

STITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

IECK APPROPRIATE BOX(ES)

] (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

IMMARY 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 spcific 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 investigating the contribution of inherited chromosome abnormalities and the susceptibility of peripheral lymphocytes to breakage (fragile sites) to the development of these diseases; lymphocytes of individuals with familial cutaneous malignant melanoma (CMM)/dysplastic nevi syndrome (DN) and their spouses were studied. As part of the investigation of the etiology of the fragile sites, we are studying the relationship between topoisomerase I and II and the expression of fragile sites. In addition, the laboratory is 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.

1. Cytogenetic studies of human neoplastic hematologic, and congenital diseases, with special emphasis on AIDS patients who develop leukemia or lymphoma. Specific disease studies include prostatic cancer, non-small cell carcinoma of lung, renal cell carcinoma, small cell tumors in childhood, acute lymphocytic leukemia, preleukemia, secondary leukemia, etc.

2. Cytogenetic studies in prostatic cancer cell lines; 8 established cell lines are being studied.

3. Localization of genes in normal chromosomes, using in-situ hybridization (^{3}H-labeled and biotinylated probes).

4. Cytogenetic studies of cell lines and normal human lymphocytes infected with HIV-1.

5. Twenty-year cytogenetic study of familial B-chronic lymphocytic leukemia in a single kindred.

6. Fragile sites studies (peripheral blood) in patients with:

   a. Small cell lung cancer (SCLC).
   b. Non-small cell lung cancer (non-SCLC).
   c. Hereditary and sporadic cutaneous malignant melanoma.

7. Study of the relationship between Topo II and fragile sites (heritable sites, constitutional sites and tumor breaking sites). The fragile sites to be studied include fragile-X site, 3p14 fragile site, bcl-2, and bcr region.

8. Study of seven KB tissue culture sublines that show variable expression of folate and are resistant to MTX, by virtue of transport mechanism, plus or minus DHFR activity. The cell line with lowest expression of HFR has lost one normal chromosome 11.

PROJECTS COMPLETED:

1. Completion and publication of cytogenetic review chapters and editorials Polycythemia vera (Pierre & Whang-Peng, in press); Non-Hodgkin's lymphoma (Whang-Peng & Lee, 1990); Double minutes (DMS) and homogeneously staining regions (HSR) (Whang-Peng, 1989); Invited editorial: small cell lung cancer (Whang-Peng, 1989).

2. Chromosomal localization of the following genes:

   a. Topoisomerase I gene, 20q12-13.2.
   b. Topoisomerase II gene, 17q21-22.

3. Cytogenetic studies of esophageal carcinoma (Whang-Peng et al., 1990).

4. Molecular and cytogenetic studies of 5 patients with extra pulmonary small cell lung cancer (Johnson et al., 1989).

5. Study of familial B-chronic lymphocytic leukemia (CLL) in a single kindred. Four siblings had documented blood and marrow lymphocytosis during the past 18 to 20 years consistent with CLL. One developed a spontaneous remission; one died secondary to subepiglotities with sepsis; one died with prolymphocytoid transformation and one remains alive with spenomegalic CLL. Cytogenetic study: one patient had several chromosomal abnormalities of the short arm of

chromosome 12, one had earlier karyotyping showed complex trisomy 12, and one had no chromosomal abnormalities seen (Caporaso et al., in preparation).

6. Study quantifying fragile site expression in individuals with familial DN/CMM and DN, as well as other individuals with sporadic DN, CMM, and unaffected spouses. No association was seen between fragile site expression and sun exposure, caffeine, alcohol intake, or skin type. All categories of familial disease had increased fragile site expression compared to sporadic affected and normal controls, in spite of a younger age distribution, age having been associated with fragile site expression in an earlier study (Caporaso et al., in preparation).

7. Induction of topoisomerase II gene expression in human lymphocytes upon PHA stimulation. Using the antisera, DNA Topo II levels of PHA stimulated human lymphocytes were measured by immunoblotting. Our results showed that the increase in intracellular topoisomerase II level parallelled with the entry of cells into proliferation. We also found that the increase in Topo II level resulted from an increase in the amount of Topo II mRNA. This study suggests that Topo I, which is constantly expressed throughout the cell cycle, might participate in the initiation of DNA replication, while Topo II is involved in solving the DNA topological problems accompanying DNA strand separation during DNA replication (Hwong et al., in press).

8. Studies of Topoisomerase-specific antitumor drugs in human lymphocytes using rabbit antisera against recombinant human topoisomerase II polypeptide. Our results showed that the intracellular topoisomerase II but not topoisomerase I level increased parallel to the entry of cells into proliferation. There was at least a 100-fold increase in topo II levels upon PHA stimulation. However, the damage induced by camptothecin also increased with PHA stimulation, while the level of Topo I remained relatively constant. Our results suggest that, in addition to cellular contents of topoisomerases, the state of cell proliferation is another important determinant of drug action (Hwang et al., 1989).

9. Cytogenetic studies in HIV-I infected cell lines. The results showed additional chromosomal abnormalities in all four infected cell lines. Chromosome 17 is the most frequently involved in abnormalities (3 lines), followed by 17 is the most frequently involved in abnormalities (3 lines), followed by chromosome 3 and 21. Six normal peripheral lymphocytes in-vitro infected with HIV-I were studied, no significant chromosomal abnormalities seen (Whang-Peng, et al., in preparation).

10. Non-randum structural and numerical changes in non-small cell lung cancer. Statistically signficiant differences in the number of chromosomal break-points occurred for regions: 1q1, 1q3, 3pl, 3p2, eq1, eq2, 5ql, 7ql, 13p1, 14p1, 15p1, 17q1, 21q1 (Whang-Peng).

PUBLICATIONS:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06513 14 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Pharmacology of Antimetabolite Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carmen Allegra	Senior Investigator	MB, COP, DCT, NCI
Others:	Bruce A. Chabner	Director, DCT	OD, DCT, NCI
	Keisuke Aiba	Guest Researcher	MB, COP, DCT, NCI
	Patrick Elwood	Medical Staff Fellow	MB, COP, DCT, NCI
	Donna Boarman	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)* Cell Biology and Metabolism Branch, NICHHD, NIH; NCI-Navy Oncology Branch, COP, DCT, NCI; Critical Care Medicine Department, Clinical Center, NIH; NCI Pediatric Oncology Branch, Clinical Center, NIH

LAB/BRANCH

Medicine Branch

SECTION

Gastrointestinal Tumor Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	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 unraduced type. Do not exceed the space provided.)*

The section for the study of gastrointestinal tumors is divided into two broad areas that include the development of strategies for the treatment of gastrointestinal 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 the mechanisms of resistance to the antimetabolite class of antineoplastic agents, specifically, 5-fluorouracil and methotrexate. The central focus of this area is to improve the therapy of gastrointestinal cancer by 1) modulating the activity of the available antimetabolite agents in an effort to improve activity and circumvent resistance mechanisms defined from both preclinical and clinical investigations 2) enhancing the dose intensity of antimetabolite agents through use of biologic agents such as interferon and colony stimulating factors and 3) investigating the activity and mechanisms of action of novel agents for efficacy in the treatment of gastrointestinal malignancy. In addition, we are investigating the autocrine growth factor requirements for colorectal carcinoma and adenoma cells in an attempt to characterize and interfere with these growth requirements using specific agents such as suramin. By investigating the progression from adenoma to carcinoma we hope to understand how various autocrine factors may be involved in malignant transformation with the ultimate goal of preventing such transformation.

The investigations of therapies for opportunistic infections is focused on the interactions of antifolate agents on the metabolic pathways in toxoplasma gondii and pneumocystis carinii . In addition to the use of basic biochemical technologies, we are using the tools of molecular biology to provide quantities of the critical enzymes for characterization and to aide in the search for new therapeutic agents.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Additional Personnel Associated with Project:

James C. Drake	Biologist	MB, COP, DCT, NCI
Sydell Zinn	Biologist	MB, COP, DCT, NCI
Edward Chu	Clinical Associate	MB, COP, DCT, NCI
Jean Grem	Medical Staff Fellow	MB, COP, DCT, NCI
Patrick Johnston	Clinical Associate	MB, COP, DCT, NCI
Pamela Daychild	Biologist	MB, COP, DCT, NCI

1. Biochemical Modulation of 5-Fluorouracil

In addition to Leucovorin, we have identified cis-Platin as an agent capable of positive interaction with 5-FU. This interaction has been characterized in several cell lines and we are presently in the process of defining the mechanisms of this potentially useful interaction. preliminary studies suggest that the locus of the interaction is at the level of DNA repair rather than at the level of protein interactions or metabolic alterations.

We have also identified sequence-dependent interactions between 5-FU and ara-azacytidine, a potent new cytidine analog with activity against adenocarcinoma of gastrointestinal origin. Finally, efforts are continuing to identify new agents for the treatment of gastrointestinal malignancies and to understand their mechanisms of action. Cyclopentenyl cytosine (CPEC) is one such agent and we are presently in the process of clarifying its mechanism of action so that it may be applied clinically in a scientifically sound fashion both alone and with other agents. CPEC has been shown to be a potent inhibitor of cytidylate synthetase. Most recently we have found that doses of CPEC that do not produce changes in nucleotide pools are nonetheless capable of cytotoxicity suggesting the drug has a second mechanism of action. We have identified the triphosphate forms of CPEC in the RNA of colon cell lines after low dose exposures to the drug. While the significance of this incorporation is unclear, the data supports RNA incorporation as a potentially important mechanism of cytotoxicity for CPEC. This second mechanism of action would make CPEC unique from other cytidine nucleotide inhibitors which appear to produce toxicity via pure enzymatic inhibition. CPEC is expected to be available for clinical trials within this fiscal year and a phase 1 study will be performed in the Medicine Branch with this new and promising compound.

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 less active or equally active antineoplastics has not resulted in a significant increase in response rate or duration of response. Acquired and de novo drug resistance appear to be the major impediments to the use of the presently available chemotherapeutic agents. Basic scientific efforts 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 "tightness" and, more importantly, 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 a consistent response rate in excess of the expected 18-20% in untreated colorectal cancer and of six randomized trials thus far reported comparing 5-FU with the combination of 5-FU plus leucovorin, 5 have shown a significantly increased response rate and two have shown a significant survival benefit.

These are the first trials to ever show a survival benefit for the treatment of patients with metastatic colorectal cancer.

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 contained biochemical endpoints demonstrating that the addition of leucovorin to 5-FU was responsible for a marked enhancement in the stability of the critical ternary complex. This was the first study to demonstrate that the addition of leucovorin had produced the desired effect of complex stability in serial tumor samples harvested from patients undergoing therapy. A critical piece of information that came from these studies was the observation that the target enzyme, thymidylate synthase, was inducible by exposure to 5-FU. Subsequent preclinical studies have shown that this induction is responsible for drug resistance in certain colon cell lines. The molecular mechanisms responsible for controlling this induction and strategies to circumvent enzyme induction are under active investigation. Using colon and rectal cell lines we have isolated the level of enzyme control to protein translation as no changes in the mRNA levels are apparent in the face of marked enzyme changes following exposure to 5FU. Subsequent studies using an in vitro translational system have revealed that control of the thymidylate synthase translation occurs via an autoregulatory interaction of the protein with its mRNA. Furthermore the binding site occupancy of the enzyme is a determinant of its ability to interact with the message. The identification of the protein-mRNA autoregulatory loop is unprecedented in mammalian systems and may provide a paradigm for other similar systems.

Because of the apparent importance of the thymidylate synthase enzyme as a therapeutic target, we have sought to develop new and more sensitive methods for its quantitation. Over the past 6 months we have developed monoclonal antibodies against the human enzyme and have adapted these antibodies to the quantitation on a cell-to-cell basis of the expression of thymidylate synthase. These immunohistochemical techniques are applicable for quantitation in fixed and paraffin embedded tissue. We are presently engaged with several major cooperative groups to investigate the prognostic significance of thymidylate synthase expression in large numbers of patients with colon, rectum and breast cancers.

2. Antifolate Projects

We have continued work on the mechanism of de novo purine and thymidylate synthesis inhibition by antifolates, including methotrexate. This work has strengthened a novel postulate that inhibition of metabolic pathways results from direct enzyme inhibition rather than via an indirect mechanism of folate depletion. These studies illustrates that de novo purine and thymidylate synthesis are inhibited by clinically relevant concentration of methotrexate without significant folate depletion in two human cell lines (breast MCF-7 and promyelocytic leukemia) and myeloid progenitors from normal human volunteers.

These studies further suggest that the mechanism of metabolic inhibiton is through direct inhibitory effects of accumulated dihydrofolate polyglutamates on the folate requiring thymidylate synthesis and AICAR transformylase. This work has been supported by corroborating the relative folate preservation during antifolate exposures suing a highly sensitive and specific assay for intracellular folates. this assay was developed by Dr. Priest and is capable of directly measuring folates without the need for radiolabels with their attendant deficiencies.

Studies directed toward a more complete understanding of leucovorin rescue may improve the clinical application of high-dose methotrexate, a strategy capable of overcoming most known

mechanisms by which neoplstic cells become resistant to methotrexate. Detailed investigations of this phenomenon have shown that concentrations of folates in vast excess of these found in untreated cells are required to affect cell rescue from methotrexate, suggesting competition at the level of folate-dependent enzymes between reduced foltes and direct enzyme inhibitors such as methotrexate and dihydorfolate polyglutamates. Further, dihydrofolate reached plateau levels in cells and this event may be interpreted as evidence for competition between dihydrofolate and the antifolate for DHFR activity as an important occurrence in the process of leucovorin rescue. Additional studies using dihydrofolate as a rescue agent have clearly illustrated that this folate is ritical to the process of rescue and that the competitive intractions of MTX and leucovorin may be explained by the competition of MTX and dihydrofolate at the site of dihydrofolate reductase. These studies have important implications for the design of new therapeutic strategies utilizing high-dose MTX.

3. Clinical Trials

The current group of intramural clinical trials for the treatment of patients with metastatic colorectal cancer have been formulated from completed clinical trials and preclinical observations made in our laboratories. Each of these trails is based on the combination of 5-FU with leucovorin plus an additional manipulation designed to further enhance the synergy of the basic combination.

Interferon is a relatively poorly understood agent with a host of cellular effects. We have combined this agent with 5-FU /leucovorin for a twofold purpose: 1) several laboratories using murine models have shown that the toxic/therapeutic ratio of 5-FU may be increased by the addition of interferon. In recognition of the steep dose-response curve for 5-FU, the addition of interferon may allow a more dose-intense regimen to be safely administered. 2) We have found that interferon and 5-FU can interact in a positive fashion. Interferon appears to inhibit the induction of the target enzyme, thymidylate synthase, with 5-FU exposure. These two effects may have important clinical implications. Indeed, a study by Wadler etal. has found that the use of interferon with 5-FU results in high response rates in patients with previously untreated colorectal cancer (60%). Preliminary results from patients treated on this trial suggests a high response rate for previously untreated patients. It has also become apparent that interferon effects the pharmacokinetics of 5-FU resulting in a 1.5-fold increase in AUC in matched sets of data taken from patients treated with or without interferon.

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.

Finally, several clinical trails have indicated that the use of PALA with 5-FU may be synergistic. In contrast to previous trials using this combination, the present trials have used a low-dose of PALA that was capable of producing the desired biochemical effect but not the MTD of the drug. Use of high-doses of PALA in previous trials required decreases in 5-FU doses and thus loss of potential benefit. We have designed a trial using PALA with 5-FU and leucovorin with the knowledge that PALA may further enhance the formation and stability of the critical ternary complex. As is true with all current colorectal trials in the Medicine Branch, this trial incorporates both clinical and biochemical/molecular endpoints. This trial has been approved and is presently accruing patients.

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. While the highly purified enzyme was unstable, methods to stabilize the activity have been elucidated. These include the inclusion of excess albumin in the enzyme preparations to prevent adherence to glass and plastic surfaces of the DHPS enzyme and the reducing reagent dithiothreatol. These additions have resulted in stabilization of the enzyme activity for up to 14 days. The purified enzyme has been characterized as having a molecular weight in its native state of 125,000 daltons and an acidic isoelectric point of 6.3. Over twenty sulfonamide and over 40 sulfone analogs have been screened for inhibitory activity against this enzyme. The sulfone compounds were unexpectedly the most potent class of analogs with typical inhibitory constants < 1 μM. The potency of dapsone, the only sulfone analog available for clinical use, was exceeded by only two new analogs which were 10-20- fold more potent inhibitors. The sulfonamide derivatives were somewhat less potent than the sulfones and their inhibitory potency varied by over 3 orders of magnitude. The inhibitors with the greatest inhibitory potential were then tested with our intact cell methodology which measures the incorporation of radio-labelled pABA into the reduced folate pools as an indicator of inhibitor potency. These methods were an outgrowth of the methods developed for the examination of folate pools in cancer cells and have been translated for use in examining folate pools in the various organisms. These studies have corroborated the findings in the cell-free experiments with respect to the interactions of sulfones and sulfonamides with T. gondii and P. carinii organisms.

In addition to serving as a measure of inhibitory ability of antifolate compounds, we have found this system to be an excellent measure of inhibitory activity of essentially any antimetabolite. As such, we have screened a number of pentamidine analogs supplied by Dr. Peter Tidwell for anti-pneumocystis activity. In addition, we have found the pABA uptake method to be a reliable measure of organism viability. Such a measure is not otherwise available for organisms such as P. carinii. This measure has allowed a careful study of potential methods for in vitro growth of the organisms.

The tools of molecular biology have been applied to the study of the P. carinii organisms as a means of understanding the basic biology of the organism and as a means of obtaining large quantities of recombinant enzyme for drug screening and enzyme characterization. In collaboration with Dr. Jeffery Edman, we have found been able to isolate and sequence the P. carinii ribosomal RNA. This sequence is well studied for a great number of organisms and allows a precise description of taxonomy. Contrary to popular opinion, the P. carinii ribosomal RNA is most closely allied with that of the fungi rather than the protozoa. The association with fungi was suspected from our previous studies indicating that the P. carinii organism contained a mono-functional dihydrofolate reductase enzyme of low molecular weight (20,000 daltons) rather than the expected high molecular weight bi-functional enzyme (containing thymidylate synthase activity) typical of the protozoa. In addition, these investigations have resulted in the isolation and expression of recombinant P. carinii dihydrofolate reductase and thymidylate synthase which are in the process of being characterized and will be available for additional drug screening.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06516 09 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Drug Resistance in Human Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth H. Cowan	Senior Investigator	MB, COP, DCT, NCI
Others:	Merrill E. Goldsmith	Microbiologist	MB, COP, DCT, NCI
	Craig Fairchild	Staff Fellow	MB, COP, DCT, NCI
	Masayuki Nakagawa	Visiting Fellow	MB, COP, DCT, NCI
	Alan Townsend	Biotechnician	MB, COP, DCT, NCI
	Jeffrey Moscow	Biotechnician	MB, COP, DCT, NCI
	Mary Jane Madden	Chemist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-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.)*

This laboratory had been investigating genetic and biochemical changes associated with drug resistance in human tumors. We have characterized an adriamycin resistant human breast cancer cell line which has developed the phenotype of multidrug resistance. Resistance is associated with decreased drug accumulation (23 fold), increased activities of glutathione peroxidase (12 fold), glutathione transferase (45 fold), decreased expression of aryl hydrocarbon hydroxylase (cytochrome P1-450). We have isolated cDNA clones from this resistant cell line which encode the gP 170 membrane glycoprotein, a gene which is often associatedwith the development of drug resistance. We have also cloned the cDNA for the anionic glutathione transferase GST-pi which is transcriptionally activated in the AdR MCF-7 cells. We have used gene transfection to analyze the roles of mdr-1, protein kinase C, and glutathione S transferase π, α, mur in development of drug and carcinogen resistance. We have also studied the mechanisms of regulation of mdr1, GST π, and GSH peroxidase gene expression in human tumor cells.

Project Description

Additional Personnel Assigned to Project:

Charles Morrow	Biotechnician	MB, COP, DCT, NCI
Kathy Dixon	Visiting Fellow	MB, COP, DCT, NCI
Lucy Gilbert	Visiting Fellow	MB, COP, DCT, NCI

Multidrug Resistance in Human Breast Cancer Cells

We have been studying the molecular genetic and biochemical changes in a multidrug resistant breast cancer cell line (AdrR MCF-7) selected in our laboratory. Resistance in these cells is associated with 2-3 fold decrease in drug accumulation, increased mdr-1 (p-glycoprotein) gene expression increased expression of glutathione peroxidase and glutathione transferase activity, and decreased expression of aryl hydrocarbon hydroxylase (cytochrome P450IA1). These changes are remarkably similar to those which are induced by carcinogens in rat hyperplastic liver nodules (Salt-Farber model) and which are associated with resistance to many structurally unrelated hepatoxins in that system. These studies suggest that the mechanisms associated with de novo and acquired drug resistance may be the same.

A. Glutathione S-Transferase

We have recently shown that multidrug resistance in MCF-7 breast cancer cells selected for primary resistance to adriamycin is associated with an increase in glutathione S-Transferase activity. This increase is due to the induction of the anionic isozyme of GST (GSTπ). We have purified this isozyme and generated polyclonal and monoclonal antibodies against it. Following affinity purification, the polyclonal antibody reacts with a single protein on Western blot analysis. This antibody is being used to screen human tumor specimens for GST π expression using immunohistochemical staining and Western blot analysis. Monoclonal antibodies against GST π purified from AdrR MCF-7 cells have been generated in collaboration with Raphe Kantor at FCRF. These antibodies have been used to screen different epitopes of anionic GST in order to compare the isozymes produced in different tissues.

Jeffrey Moscow has isolated GSTπ cDNA clones from a library constructed by Merrill Goldsmith from AdrR MCF-7 RNA. This gene encodes a 750 bp mRNA which is overexpressed in AdrR cells. Mary Jane Madden has sequenced this gene and shown that its sequence is remarkably homologous to the rat anionic human GST P gene but differs markedly from the human basic GST genes. This was reported in Cancer Res, 1988.

The human GST π cDNA has been used to screen RNA from normal and malignant human tissues. GST π is expressed in 24/26 human colon cancers but in only 2/10 normal colon tissue samples. This gene may thus represent a marker of the de novo resistance of chemotherapy, in this carcinogen-induced tumor. Other studies have shown that GST π expression is increased in 2 patients with recurrent pre B cell ALL relative to the expression in 3 patients with pre B cell ALL at initial presentation. Thus, GST π expression may be a useful marker in acquired resistance to chemotherapy.

We have also found a relation between GST π expression and the absence of estrogen receptors in breast cancer cell lines using Western and Northern blot techniques. Similarly, GST π expression is also inversely related to expression of estrogen receptors in primary breast cancer. GST π RNA

is moderately high in ER negative (<10 fmoles lung) breast cancers but is present in low or undetectable levels in ER positive breast cancers. Thus, this gene may be a marker of hormone resistance in breast cancer and may be a useful prognostic marker in this disease. Whether this reflects any difference in chemosensitivity is unclear. Dr. Lucy Gilbert has developed a sensitive immunohistochemical assay for GSTπ and is using it to screen a variety of human tissues and tumors for the expression of this isozyme.

Dr. Moscow has developed eukaryotic expression vectors which express GST π in transfected cells. MCF-7 cells transfected with this vector, over expressing GST π are resistant to the carcinogens benzo(a)pyrene and benzo(a)pyrene antidiol epoxide. These cells display little change in sensitivity to adriamycin, cisplatin, and phenylalanine mustard. Thus, the role of this gene is antineoplastic drug resistance is not clear. This work appeared in **Mol. Pharm.**, 1989.

Dr. Alan Townsend in our laboratory has been studying the role of mu class glutathione transferases in drug and carcinogen resistance. He has cloned two murine mu class GST's from a mouse fibroblast cell line. Sequence analysis of the mu class murine gene has shown that they are remarkably similar to the rat mu class genes Yb1 and Yb2. Dr. Townsend has created prokaryotic expression vectors for each of these murine mu class genes. He has used these prokaryotic expression vectors to purify isozyme specific murine GST's and has characterized the biochemistry of each of each of these isozymes and compared them to rat Yb1 and Yb2 isozymes as well as the human GST mu gene. Substrated and inhibitor analyses have shown some interesting differences between the different mu class isozymes. The cloning of the murine mu class GST's appeared in JBC, 1989. A second manuscript on the biochemical analysis of the GST mu class isozymes is currently in preparation.

Drs. Bryan Leyland Jones, Merrill Goldsmith, and Alan Townsend have examined the relationship between alpha and mu class GST expression and drug and carcinogen resistance. They have developed eukaryotic expression vectors for the human GT1 and GT2 cDNA's (human alpha class cDNA's). In addition they have made expression vectors for the human GT mu cDNA (mu class gene). These GST expression vectors have been transfected into MCF-7 human breast cancer cells. Following transfection individual clones containing elevated levels of glutathione transferase were selected and analyzed for drug resistance. The alpha and mu class genes resulted in resistance to ethacrynic acid, a known substrate for glutathione transferase. However, we could demonstrate no resistance to other anticancer drugs including alkylating agents, cis DDP, adriamycin, or other drugs associated with multidrug resistant phenotype. Furthermore the cells were not cross-resistant to benzo(a)pyrene or benzo(a)pyrene diol epoxide.

B. MDR Gene Transfection and Regulation

Craig Fairchild, and Merrill Goldsmith have isolated p-glycoprotein cDNA sequences from AdrR MCF-7 cells. Dr. Fairchild has sequenced the mdr 1 which is overexpressed in adriamycin-resistant MCF-7 cells and found a nucleotide change resulting in an amino substitution in a transmembrane domain of the mdr-1 gene in adriamycin-resistant MCF-7 cells. This is of interest since previous studies by Roninson and coworkers have shown that a single amino acid change in the mdr gene in another region apparently encodes a p-glycoprotein with a different resistance pattern. Dr. Fairchild has created an expression vector using mdr 1 gene sequences isolated from AdrR MCF-7 cells. These were fused to a variety of different promoters and each of the expression vectors have been transfected into wild type MCF-7 cells. The mdr 1 expression vectors do result in a multidrug resistant phenotype when transfected into drug sensitive cells. The phenotype is similar to that present in the parent cell line (AdrR MCF-7). We have also transfected cells with an mdr-1 gene without that single amino acid subsitution. Analysis of the MDR pattern suggests that both of these normal human alleles give rise to similar patterns of mdr.

Since we have found overexpression of both the p-glycoprotein and the GST π gene in multidrug resistant MCF-7 cells, we have also examined whether these two genes can function together. Cells transfected with the mdr 1 vector only were then subsequently transfected with the GST π expression vector. Clones were selected by co-transfected with PSV-Neo and surviving colonies were screened for glutathione transferase activity. Subclones expressing high levels of GST π were then grown and studied for drug resistance. Overall, we could find no difference between the pattern and level of multidrug resistance in mdr 1 transfected cells and mdr + GST transfected cells. Thus GST π does not apparently interact with p-glycoprotein. This was published in Mol. Pharm. 1990.

In order to study the regulation of the *mdr-1* gene, Mary Jean Madden has cloned the promoter and 5' flanking sequences of the human *mdr-1* gene. Dr. Masayuki Nakagawa has sequenced the *mdr-1* promoter regent and 4.7 kb of upstream sequences. These sequences have been fused to the bacterial chloramphenol acetyl transferase gene and the study of the *mdr-1* promoter has been accomplished by transfection of the *mdr-1-CAT* fusion genes into drug sensitive and multidrug resistant human tumor cell lines. These studies have indicated that the *mdr-1* promoter is, in contrast to previous suggestions, a relatively strong promoter. Following transfection CAT expression in cells transfected with the *mdr*CAT vector is comparable to the CAT expression in cells transfected with SV2CAT and RSVCAT expression vectors. Thus the *mdr-1* promoter is comparable in strength to these relatively strong viral promoters. A series of deletion mutants in which portions of the 5' flanking region of the *mdr-1* gene were deleted from the *mdr*-CAT fusion vectors. Analysis of transfection of each of these deletion constructs has indicated that there are two possible regulatory regions in the 5' flanking regions. There is a 500 base pair region around 2kb upstream from the promoter which appears to have negative regulatory control and a region between 2040-700 base pairs upstream which has a mild (two fold) positive regulatory effect. Furthermore, deletion of sequences down to 133 base pairs can be done without losing a significant amount of the *mdr* promoter strength. Thus much of the regulation of basal expression of the *mdr* gene is encoded within these sequences.

Dr. Masayuki Nakagawa has taken these *mdr* CAT expressions vectors and transfected them into the human colon carcinoma cell line SW620. Dr. Fojo in the Medicine Branch has recently shown that treatment of SW620 cells with sodium butyrate enhances *mdr* gene expression. Dr. Charles Morrow in our laboratory has done nuclear run on analysis of the SW620 cells before and after butyrate. Analysis of these studies have shown that the increase in *mdr* -1 gene expression in these cells following treatment with sodium butyrate is associated with enhanced *mdr* - gene transcription. Dr. Nakagawa has shown that CAT expression of SW620 cells is markedly increased on treatment with *mdr*-CAT expression in SW620 cells treated with sodium butyrate this is consistent with the *mdr-1* gene being transcriptionally upregulated following sodium butyrate treatment of SW620 cells. Deletion mutant analysis suggest that much of the upregulation of CAT expression induced by sodium butyrate is also encoded within the first 133 base pairs 5' to the start of transcription. Two manuscripts on *mdr* gene regulation are currently in preparation.

C. GST π Gene Regulation

Since pi class GST is overproduced in two models of drug resistance, the multidrug resistant MCF-7 breast cancer cell line and the Salt-Farber resistant hyperplastic liver nodule system, our laboratory has focused attention on what regulates the expression of this gene. In order to do this Charles Morrow in our laboratory has cloned the human genome GST π sequenced the entire gene as well as 2500 base pairs of 5' flanking sequence. To study the elements involved in transcriptional regulation of this gene, Dr. Morrow has fused the 5' flanking sequences of the human genomic GST gene to the CAT gene and made a series of 5' end deletions and internal deletions of the GST π CAT fusion constructs. These constructs were transfected into a series of cell lines and Dr. Morrow has been able to identify at least two different elements which are

involved in the transcriptional regulation of this gene. Furthermore, Dr. Morrow has found that the human GST π gene like the rat GST-P gene, has a consensus sequence which corresponds to the binding site for AP-1 transcription factors, which include the c-jun oncogene. These sequences are normally upstream from genes which are regulated by phorbol esters. In order to study whether this sequence is indeed involved in the regulation of the human GST π gene, Dr. Morrow has co-transfected the human GST π-CAT fusion constructs with expression vectors containing the jun oncogene with or without vectors expressing the fos oncogene, which also augments expression of genes with AP-1 consensus site. These experiments have shown that the AP-1 site in the human GST π gene is not recognized by the cjun oncogene. The work on cloning and sequencing of the human GST π gene has been published in Gene, 1989 and a second manuscript on the regulation of expression of the GST π gene appeared in Gene 1990.

D. Glutathione Peroxidase Gene Regulation

Dr. Jeffrey Moscow in our laboratory has cloned the human glutathione peroxidase genomic gene. Our intrest in this gene stems from the observations that anticancer drugs like adriamycin can generate hydrogen peroxide in normal and tumor cells. Furthermore our adriamycin resistant MCF-7 breast cancer cells contain elevated levels glutathione peroxidase. Drs. Moscow and Townsend have shown that there is a correlation between estrogen receptor and glutathione peroxidase gene expression in human breast cancer cell lines, a relationship that we previously described with respect to glutathione transferase π gene expression. Dr. Moscow has sequenced the glutathione peroxidase gene promoter region and 2 kb of upstream flanking region. He has also generated a GPXCAT expression vector and used it analyze the upstream sequences of peroxidase gene which are involved in it's intercellular regulation. He has identified a very potent upstream enhancer of the glutathione peroxidase gene.

E. Protein Kinase C and MDR

Previous studies by Dr. Robert Fine and Dr. Bruce Chabner have demonstrated that in our adriamycin resistant breast cancer cells, there was a marked increase in protein kinase C activity. In order to study the role of protein kinase C in the development of drug resistance we have first transfected MCF-7 cells with an expression vector for the *mdr-1* gene. These studies were described earlier and resulted in the transfected MCF-7 displaying multidrug resistance. However despite having comparable *mdr-1* RNA levels and comparable p-glycoprotein levels (determined by flow cytometry in collaboration with Dr. Jane Trepel of the Medicine Branch) the *mdr-1* gene transfected MCF-7 cell still had approximately 10 fold lower level of resistance than the adriamycin resistant MCF-7 cells. Dr. Fairchild then took two clones of *mdr-1* gene transfect MCF-7 cells and subsequently transfected them with an expression vector for protein kinase alpha. These studies were done in collaboration with Drs. Robert Glazer and Gang Yu of the Laboratory of Biological Chemistry. These studies have shown that the clones that were subsequently transfected and expressing higher levels of protein kinase C developed 3 to 10 fold higher levels of multidrug resistance than cells transfected with *mdr-1* alone. This was associated with increased p-glycoprotein phosphorylation in the protein kinase C transfected cells. These studies suggest that protein kinase C does have a role in the development of multidrug resistance. A manuscript on this work is currently submitted for publication.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06523 06 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Metabolism, Irreversible Binding 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)*

PI:	Birandra K. Sinha	Head, Medical Pharmacology Section	MB, COP, DCT, NCI
Other:	Pedro Politi	Visiting Associate	MB, COP, DCT, NCI
	Helen M. Eliot	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Medicine Branch

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

VP-1-16 undergoes O-demethylation to generate active intermediates that bind to proteins and DNA. The 0-demetylation is P450 dependent. Peroxidases, such as horseradish or prostaglandin synthetase also activate VP-16 and VM-26 to their O-Quinone derivatives, and catalyze binding of reactive intermedites 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 topo-II-DNA complex for these O-demothylted drugs are similar to 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.

HS 6040 (Rev. 1/84) GPO 914-918

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 for its cytotoxicity. Recently, we have proposed that the cellular damage induced by VP-16 may result from the formation of a reactive immediate 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 it was shown 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 0-demethylation was also P-450 dependent.

Incubation of [^{3}H] VP-16 with microsomes containing NADPH and DNA resulted in irreversible binding of the drug to DNA and 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 0-demethylated to 0-Quinone (Ar-VP-16-Q). Inhibition studies suggest that the protein binding species result from 0-demethylation reactions (VP-16-Q and AR-VP-16-Q), and that DNA binding species are positively charged.

Recent studies also indicate that a VP-16 metabolite, dihydrxy VP-16, formed from 0-demethylation of VP-16 chelates Fe^{3+} ions and in the presence of H_2O_2 or reduced gluatathione forms hydroxyl radicals which induce topo-II independent DNA cleavage. In addition, Cu^{2+} is also excellent in inducing DNA cleavage in an oxygen radical-dependent pathway. We have shown that O-demethylated compounds bind to topo-II and the binding is similar to VP-16, and induce DNA cleavage.

Using alkaline elution studies in a sensitive and resistant MCF-7 cells recently, 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 two-fold difference in VP-16 induced DNA strand breaks between the sensitive and 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 mechanics of cell kill by VP-16.

Reistance to VP-16 and other antitumor drugs results in a decreased drug accumulation and in multidrug 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 it 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 very low binding affinity for the protein, indicating that P-170 is not the major mechanism for VP-16 resistance.

PUBLICATIONS

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Sinha BK, Antholine W, Kalyanaraman B, Eliot H. Copper ion-dependent oxyradical-mediated DNA damage from dihydroxyderivative of etoposide. Biochem Biophys Acta, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06716 03 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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
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	Freida Bostick-Bruton	Biologist	MB, COP, DCT, NCI
	Meenakshi Dabholkar	Visiting Fellow	MB, COP, DCT, NCI
	Otis Brawley	Medical Staff Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Cellular Carcinogenesis and Tumor Promotion, DCE, NCI; Laboratory of Molecular Pharmacology, DTP, DCT, NCI; U.S.C. Cancer Center, Los Angeles, CA

LAB/BRANCH

Medicine Branch

SECTION

Clinical Pharmacology Branch

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

This unit has conducted clinical and basic science studies on clinical resistance to platinum compounds. Work published by this group during the indicated period shows that in patients with ovarian cancer, platinum-DNA adduct formation in peripheral blood cell DNA is more closely related to disease response than any previously identified prognostic variable.

Major Findings:

We have begun to develop methods to assess DNA repair capability in patients receiving platinum therapy. Publications listed below include our initial report of assay development of a host cell reactivation assay for cisplatin-damaged DNA, and the observation that the human DNA repair gene ERCC1 may have a clinically important role in platinum drug resistance.

Since cisplatin is also a potent carcinogen, we have also studied the occurrence of secondary leukemia in ovarian cancer patients as well as the mechanism of cisplatin-induced malignant conversion of skin tumors in living mice.

PUBLICATIONS

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Reed E, Jacob J. Carboplatin and renal dysfunction. Ann Intern Med 1989;110:409.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06717 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Genetic and Biochemical Differences of Glucose Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles E. Myers	Chief, Medicine Branch	COP, DCT, NCI
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2.0	2.0	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 major obstacle in cancer chemotherapy is the development of drug resistance in tumor cells. The biochemical and molecular mechanisms associated with multidrug resistance (MDR) are know to be diverse. The major changes are (a) over expression of MDR gene associated with drug efflux pump plasma membrane glycoprotein 170 (gp 170), (b) decreased generation of cytotoxic drug moiety and (c) increased mechanisms for drug detoxification. We found the major changes in our independently selected adriamycin resistant human breast cancer MCF-7 cells are the over expression of gp 170 and decreased glucose-6-phosphate dehydrogenase activity in early, intermediary and late stages of resistance.

Major Findings:

The cytotoxicity effect of adriamycin in tumor cells may be directly related to the drug activation, detoxification and uptake/efflux mechanisms. The biochemical changes of adriamycin (Adr) resistant MCF-7 cells has been intensively studied in our Branch. A decrease in oxygen radicals formation and increases in both detoxification enzymes and drug efflux associated membrane glycoprotein 170 were reported by several investigators. We reported previously that glucose-6-phosphate dehydrogenase activity was markedly decreased in Adr resistant compared to the sensitive MCF-7 wild type cells. The marked changes were observed in early, intermediate and late stages of drug resistant cells in enzyme Vmax but not Km activities. Membrane associated gp170 has two ATP binding sites and the major cytosolic ATP generation in tissue culture cells is the glycolysis pathway. Therefore, we examined the glycolysis major enzyme activities. We measured hexose kinase, phosphofructokinase and lactate dehydration activities in Ada sensitive and resistant cells and found only one to two fold changes in those enzyme activities but the flux of glucose through glycolysis by measuring the lactate production was 45X higher in resistant than sensitive cells.

The biochemical changes in adriamycin (Adr) resistant human breast cancer MCF-7 cells may limit the availability of NADPH for activation of Adr. When compared to drug-sensitive cells, drug-resistant cells had decreased NADPH-generating capacity with a 60X lower glucose-6-phosphate dehydrogenase (G6PD) activity and a 50X higher rate of lactate production. We now report that pyrroline-5-carboxylate (P5C), a physiologic NADP/NADPH regulator, produced a 8X increase in pentose phosphate shunt activity in Adr resistant cells and less than 1X in wild type cells. The differential response to P5C was seen despite the markedly lower G6PD activity in resistant cells. The P5C-mediated effect was independent from that of peroxides. However, P5C produced no increase over that with saturated concentrations of methylene blue. Our results suggest that P5C, a physiologic redox regulator, may play an important role in the resistance to Adr. Both the reduction of P5C and activation of Adr are NADPH-dependent. By competing for available NADPH in cells already limited by low G6PD activity, P5C can further decrease the activation of Adr thereby augmenting cellular resistance. The marked increase in lactate production in drug resistant cells leads to increasing ATP generation thereby enhancing drug efflux mechanism through the regulation of membrane glycoprotein 170. Whether the changes in both pathways are independent or complementary is under our current investigation.

PUBLICATIONS

Yeh GC, Occhipinti SJ, Myers CE. Biochemical changes of glucose metabolism in adriamycin sensitive and resistant human breast cancer MCF-7 cells. Proceedings of American Association for Cancer Research 1990;31:2235.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06718 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Human Folate Binding/Transport Proteins (FBPs)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Patrick C. Elwood	Senior Investigator	COP, DCT, NCI
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	Yutaka Saikawa	Visiting Fellow	COP, DCT, NCI
	Clement Knight	Visiting Associate	COP, DCT, NCI

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LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The Molecular Cell Biology Section has investigated:

1. The structure, function, and molecular biology of human folate binding/transport proteins.

2. The role of these FBPs in the transport of folates and folate analogues and in the development of methotrexate resistance in vitro.

3. The expression of these FBPs.

Major Findings:

1. The structure, function, and molecular biology of human folate binding/transport proteins.

We have previously isolated and characterized the human folate binding proteins (FBP) from placenta, milk, and tissue culture cells (KB cells); and, based on in vitro ligand inhibition and antibody inhibition transport experiments, have shown that the membrane bound form of the human FBP, or folate receptor (FR), mediates folate and methotrexate (MTX) transport at physiologic (ortherapeutic) concentrations of ligand in these systems. We have recently reported the cloning of the human FR receptor cDNA isolated from KB cell and placental cDNA libraries. Characterization of this FR cDNA1 clones has demonstrated that (1) the soluble and membrane form of the FR are encoded by a single 1100 pb mRNA; (2) the cDNA nucleotide sequence encodes a 257 residue peptide (MW=29,918) which contains a 26 residue amino terminal leader sequence, a hybrophobic carboxyl terminus, and 3 N-linked glycosylation and multiple phosphorylation consensus sites; and (3) is variably expressed in normal human tissues.

Others recently published a second human FR cDNA2 nucleotide sequence isolated from a human placental library which was approximately 70% homologous to the FR cDNA1 reported from our laboratory. Reanalysis by dideoxynucleotide sequence analysis and restriction mapping of 18 cDNA clones that we isolated from a placental library confirmed the presence of two closely related (70% overall homology) FBP cDNAs (referred to as cDNA1 or cDNA2) approximately 1100-1200 in length. Compared to the reported FR cDNA2, the coding region and 3' untranslated regions from the FR cDNA2 isolated in our laboratory were identical except for several base substations, only one of which resulted in a different amino acid. However, the 5' nucleotide sequence was shorter and contained a different nucleotide sequence; the significance of this observation is not known (see below). Due to extensive nucleotide homology radiolabeled full length cDNA probes cross hybridized with each other. However, significant differences in nucteotide sequences were present at the 5' and 3' termini such that radiolabeled cDNA subfragments specifically identified respective cDNA clones. By means of radiolabeled specific subfragments, we have shown that 80% (16/18) of the placental cDNAs cloned represented FR cDNA2 clone and 20% (2/18) represented FR cDNA1 clones whereas all cDNAs cloned from a KB cell library represented FR cDNA1. Interestingly, the proteins encoded by these independent cDNAs are similar in length (257 and 255 amino acids), contain typical leader sequences, contain an extremely hydrophobic carboxyl terminus, and share 17 cysteine residues (suggesting very similar and conserved secondary structure, and similar functions). These results indicate that human FBPs are heterogenous, are products of a gene family (see below), and may be differentially expressed in different tissues or at different stages in embryonic development. We are currently screening Northern blots containing RNA from different adult and fetal tissues to address this question. Based on the cDNA predicted amino acid sequence, synthetic peptides have been constructed in collaboration with Henry Crutch, Ph.D. of DCDB to homologous and nonhomologous regions of these two proteins for the development of polyclonal antibodies for epitope mapping, for immunohistochemical studies, and to map functional domains of the FBPs (specifically the ligand and detergent binding sites and the membrane anchor).

To determine the molecular structure and regulation of the FR genes and to characterize the FR gene family, approximately 40 genomic FR clones have been isolated from a normal human leukocyte genomic library. Based on restriction mapping, hybridization analysis, and

sequence data, four distinct clones have been isolated. The genomic clone representative of the FR cDNA2 is approximately 7b long and consists of 5 exons ranging from 145 to 480 bp in length. The genomic DNA sequence representative of the cDNA 5'UTR was identical to the cDNA2 sequence determined in our laboratory (see above). Studies to determine the transcription start site by primer extension and to study the regulatory regions (promoters, enhancers, or suppressors) are ongoing. DNa sequence and Southern blot analysis of the remaining 3 genomic clones indicated that the FR cDNA1 genomic clone was not represented. We have constructed a KB cell ecoR1 subgenomic library which we will screen in parallel with a normal human genomic library with cDNA subfragments specific for the KB cell FR cDNA1 (see above). Once isolated, the clones will be characterized as described. These data indicate that human FBPs are constituents of a FR receptor gene family which contains at least two transcriptionally active genes, and that the gene encoding FR cDNA2 is complex consisting of 4 introns and 5 exons.

In collaboration with Drs. Viswanadhan and Weinstein of the Laboratory of Mathematical Modeling, NCI, we have recently reported the secondary structure of the human KB cell M-FBP (FR) as predicted by computer analysis using a unique data base composed of determined structure characteristics of human membrane proteins. This model predicts 2 transmembrane domains at the amino and carboxyl termini. The former represents a leader peptide whereas the latter was predicted to anchor the protein to the membrane such that the amino terminus was extracellular in location. Based on the absence of intracytoplasmic tail and on the release of M-FBP from membranes by PI-specific PLC, two laboratories have hypothesized that M-FBP is anchored by a glycosylphosphotidyl inositol linkage. We have recently submitted a manuscript which describes conditions under which the M-FBP (FR) is released from membranes and converted to the soluble FBP (S-FBP). Preliminary characterization of metalloprotease indicates that a divalent location is required, that conversion is inhibited by EDTA and 1,10-phenanthroline and that the metalloprotease is contained in the membrane fraction of KB cells. Based on changes in specific activity of purified endogenously labeled M-FBP during conversion to S-FBP, this metalloprotease cleaved M-FBP between residue 226 and residue 229 releasing the S-FBP and an extremely hydrophobic carboxyl terminal fragment. Thus, the soluble form of human KB cell FBP is a proteolytic fragment of M-FBP. Furthermore, this data suggests that the carboxyl terminus of the M-FBP contains the detergent binding and membrane anchor characteristic of the membrane form. Release of the membrane FR by a membrane associated metalloprotease may represent a generalized biologic phenomena relative to membrane proteins and provides a means by which human cells may regulate surface expression of the FR and thus net intracellular accumulation of folates or more importantly MTX.

In collaboration with Dr. Wes McBride (DCBD, NCI), we have probed Southern blots of well characterized human-mouse hybridoma cell lines using full length cDNA probes as well as specific cDNA subfragment probes, and we have probed Southern Blots containing normal human DNA from 30 or more individuals. These experiments have demonstrated that both FR cDNA1 and cDNA2 as well as the other homologous DNA fragments (genomic clones (see above) reside on chromosome 11. In situ hybridization studies localize both cDNA1 and cDNA2 to distinct loci on chromosome 11q22.1 to 11q23.1. Other findings from these studies include the demonstration of at least two allelic forms of KB cell cDNA1 and one allelic form of cDNA2. Linkage analysis to localize the gene to each other and to other genes on chromosome 11 are ongoing.

Earlier studies have demonstrated that the KB cell FBP are extensively processed including glycosylation and removal of a signal peptide such that the MW of the protein increases from

approximately 29,800 to 43,000. The cDNA predicted amino acid sequence contains multiple potential phosphorylation sites including a PKC consensus site. Incubation of intact KB cells or KB cell membranes with P^{32} results in phosporylation of M-FBP analysis, and immunoprecipitation with polyclonal antisera. Furthermore, purified M-FBP is phosphorylated in vitro in the presence of PKC. These data demonstrate that the FR is phosphorylated, at least in part, by PKC. The functional significance of this observation is unknown but may be related to binding or release of ligands, or to cycling of the receptor or receptor-ligand complex.

2. The role of the M-FBP (FR) in Mtx transport and resistance.

We have addressed the role of the FR in Mtx transport in KB cells using an affinity-labeled form of Mtx (N-hydroxysuccinamide ester of Mtx or NHS-Mtx). These studies demonstrated that the K_d for Mtx was 200 nM; that the Fr receptor bound significant quantities of Mtx mono- and polyglutamates throughout a 24 hr. incubation period; that NHS-Mtx specifically and covalently labeled only FR which resulted in inhibition of Mtx transport by > 90% and inhibition of Mtx or folate binding to Fr; and demonstrated that the Fr-ligand complexed cycled from the exterior membrane to an interior compartent in a manner analogous to receptor mediated endocytosis.

To study the role the FR plays in the development of Mtx resistance, we have developed a set of methotrexate resistant KB cell lines selected by Mtx exposure to standard media or in media containing fetal calf serum as the sole source of folates. The former method is comparable to other reports whereas the latter method represents a more physiologic system relative to extra- and intracellular folates. Of the 12 Mtx resistant clones isolated from standard media, 8 clones (66%) contained reduced quantities (ranging from 2 to 75% of control) of the FR whereas the FR expression of 1 clone was increased by three-fold. Six clones, including the clone with increased expression of the FR, were further characterized relative to the level of Mtx and trimetrexate resistance; karyotypic analysis; DHFR activity and gene amplification; Ts binding and activity; Mtx polyglutamatin; Mtx efflux; Mtx and folate transport; and Fr expression by functional assays, immunologic assays (Western blot analysis), and Northern blot analysis. The major findings include (1) the level of Mtx resistance was 50-100 fold relative to control cells; (2) V_{max} of Mtx transport paralleled V_{max} of 5MTHF and both correlated with the FR expression; (3) no differences were noted in theK_t; (4) the Fr function (ligand binding) correlated with the results of FR expression by Western and Northern blot analysis suggesting that the FR proteins were not significantly altered (or mutated); (5) all clones contained markers characteristic of KB cells and the clone with only 2% of the FR receptor had lost 1 chromosome 11 and contained mutations within the complementary chromosome 11; (6) 2 clones represented purely transport deficient mutants resulting from decreased expression of the FR; 2 clones appeared to be resistant as a result of their inability to internalize externally bound drug (e.g. translocation defective mutants); and 2 clones were resistant as a result of amplified DHFR activity combined with mild transport and polyglutamation defects; (7) trimetrexate IC_{50} of transport resistant cell lines were equal to the trimetrexate IC_{50} of controls; and (8) the expression of the FR under these conditions appears to be at a transcriptional level. Studies are ongoing to determine the Ka of FR and the Km of DHFR from each of these cell lines. The cDNAs and genomic regulatory elements will be PCF amplified and cloned for further characterization to understand the mechanism of decreased expression in these resistant clones. We are currently characterizing 9 different KB cell Mtx resistant clones selected in media containing physiologic concentrations of folate to determine if the method of selection changes the

pattern of resistance mechanisms, if decreased (or increased) expression of Fr is operative under these conditions, or if the FR is altered in these cell lines.

3. Regulation of Expression of the FR.

See above.

PUBLICATIONS

Elwood PC. Molecular cloning and characterization of the human folate-binding protein cDNA from placenta and malignant tissue culture (KB) cells. J Biol Chem 1989;264:14893-14901.

Viswanadhan VN, Weinstein JN, Elwood PC. Secondary structure of the human membrane-associated folate binding protein using a joint prediction approach. J of Biomolecular Structure and Dynamics 1990;7:985-1001.

Knight CB, Chabner BA, Elwood PC. The human folate receptor in KB cells is altered in transport mediated, acquired methotrexate resistance. Blood 1989;74:917A.

Deutsch JC, Elwood PC, Portillo RM, Macey MG, Kolhouse JF. Role of the membrane-associated folate binding protein (folate receptor) in methotrexate transport by human KB cells. Archives of Biochemistry and Biophysics 1989;274:327-337.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06719 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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)*

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	Yung Jue Bang	Visiting Fellow	MB, COP, DCT, NCI
	Farzaneh Pirnia	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Prostate cancer is the second most common cause of cancer death in men in the United States. The only treatment for advanced disease is hormonal therapy, which is not curative. Hormonal therapy is severely limited because androgen-dependent tumor typically becomes androgen independent. Although there has been a considerable amount of work on the regulation of prostatic cell growth by androgen and the molecular events following androgen withdrawal, relatively little is known about the receptors expressed by androgen-independent prostate carcinoma cells. We have been working on new approaches to the treatment of this neoplasm through identification of novel cell surface receptors that transmit a growth inhibitory signal through activation of the phosphatidylinositol signal through activation of the phosphatidylinositol signal transduction pathway and mobilization of intracellular Ca^{2+}. Twelve hormones and neurotransmitters stimulated an increase in cytoplasmic free Ca^{2+} in human androgren-independent prostate carcinoma cell lines. Stimulation of the plasma membrane receptor for adenine nucleotides, the P_2 purinergic receptor, consistently caused a massive increase in Ca^{2+} release, close to the total intracellular releasable Ca^{2+} store. Studies with Ca^{2+} channel blockers and EGTA demonstrated that this response derived approximately 50% from release from internal stores and 50% from the opening of dihydropyridine-sensitive plasma membrane Ca^{2+} channels. High pressure liquid chromatographic analysis of inositol phosphate isomers showed a purinoceptor-linked increase in phosphatidylinositol turnover. Treatment with ATP or the non-hydrolyzable analog adenylimidodiphosphate induced a marked change in cell morphology, including chromatin condensation and nucleolar degeneration, and significantly inhibited cell growth *in vitro*. Normal prostatic cells are triggered to undergo programmed cell death in response to androgen withdrawal. This response can be inhibited by Ca^{2+} channel blockers. Our data suggest that it may be possible to circumvent the absence of androgen receptors in androgen-independent prostate carcinoma cells, and trigger a cytotoxic response through activation of a Ca^{2+} dependent signal.

GPO 914-918

Major Findings:

1. Human androgen-independent prostate carcinoma cells express phospholipase C-linked P_2 purinergic receptors.

2. Stimulation of this receptor induces a massive release of Ca^{2+} from intracellular stores. The P_2 purinergic receptor Ca^{2+} response is prolonged by the opening of dihydropyridine-sensative Ca^{2+} channels.

3. Treatment with purinergic receptor agonists markedly inhibits the growth of androgen-independent prostate carcinoma cells.

4. These data are the first demonstration of a Ca^{2+} transient or phosphatidylinositol turnover in androgen-independent prostate cancer, and may represent a new approach to prostate cancer treatment.

PUBLICATIONS

Lebacq-Verheyden AM, Trepel JB, Sausville EA, Battey JF. Bombesin and gastrin releasing peptide: neuropeptides, secretogogues, and growth factors. In: Roberts AB, Sporn M, eds. Peptide Growth Factors and Their Receptors. Handbook of Experimental Pharmacology ,Vol 95II, 1990.

Sausville EA, Trepel JB, Moyer JD. Inhibitors of bombesin-stimulated intracellular signals: Interruption of an autocrine pathway as a therapeutic strategy. International Symposium on the Biology and Kinetics of Surviving Tumor. Liss AR, 1990.

Sharoni Y, Viallet J, Trepel JB, Sausville EA. Effect of guanine and adenine nucleotides on bombesin-stimulated phospholipase-C activity in membranes from Swiss 3T3 and small cell lung carcinoma cells. Cancer Res, in press.

Myers CE, Trepel JB, Neckers L, Linehan M. Potential roles of growth factors, their agonist and antagonists in adjuvant therapy. In: Sixth International Conference on the Adjuvant Therapy of Cancer. Saunders WB, Philadelphia, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06721 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Analysis of Drug Resistance by Flow Microfluorocytometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jane B. Trepel	Biologist	MB, COP, DCT, NCI
Others:	F. Wei-Gang	Visiting Fellow	MB, COP, DCT, NCI
	L. M. Neckers	Research Chemist	MB, COP, DCT, NCI
	K. H. Cowan	Senior Investigator	MB, COP, DCT, NCI
	P. Elwood	Senior Investigator	MB, COP, DCT, NCI
	A. T. Fojo	Senior Investigator	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Pediatrics Branch, COP, DCT, NCI; Navy Medical Oncology Branch, NCI; Japanese Foundation for Cancer Research

LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Since the discovery of the importance of the mdr-1 gene product in multidrug resistance, most of the studies of the multidrug resistance phenotype have been performed at the nucleic acid level. We have established assays to examine drug resistance at the protein level by using flow cytometry to look at intact single cells. There are significant advantages to this approach, especially in probing the role of P-glycoprotein expression in innate and acquired multidrug resistance in clinical specimens. The assays we have developed allow us to measure P-glycoprotein expression and adriamycin content in each of thousands of cells, within hours of receiving a specimen. We have developed a two-parameter technique that allows us to correlate expression and adriamycin content in individual cells in a tumor specimen or cell line. Using this technique, we have been able to identify a subpopulation of innately resistant cells (high P-glycoprotein expression, low Adr content) in a CML-blast crisis cell line that was not detectable by previously available single parameter techniques. conversely, we were able to detect a drugsensitive subpopulation in a CML-blast crisis cell lines selected for Adr resistance and in MCF-7 cells transfected with the mdr-1 gene.

In addition, we are working on flow cytometric analysis of other measures of drug resistance, including single cell detection of dihydrofolate reductase by fluoresceinated methotrexate and on the use of flow cytometry for the rapid screening of resistance reversal agents. We will use the techniques outlined to study drug resistance and its reversal in a variety of tumors. A project utilizing the unique multiparameter capabilities of flow analysis has been initiated to study the relationship of drug resistance to N-myc expression.

Major Findings:

Flow cytometry is a highly sensitive method for detection of P-glocoprotein expression. The techniques we have developed are particularly useful in detecting clinical levels of drug resistance and for analyzing and quantifying the heterogeneity of P-glycoprotein expression within tumor populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06722 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Characterization of IL6-Mediated Tumor Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.P. Nordan	Senior Staff Fellow	MB, COP, DCT, NCI
Other:	G. Schwab	Visiting Fellow	MB, COP, DCT, NCI
	F. D'Allesandro	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, DCBD, NCI, NIH (C. Segall); Laboratory of Cellular and Developmental Biology, NIDDK, NIH (A. Greenberg, C. Landos, A. Kimmel); Red Cross Blood Transfusion Service, Amsterdam (L. Arden).

LAB/BRANCH

Medicine Branch

SECTION

Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

A primary goal of this laboratory is to increase our understanding of how growth factor-dependent cells escape the control of regulatory growth factors thus becoming autonomous tumor cells. One example of this process is the murine myeloma model. Previous studies by R. Nordan identified and characterized a cytokine, interleukin 6 (IL6), that supported the growth of early murine plasma cell tumors. These early tumors also require an inflammatory peritoneal oil granuloma for in vivo growth. Without this microenvironment, the cells fail to grow in vivo. 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 IL6 (supplied by the local microenvironment) for in vivo growth with the subsequent loss of IL6-dependence representing a key step in the progression to a fully malignant tumor. Since the establishment of this laboratory in the Tumor Biology Section in January, 1989, we have performed studies aimed at (1) elucidating the role of IL6 in the growth of human and murine tumor cells and (2) analyzing the mechanisms utilized by these cells escape the requirement for this growth factor. Although initially focusing on myeloma, we have also expanded our studies to other tumors as well.

One approach we are taking is to ask if autocrine loops are responsible for the transition to autonomous tumor cell growth and if so to analyze the events leading to this transition. We have identified human and murine myeloma cell lines that constitutively produce IL6. In addition, we found that the in vitro growth of the human myeloma cell line, U266, can be partially inhibited with a neutralizing monoclonal antibody to human IL6 thus providing evidence for the existence of an IL6-based autocrine loop. We have since demonstrated that downregulating the endogenous production of IL6 with IL6-antisense oligonucleotides also interrupts the autocrine loop in U266 and other myeloma cell lines. Using this antisense technique, we are evaluating other types of tumors for IL6-mediated autocrine loops. Of particular interest is our recent finding that 3 of 3 prostate cancer lines tested produce IL6 and express IL6 receptors. Treatment with IL6 antisense oligos also inhibits proliferation of the prostate cell lines. This effect is presumably also the result of an interruption of an IL6 autocrine loop since a concomitant reduction in IL6 synthesis is also observed. This the first report suggesting a possible role for IL6 in the growth of prostate cancer. In order to investigate the long term effects of interrupting the autocrine loop, we are initiating transfection experiments with IL6 antisense expression vectors. Projected studies include an accelerated investigation into the possible role of IL6 in prostate cancer, including an evaluation of (1) the possible requirement of primary prostate tumor cells for IL6 and (2) the relationship of the androgen dependence of prostate cancer cells and their production of or growth response to IL6.

The progression of tumors to factor-independent growth may also involve a constitutive activation of some part of the signal transduction pathway which for IL6 is unknown. We have initiated the characterization of the starting point of this pathway: the IL6 receptor. We have identified three distinct membrane protein-IL6 complexes that appear to contribute to the putative receptor complex. We have generated a monoclonal antibody to a 130Kd component of the receptor and are proceeding with the biochemical characterization of the receptor complex. We have also initiated studies to evaluate the ability of an antibody-toxin conjugate directed to the 130Kd molecule to selectively kill tumor cells bearing the IL6 receptor.

In addition to our studies on IL6 as a tumor growth factor we are participating in studies which evaluate the potential role of IL6 in cachexia. Our initial observations revealed that elevated serum levels of IL6 are found in cachectic patients and tumor bearing cachetic mice. We have since found that, when cultured with IL6, freshly isolated adipocytes and cultured 3T3-L1 adipocytes display decreased lipoprotein lipase activity resulting in decreased lipid storage. This finding has been extended to adipocytes isolated from animals administered IL6. In addition, TNF stimulated the production of IL6 by adipocytes suggesting an autocrine role for IL6 in fat tissue. These findings support the idea that increased levels of IL6 may play a role in diseases manifested by deficient fat storage.

Major Findings:

1. We have identified an IL6-based autocrine growth mechanism in the human myeloma cell line, U266.

2. We have identified an IL6-mediated autocrine growth loop in 3 of 3 prostate cancer cell lines.

3. We have identified three distinct membrane proteins-IL6 complexes on the cell surface which presumably constitute the IL6 receptor. We have developed a monoclonal antibody to the 130 Kd chain of the IL6 receptor.

4. IL6 appears to be involved in the regulation of liproprotein lipase activity in adipocytes and thus may play a role in cachexia.

PUBLICATIONS:

Mock BA, Nordan RP, Justice MJ, Kozak C, Jenkins NA, Copeland NG, Clark S, Wong G, Rudikoff S. The murine IL-6 gene maps to the proximal region of chromosome 5. J Immunol 1989;142:1372-1376.

Bauer SR, Piechaczyk M, Nordan RP, Owens JD, Nepveu A, Marcu KB, Mushinski JF. Altered myc gene transcription and intron-induced stabilization of myc RNAs in two mouse plasmacytomas. Oncogene 1989;4:411-418.

Plaut M, Pierce J, Watson C, Hanley-Hyde J, Nordan RP, Takaki S, Takatsu K, Paul WE. Mouse mast cell lines produce interleukins in response to cross linkage of FceRI or to calcium ionophores. Nature 1989;339:64-74.

Nordan RP, Mock BA, Neckers LM, Rudikoff S. The role of plasmacytoma growth factor in the in vito responses of murine plasmacytoma cells. Annals New York Academy of Sciences 1989;557:200-205.

Morrissey PJ, Goodwin RG, Nordan RP, Anderson D, Grabstein KH, Cossman D, Sims J, Lupton S, Acres B, Reed SG, Mochizuki D, Eisenman J, Conlon PJ, Namen AE. Recombinant IL7, pre B cell growth factor, has costimulatory activity on purified mature T-cells. J Exp Med 1989;169:707-716.

Hilbert DM, Cancro MP, Scherle PA, Nordan RP, Van Snick J, Gerhard W, Rudikoff S. T cell derived IL-6 is differentially required for antigen-specific antibody secretion in primary versus secondary B-cells. J Immunol 1990;143:4019-4024.

Siegall CB, Nordan RP, Fitzgerald DT, Pastan I. Cell-specific toxicity of a chimeric protein composed of interleukin-6 and pseudomonas exotoxin (IL6-PE40) on tumor cells. Mol Cel Biol 1990;10:2443-2447.

Colamonici OR, D'Alessandro F, Diaz MO, Gregor SA, Neckers LM, Nordan RP. Characterization of three monoclonal antibodies that recognize the 130 kD IFNa2/IFNa-receptor complex of the interferon-a receptor. PNAS, in press.

Pluznick DH, Frappier N, Nordan RP. Differentiation of murineleukemic myeloid M1 cells by post-endotoxin serum is partially neutralized by anti-IL6 antibodies. Exp Hematol, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06723 02M

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Subunits of the Interleukin 2 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	O.R. Colamonici	Visiting Associate	MB, COP, DCT, NCI
Others:	L.M. Neckers	Research Chemist	MB, COP, DCT, NCI
	A. Rosolen	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

NCI-Navy Oncology Branch, COP, DCT, NCI; Pediatric Branch, COP, DCT, NCI

LAB/BRANCH
Medicine Branch

SECTION
Tumor Cell Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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.)*

Three forms of the IL2 receptor have been reported: low, intermediate and high affinity. Each form corresponds to the expression of two different receptor subunits: p55 α chain and p75 β chain. Sole expression of p55 yields low affinity receptors, sole expression of p75 yields intermediate affinity receptors, and co-expression of both subunits yields high affinity receptors. Resonance energy transfer studies have suggested the presence of another peptide subunit of the IL2 receptor with an approximate molecular mass of 95,000 Daltons. The requirement of another protein to form a functional IL2 receptor is supported by experiments in which fibroblasts transfected with p75 cDNA express the protein on the surface but do no bind IL2, whereas transfected T cells express p75 and bind IL2.

We have discovered and characterized the presence of a putative new IL2 receptor subunit with a molecular mass of 95,000 - 110,000 Daltons. This subunit is present in low, intermediate and, perhaps, in high affinity receptor complexes. It's presence is required to form intermediate affinity receptors with p75, but it is not necessary to obtain IL2 binding to p55. The p95 protein, termed the γ subunit of the IL2 receptor, is not ICAM-1 as determined by monoclonal antibody competitor studies.

Major Findings:

1. We have described a novel third subunit of the IL2 receptor, designated the γ subunit.

2. The γ subunit of the IL2 receptor is required for binding of IL2 to intermediate affinity, but not low affinity IL2 receptors.

PUBLICATIONS

Colamonici OR, Neckers, LM, Rosolen A. Putative γ subunit of the IL2 receptor is detected in low, intermediate and high affinity IL2 receptor-bearing cells. J Immunol, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06724 02 M

PERIOD COVERED

October 1, 1989 - September 30, 1990

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

Mechanism of Cellular Oligonucleotide Uptake and use as Anti-Tumor Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.M. Neckers	Research Chemist	MB, COP, DCT, NCI
Other:	D. Geselowitz	Biotechnology Fellow	MB, COP, DCT, NCI
	A. Rosolen	Visiting Fellow	MB, COP, DCT, NCI
	L. Whitesell	Biotechnology Fellow	PB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Pediatric Branch, COP, DCT, NCI

LAB/BRANCH

Medicine Branch

SECTION

Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	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.)*

In order to be able to rationally design oligonucleotides which are stable in culture and penetrate cells more efficiently than normal oligos, we have investigated the mechanism by which cells transport normal oligomers. Using acridine-labeled oligos and flow cytometry, we found that transport is active, receptor-mediated and energy dependent. We elucidated the characteristics of an oligonucleotide which are critical for uptake and studied the ability of certain oligonucleotide derivatives to compete for this uptake process. We found that methylphosphonates do not enter cells via this mechanism, but that phosphorothioates do, although much less efficiently than normal oligos. Uptake is by endocytosis and generally results in the occurrence of oligo-containing vessicles in the cytoplasm. In general, oligos are localized to cytoplasm and not the nucleus following uptake. By fluoresceinating novel oligo derivatives, one can easily follow their rate of accumulation, or lack thereof, by cells. In this way, more efficient oligos can be rapidly designed and tested.

We have observed a novel intracellular oligonucleotide binding protein, different from the one we characterized on the cell surface, which may be involved in determining the intracellular fate of exogenously administered oligos.

We are currently utilizing micropumps implanted subcutaneously in nude mice to explore the efficacy of constantly delivered antisense oligos as anti-tumor agents. To date, no toxicity due to oligo administration has been noted.

Major Findings:

1. We are the first to describe the nature of the cellular uptake system for oligomers.

2. We have identified an 80 kD membrane protein which may mediate this process.

3. We have shown that, using liposomal fusion, targeted delivery of both normal and substituted oligos to cells is possible.

4. We have demonstrated the potential utility of phosphorothioate oligos as anti-oncogene compounds.

5. We have described a novel, intracellular protein which binds oligos and may play a role in their intracellular trafficking.

6. We have devised a system to deliver a constant supply of oligo over a one week period to nude mice to test antisense oligo effects on tumor growth in vivo.

7. To date, we have observed no ill effects of oligo delivery on the mice.

PUBLICATIONS

Loke SL, Stein CA, Zhang XH, Mori K, Nakanishi M, Subasinghe C, Cohen JS, Neckers LM. Characterization of oligonucleotide transport into living cells. Proc Natl Acad Sci USA 1989;86:3474-3478.

Neckers LM. Antisense oligonucleotides: Mechanism of cellular uptake and utility as inhibitors of oncogene expression. In: Cohen JS, ed. Antisense inhibitors of gene expression. London: Macmillan Press, 1989;211-231.

Cazenave C, Stein CA, Loreau N, Thuong NT, Neckers LM, Subasinghe C, Helene C, Cohen JS, Toulme JJ. Comparative inhibition of rabbit globin mRNA translation by modified antisense oligodeoxynucleotides. Nuc. Acids Res. 1989;17:4255-4273.

McManaway ME, Neckers LM, Loke SL, Al-Nasser AA, Redner RL, Shiramizu BT, Goldschmidts WL, Huber BE, Bhatia K, Magrath I. Tumor-specific inhibition of tumor growth by an antisense oligodeoxynucleotide. Lancet 1990; 335:808-811.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06725 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Inhibition of N-myc Expression in Neuroblastoma Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leonard M. Neckers	Research Chemist	MB, COP, DCT, NCI
Others:	Angelo Rosolen	Visiting Fellow	MB, COP, DCT, NCI
	Luke Whitesell	Biotechnology Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Pediatric Branch, COP, DCT, NCI

LAB/BRANCH

Medicine Branch

SECTION

Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	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.)*

N-myc is a nuclear oncogene which is generally overexpressed in neuroblastoma. Although N-myc protein is DNA binding and is structurally similar to c-myc, its function in both normal and neoplastic cells is unknown. We are making use of the "antisense concept" to study the role of N-myc in neuroblastoma and neuroepithelioma cell lines whch overexpress this oncogene. This approach is two-fold: 1) addition to cell cultures of small single-stranded oligonucleotides complementary to a portion of N-myc mRNA; and 2) transfection of cells with expression vectors containing a portion of the N-myc gene in antisense orientation. Using the former approach, we have been able to show that oligonucletoide 15-mers complementary to an area containing the initiation site of N-myc mRNA are capable of concentrating in cells and inhibiting production of N-myc protein. In addition, these oligos inhibit cellular DNA synthesis and production of a nuclear protein termed Ki67. This protein may be a co-factor for DNA polymerase alpha and is required for DNA synthesis in isolated nuclei. Continued administration of antisense oligomer to neuroblastoma cells leads to reduced cell growth. This growth inhibition is not accompanied by a reduction in c-myc protein and may be secondary to loss of the typical mixed morphologies normally seen in neuroectodermal cultures.

In order to confirm these findings, we utilized the second approach, vector transfection, by constructing an antisense N-myc containing episomal vector which replicates extra-chromosomally at high copy number. Results obtained with this vector support the findings made with antisense oligos - namely, that N-myc suppression leads to a reduced growth rate of the culture; not due to a direct affect on growth, but to an alteration in the morphologic heterogeneity normally observed in neuroectodermal cultures in vitro.

These results have implications concerning the mode of generation and maintenance of the mixed morphology cultures and the involvement of N-myc in this process.

PHS 6040 (Rev. 1/84) GPO 914-916

Major findings:

1. Addition of N-myc antisense oligomers to neuroblastoma cells in culture reduce the amount of N-myc protein which is detectable.

2. DNA synthesis is also reduced by this treatment and cell growth is slowed.

3. The nuclear protein, Ki67, is also reduced by N-myc antisense treatment. This protein is required for DNA synthesis in isolated nuclei.

4. N-myc protein may regulate Ki67 gene expression in neuroblastoma cells.

5. N-myc suppression results in loss of morphologic heterogeneity characteristic of neuroectodermal cultures in vitro.

6. N-myc suppression following transfection of an antisense construct in an episomal vector relicates the findings made with exogenous addition of small antisense oligos.

7. N-myc plays a role in generation and maintenance of morphologic heterogeneity in both N-myc amplified and un-amplified neuroectodermal cultures.

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. Progress in Clin Biol Res, in press.

Whitesell L, Rosolen A, Neckers LM. Episome generated N-myc antisense restricts the differentiation potential of neuroectodermal cell lines. Progress in Clin Biol Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06726 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fd on one line between the borders.)

Study of the Role of RNAse H in vivo in Modulation of Antisense Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Leonard M. Neckers	Research Chemist	MB, COP, DCT, NCI
Others:	Angelo Rosolen	Visiting Fellow	MB, COP, DCT, NCI
	Gisela Schwab	Visiting Fellow	MB, COP, DCT, NCI
	Edward Kyle	Microbiologist	MB, COP, DCT, NCI

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics, NICHHD (R. Crouch)

LAB/BRANCH

Medicine Branch

SECTION

Tumor Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	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.)

The intracellular mechanism of action of exogeneously administered antisense oligonucleotides is not known. Two mechanisms have been suggested based on studies in cell free systems. These are: 1) inhibition of ribosome attachment or movement along mRNA; and, 2) creating a substrate for RNAse H degredation of targeted mRNA. RNAse H is an enzyme which destroys the RNA portion of an RNA/DNA hybrid complex and is present in the cytoplasm of all proliferating cells. Its natural function in cells is unknown. We have obtained a full-length cDNA coding for bacterial RNAse H. We have succeeded in constructing a mammalian episomally replicating expression vector which contains the bacterial RNAse H gene in a sense orientation.

We have demonstrated by in situ gel assay that the bacterial RNAse H gene is expressed at high levels in the transfected cells and that this activity can be induced with cadmium. Cells transfected with this construct and sham-transfected cells are being compared for their responsiveness to antisense oligonucleotide addition. We are utilizing a model system which makes use of the fact that U937 cells apparently possess an autocrine growth loop regulated by IL-6. If IL-6 antisense is added to these cells, their growth is markedly inhibited. If RNAse H is involved in the intracellular functioning of antisense oligos, then cells with high levels of RNAse H should respond better to addition of these compounds.

PHS 6040 (Rev. 1/84) GPO 914-918

Major Findings:

1. RNAse H potentiates the efficacy of antisense oligos in a cell free system.

2. U937 cells possess an IL-6 mediated autocrine growth loop blockable with IL-6 antisense oligos.

3. Expression of excess RNAse H activity in U937 cells increases the efficacy of exogenously added antisense oligos without affected nonspecific oligo toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06727 02 M

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncogenes Activation in Human Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Maria Zajac-Kaye	Senior Staff Fellow	MB, DCT, NCI
Others:	Bennett Yu	Clinical Associate	MB, DCT, NCI
	Noa Ben-Baruch	Clinical Associate	MB, DCT, NCI
	Melissa Blake	Technician (Stay-in -School)	MB, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pathology, DCBD, NCI, NIH (D. Levens)

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.2	0.3

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.)*

We have investigated the mechanisms of oncogenes regulation in human malignancies. Deregulation of the c-myc gene accompanies all cases of human Burkitt's lymphoma (BL) and therefore the mechanism underlying the transcriptional regulation of the c-myc gene in lymphoid tumors is of great importance. We have discovered a cis element located in the intron I of the human c-myc gene which binds a novel nuclear protein and we showed that this binding was abolished by point mutation present in the corresponding region in most BL DNA. We have purified and identified this Myc Intron Factor (designated MIF-1) to be a 138 kD protein. We have also demonstrated that the 138 kD MIF-1 is a phosphoprotein and that phosphorylation of MIF-1 is required for the interaction with its recognition sequence. Functional analysis of MIF-1 led as to the identification of additional cis element adjacent to MIF-1 binding site. We showed that the interaction between these two regions and its binding factors may be important in the control of c-myc expression and it may be perturbed in BL due to mutations frequently observed in MIF-1 binding site. Our results suggest that the intron I cis elements, the binding factors MIF-1 and MIF-2 and the kineses which phosphorylates MIF-1 may comprise and important physiological circuit, alteration of which may perturb c-myc expression and may have malignant consequences.

In addition we have studied the effect of conventional and novel cancer agents as well as differentiating agents on the function of transcription factors which regulate expression of the c-myc gene. We have shown that treatment of cells with certain pharmacological agents alters dramatically MIF-1 binding pattern to the intron I cis element. Understanding the process by which transcription factors regulate gene expression will allow development of reagents which could turn off uncontrolled expression of genes implicated in malignant transformation.

GPO 914-918

The mechanism of transcriptional deregulation of oncogenes in human malignancies was investigated. The major projects were the following:

1. Mechanism involved in the transcriptional regulation of c-myc oncogene in lymphoid tumors. The c-myc oncogene is deregulated in many types of human tumors and therefore is an excellent model system for studying the effects of transcriptional deregulation in human tumorigenesis. In studying c-myc deregulation in a Burkitt's lymphoma (BL) cell line derived at the NIH from a AIDS patient, a 20 bp region located in the first intron of the normal c-myc gene was identified as a binding site for a novel nuclear protein. We showed that this binding was abolished by a point mutation in a corresponding region of the c-myc gene derived from the BL DNA. Moreover, this region is mutated in the majority of BL.

To understand the role of the intron I sequence in the regulation of the c-myc gene purification and identification of this protein was undertaken. A 138 kD nuclear protein was identified as the factor which binds to the previously identified 20 bp cis element located in the intron I of the c-myc gene. This myc intron factor (termed MIF-1) binds to the wild type c-myc sequence but does not bind to the c-myc from BL which contain point mutations in this binding region. We have also demonstrated that the 138 kD MIF-1 is a phosphoprotein and that treatment of the purified MIF-1 with potato acid phosphatase abolished binding to its 20 bp c-myc recognition sequence. Binding activity was protected by inclusion of phosphatase inhibitors. This results suggest that phosphorylation is required for the specific DNA:MIF-1 interaction in vitro and that the phosphorylation state of MIF-1 may be an important factor in controlling c-myc expression in vivo. The observed effect of dephosphorylation of MIF-1 is similar to that of the BL mutation within its recognition sequence, namely reduced MIF-1 binding to DNA. We plan to determine, therefore, whether mutations in MIF-1 or the serine kineses which phosphorylates MIF-1 could mimic the BL- point mutations in lymphoid and perhaps other malignancies. The effect of protein kineses and phosphatases on MIF-1 phosphorylation at different stages of cell cycle would be evaluated.

In addition, we have identified a second factor located in the intron I of the c-myc gene and we localized its binding site to the palindromic sequence 120 bp downstream from MIF-1 binding region. We showed, that the second factor (termed MIF-2) has a specificity for MIF-1 binding sequence. Thus, the effect of MIF-1 binding to its recognition sequence on the control of c-myc expression may be dependent on the MIF-2 element located in intron I of the c-myc gene. The relative balance between active and inactive positive and negative regulators of gene expression such as MIF-1 and MIF-2 may be shifted by protein phosphorylation and may influence oncogenesis. Production of antibody to MIF-1 and MIF-2 as well as cloning of the genes which encodes these proteins will allow us to better understand their role in the regulation of the c-myc gene and there involvement in neoplastic disease.

2. Effect of cancer agents on the function of transcription factors which regulate expression of cellular oncogenes. HL60 cells provide an experimental system where c-myc expression (linked with cell growth and differentiation) can be physiologicaly modulated and are easily examined. In response to agents which differentiate HL60 cells c-myc expression dramatically decreases. We have found that treatment of HL60 cells with 100μg/ml suramin, a polysulfonated naphtylurea, is associated with a decrease of c-myc oncogene expression 24-48 hr after treatment, followed 24 hr later by 50% inhibition of cell growth. Parallel experiments with 1μM retinoic acid in both cell lines resulted in a time dependent decrease in c-myc expression.

To determine whether nuclear regulatory proteins that bind to the c-myc gene are affected by suramin or retinoic acid treatment, we examined binding of the Myc Intron Factor (MIF-1) to its

20 bp binding region located in the first intron of the c-myc gene. Comparison of the DNA-protein complex between suramin or retinoic acid treated and control cells revealed a consistent difference in the mobility of this complexes on a gel retardation assay. The untreated HL60 cell extracts appeared as a diffuse smear which upon suramin or retinoic acid treatment changed to two distinctive bands of slower mobility. We have demonstrated that a 138 kD protein is the essential component of the slower migrating complex seen on the mobility assay. Purification of the protein from treated and untreated HL60 cells will allow us to determine whether the differences in the MIF-1:DNA complexes are due to post-transcriptional modification of the protein such as changing phosphorylation state (by inhibiting phosphatases or activating kineses) or whether the 138 kD protein is induced following treatment. In summary, our results suggests that nuclear regulatory proteins that bind to c-myc may be modulated by pharmacologic agents, allowing us to develop reagents that could influence uncontrolled expression of regulatory cellular genes.

PUBLICATIONS

Zajac-Kaye M, Levens D. Phosphorylation-dependent binding of a 138-kDa Myc intron factor to a regulatory element in the first intron of the c-myc gene. J Biol Chem 1990;265:4547-4551.

Zajac-Kaye M, Yu B, Ben-Baruch N. Downstream regulatory elements in the c-myc gene. In: Potter M, Melchers F, eds. Current Topics in Microbiology and Immunology; Mechanism in B cell neoplasia. Springer-Verlag, Berlin, Heidelberg, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06728 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Biochemical Regulation of Tyrosine Kinases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ivan D. Horak	Senior Investigator	MB, COP, DCT, NCI
Others:	Anne L. Burkhardt	Microbiologist	MB, COP, DCT, NCI
	Zhen-hong Li	Fogarty Fellow	MB, COP, DCT, NCI
	B. Matoskova	Fogarty Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Tumor Virus Biology, DCE, NCI (J. B. Bolen, E. M. Horak); Metabolism Branch, DCBD, NCI (T. A. Waldmann); Experimental Immunology Branch, DCBD, NCI (R. E. Gress);

LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

During the past year the laboratory has continued to investigate the regulation and function of the src family of tyrosine protein kinases (TPK) in a variety of human cellular systems. We have prepared numerous unique molecular and biochemical reagents for those studies and have analyzed in detail the expression of c-src, c-yes, fyn, lck, hck, lyn and c-gar in hematopoietic cells. The results of these studies have defined expression of src family of tyrosine protein kinases in fresh human cells. Protein kinases of this gene family, such as the lck gene product, have been shown to be important for generation of IL-2 dependent proliferation signals and for activation signals following interaction between T-cells and antigen presenting cells. Using IL-2 dependent human T-cell lines we further characterized signal transduction pathway initiated by binding of IL-2 to the high affinity IL-2 receptor. We showed that IL-2 induced signal transduction in T cells is mediated by the lymphocyte tyrosine protein kinases $p56^{lck}$.

GPO 914-818

Cooperating Units:
Laboratory of Immunology, NIDR (L. M. Wahl); Laboratory of Molecular Carcinogenesis, DCE, NCI (T. R. Burke); Laboratory of Tumor Cell Biology, DCE, NCI (M. Popovic);

Major Findings:

Using IL-2 dependent and independent acute T-cell Leukemia/Lymphomas (ATLL) cell lines we have found that normal pattern of tyrosine protein kinase expression is disrupted in T-cell lymphomas during the transition from IL-2 dependence to IL-2 independence. These observations suggest that deregulated phosphorylation by the src family members may be function to bypass T-cell proliferation control points leading to uncontrolled cell growth. Preliminary results suggested positive correlation between the TPK's expression and response to anti-TAC antibody in patients with ATLL.

Recently we started to investigate the utility of tyrosine protein kinase inhibitors to block T-cel proliferation and activation signals. Specific inhibitors of TPK's not only provide useful tools for studying the structure and function of these enzymes, but also offer new therapeutic approaches for the treatment of certain cancers.

PUBLICATIONS

Veillette A, O'Shaughnessy J, Horak ID, Israel MA, Yee D, Rosen N, Fujita DJ, Kung HJ, Biedler JL, Bolen JB. Coordination alterations of $pp60^{c\text{-}src}$ abundance and c-src RNA expression in human neuroblastoma variants. Oncogene 1989;4:421-427.

Horak ID, Kawakami T, Gregory F, Robbins KC, Bolen JB. Association of $p60^{fyn}$ with middle tumor antigens in murine polyoma virus transformed rat cells. J Virology 1989;63:2343-2347.

Salen G, Shore V, Tint S, Forte T, Shefer S, Horak ID, Horak E, Dayal B, Nguye L, Batta AK, Lingren FT, Kwiterovich PO Jr. Increased sitosterol absorption, decrease removal and expanded body pools compensation for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. J Lipid Res 1989;30:1319-1330.

Horak ID, Corcoran ML, Thompson PA, Wahl LM, Bolen JB. Expression of $p60^{fyn}$ in human platelets. Oncogene 1990;5:597-602.

Bolen JB, Thompson PA, Eisman E, Horak ID. Expression and interaction of the SRC family of tyrosine protein kinases in T lymphocytes. Advances in Cancer Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06730 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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)*

PI:	Charles E. Myers	Chief, Medicine Branch	COP, DCT, NCI
Others:	Michael Cooper	PRAT Fellow	COP, DCT, NCI
	Romano Danesi	Visiting Fellow	COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The use of polyanions as anti-neoplastic and anti-HIV agents was investigated. Examples of these types of compounds are phosphorothioate oligeodeoxynucleotides and the bis-naphthalene sulfonic acids (e.g. suramin). Suramin administration has been shown to cause elaboration of a heparin sulfate which is excreted in the urine. This was isolated and its biologic effects characterized.

PHS 6040 (Rev. 1/84) GPO 914-918

Major Findings:

The heparin 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 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 heparin sulfate may play an important role in the anti-tumor activity of this drug.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06731 02 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Expression and Regulation of the mdr1 Gene and Transforming Growth Factor Alpha

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Susan Bates	Senior Investigator	MB, COP, DCT, NCI
Others:	Liz Deutsch	Biologist	MB, COP, DCT, NCI
	Yi-Nan Chen	Visiting Fellow	MB, COP, DCT, NCI
	Gi-Ming Lai	Visiting Associate	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pathology, DCBD, NCI (Maria Tsokos)

LAB/BRANCH

Medicine Branch

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-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 efforts this past year were subdivided between studies of multidrug resistance mediated by P-glycoprotein and the role of growth factors in drug resistance as well as their regulation in normal tissues, tumors and in culture with differentiating agents.

GPO 914-918

The accomplishments of this past year can be summarized as follows:

Studies of P-glycoprotein Mediated Drug Resistance

1. Previous observations from our laboratory had established a correlation in cell culture models of human neuroblastoma between expression of P-glycoprotein and degree of differentiation. Work performed this past year in collaboration with Maria Tsokos of the Pathology Department extended these observations to human neuroblastoma tumors at the cellular level by utilizing the techniques of in situ hybridization and immunohistochemistry. A correlation between expression and differentiation was observed, as well as documentation of focal heterogeneity which might represent potential clones for expansion after treatment with chemotherapy. These observations will now be extended back to the cell culture models to further investigate the mechanism of regulation of function of this protein as a function of differentiation.

2. Modulation of P-glycoprotein by various agents including several of the agents previously identified as potential blockers of its function was investigated with documentation that some of these agents can increase P-glycoprotein expression. These investigations which have great potential clinical import are currently being actively pursued to further understand the mechanisms responsible for this increase.

3. In collaboration with Tito Fojo, we have studied the mechanism responsible for the lack of P-glycoprotein function in cell culture models of differentiation. These studies have demonstrated that the protein has some transport functions, but it appears that alterations in protein phosphorylation alter this function and that modulation of phosphorylation can increase the drug efflux activity of this pump.

4. In depth studies were carried out to establish the utility of the polymerase chain reaction as a tool for measuring P-glycoprotein expression in clinical samples. Careful studies demonstrated the value of this technique, its sensitivity and its limitations in this system but with broader clinical applications.

5. Collaborative studies with Tito Fojo have evaluated mitotane as a P-glycoprotein antagonist, offering potential treatment of adrenocortical cancer in combination with natural products. Mitotane effectively blocks P-glycoprotein, resulting in enhanced drug accumulation in cell lines selected from drug-resistance in vitro and in an unselected adrenocortical cancer cell line.

6. A clinical trial combining continuous infusion adriamycin with amiodarone as a P-glycoprotein antagonist has been underway for the past year for treatment of metastatic, refractory breast cancer. The regimen is well-tolerated, but the effectiveness of amiodarone in enhancing drug efficacy is not dramatic. However, biopsies of both immunohistochemical assays and RNA in situ hybridization, suggesting a need for P-glycoprotein reversal in refractory breast cancer.

Studies of EGFR and Estrogen Receptor Regulation in Drug Resistant MCF-7 Breast Carcinoma Cells

An MCF-7 cell line selected for adriamycin resistance in the presence of the P-glycoprotein antagonist verapamil was studied. This cell line has been previously characterized and demonstrates increased levels of a 95 kilodalton protein without changes in P-glycoprotein expression. Studies completed this year demonstrated increased expression of epidermal

growth factor receptor (EGFR) with decreases in both estrogen receptor (ER) and progreterone receptor (PR) levels. These findings are currently being followed with the hopes of exploiting this apparent growth factor dependence in increasing the sensitivity of cells to chemotherapy.

Role of the TGFα/EGFR Loop in Normal Tissue and Human Cancer

Examination of TGFα and EGFR in colon cancer yielded results supporting a role for this loop in differentiated colon cancer. Treatment of the human colon cancer cell lines with the differentiating agent, sodium butyrate, results in enhanced expression of both TGFα and the EGF receptor. While differentiation accomplished by sodium butyrate is accompanied by growth inhibition so that EGF effects are not discernible, it is known that the lines which are inherently well differentiated are the ones which respond to EGF with increased growth. While these studies are far from demonstrating the presence of an autocrine loop, they are consistent with the presence of an autocrine TGFα/EGFR loop in well differentiated colon cancer. Current directions in this field include studies with drug resistant cell lines to examine the response of these lines to differentiating agents.

Publications Accepted Pending Revisions:

Bates SE, Shieh CY, Mickley LA, Dietich H, Lauriaux L, Fojo AT. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/Pgp) found in adrenocortical carcinoma. J Clin Endocrinology & Metabolism.

Deutsch LA, Valverius EM, Mickley LA, Rosen N, Bates S. Modulation of TGFα/EGF receptor expression in human colon carcinoma cells by differentiating agents. Cancer Communications.

Deutsch LA, Rudick JR, Fojo AT, Bates SE. Quantitation of mdr-1 mRNA by polymerase chain reaction. Biochemistry.

Bates SE. Clinical applications of serum tumor markers. Annals of Internal Medicine.

Publications Submitted:

Lai GM, Chen YN, Mickley LA, Fojo AT, Bates SE. Intrinsic and acquired drug resistance in human colon cancer: Role of P-glycoprotein and the glutathione redox cycle. Cancer Research.

Chen YN, Valverius EM, Murphy LD, Pearson J, Mickley LA, Schwartz AM, Saceda M, Martin MB, Bates SE. Induction of EGF receptor in an estrogen-responsive adriamycin-resistant MCF-7 cell line. Molecular Endocrinology.

PUBLICATIONS

Bates SE, Valverius EM, Ennis BW, Bronzert DA, Sheridan JP, Stampfer MR, Lippman ME, Dickson RB. Expression of the TGFα/EGF receptor pathway in normal human breast epithelial cells. Endocrinology 1990;126:596-607.

Bates SE, Mickley LA, Chen YN, Richert N, Rudick J, Fojo AT. Expression of a drug-resistance gene in human neuroblastoma cell lines: Modulation by retinoic acid-induced differentiation. Molecular and Cellular Biology 1989;9:4337-4344.

Ennis BW, Valverius EM, Bates SE, Lippman ME, Bellot F, Kris R, Schessinger J, Masui H, Goldenberg A, Mendelsohn J, Dickson RB. Monoclonal anti-EGF receptor antibodies inhibit the growth of malignant and nonmalignant mammary epithelial cells. Molecular Endocrinology 1989;3:1830-1838.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06732 02 M

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modulation of the Expression of a Multidrug Resistance Gene (mdr-1)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Antonio T. Fojo	Senior Investigator	MB, COP, DCT, NCI
Others:	Lyn A. Mickley	Biologist	MB, COP, DCT, NCI
	Yi-Nan Chen	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The focus of the laboratory continues to center around the problems of drug resistance. As in the past, three major areas continue to be actively investigated. They are: multidrug resistance mediated by P-glycoprotein, adriamycin resistance associated with overexpression of a 95 kilodalton membrane protein and mechanisms of cisplatin resistance.

Multidrug Resistance Mediated by P-glycoprotein

This field has been of interest to the principal investigator for seven years. Work in this field is continuing. Emphasis this year has been placed on understanding several aspects of this field, with a final goal of applying the findings to clinical trials. To this end several projects were embarked upon, most of which have been completed and have yielded offspring projects:

1. The primary sequence of over 100 P-glycoprotein from a wide variety of sources was determined. To achieve this, the technique of RNAse protection was utilized. There is a high degree of conservation of P-glycoprotein structure. Different alleles were identified. The regulation of these alleles both under "normal" conditions and with selection for drug resistance was studied. The control of expression from individual alleles with gene amplification was also investigated. Based on these findings we have formulated concepts on how drug resistance develops in a population of cells. Attempts were also made to correlate expression with cross resistance patterns given the belief that different patterns are mediated by different P-glycoprotein. Evidence from these studies convincingly demonstrates that such changes are highly infrequent and that other explanations need to be proposed.

2. A large study was undertaken to compare the various methods of P-glycoprotein expression in collaboration with Susan Bates and Jane Trepel of the Medicine Branch. This approach compared eight different approaches and established the sensitivity, specificity, advantages and disadvantages of the various approaches. In addition, the study examined the cross resistance patterns observed in cell lines with levels of P-glycoprotein similar to those found in the majority of clinical samples by studying both selected and unselected cell lines. Broad cross resistance was observed and the reversibility of this resistance after the addition of verapamil and other P-glycoprotein antagonists was investigated.

3. The utilization of differentiating agents for reversal of drug resistance was also investigated with cell lines made resistant to both adriamycin and vinblastine. These studies clearly demonstrate that one can partially reverse multidrug resistance with differentiating agents and that this reversal is additive and possibly synergistic with the effect of verapamil. Work done in collaboration with Susan Bates implicates that alternations in the phosphorylation of P-glycoprotein is likely responsible for this effect and suggests that these agents should be further investigated as potential resistance modifying agents.

Adriamycin Resistance Associated with Overexpression of a 95 Kilodalton Membrane Protein

Attempts continued to further investigate this mechanism. Work was begun on attempts to clone the cDNA encoding this protein by screening a cDNA library with the polyclonal antibody raised earlier against this protein. This work is currently in progress.

Mechanisms of Cisplatin Resistance

An increased effort was devoted to this field with several leads currently under investigation. The studies have been carried out utilizing 14 cell lines isolated in the laboratory, including platinum resistant cell lines as well as drug sensitive revertants. Studies to date demonstrate that there is a very tight correlation in several of the cell lines between the level of a 55kd protein and a 42kd protein and the degree of platinum resistance. Both of these proteins are seen to disappear with selection for platinum resistance and reappear when platinum sensitivity is restored. In addition, a tight correlation was also observed between constitutive metallothionein expression and platinum resistance. In collaboration with Eddie Reed, the correlation between platinum resistance and accumulation was investigated and shown to be an

early and frequently occurring step in the generation of cisplatin resistant cell lines. The broad cross resistance of these cell lines to all platinum analogs trested and the observed changes are all encouraging as to the future studies with these models.

PUBLICATIONS

Rothenberg ML, Mickley LA, Balis FM, Cole D, Gillespie A, Poplack DG, Fojo AT. Modulation of the mdr-1/P-170 gene and the dihydrofolate reductase gene in patients with acute lymphoblastic leukemia. Blood 1989;74:1388-1395.

Mickley LA, Bates SE, Richert ND, Foss F, Rosen N, Fojo AT. Modulation of the expression of a multidrug resistance gene (mdr-1/P-glycoprotein) by differentiating agents. J Biol Chem 1989;264:18031-18040.

Bates SE, Mickley LA, Richert ND, Rudick J, Fojo AT. Expression of a drug resistance gene in human neuroblastoma cell lines: Modulation by retinoic acid-induced differentiation. Molecular and Cellular Biology 1989;9:4337-4344.

Chen YN, Mickley LA, Hwang JL, Acton E, Fojo AT. A novel resistance-related membrane protein is overexpressed in an adriamycin-resistant MCF-7 cell line. JBC, in press.

Travis WD, Schmidt K, MacLowry MC, Masur H, Condron KS, Tsokos M, Fojo AT. Respiratory cryptosporidiosis in a patient with malignant lymphoma: Report of a case and review of the literature. Arch Pathol, in press.

Bates SE, Shieh CY, Mickley LA, Dichek H, Loriaux DL, Fojo AT. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein (Pgp) found in adrenocortical carcinoma. J Clin Endo and Metab, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z 01 CM 06733 01 M

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Triplex Formation to Specifically Inhibit Gene Transcrition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leonard M. Neckers	Research Chemist	MB, COP, DCT, NCI
Others:	Daniel Geselowitz	Biotechnology Fellow	MB, COP, DCT, NCI
	Vinay Jain	Medical Staff Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Pediatric Branch, COP, DCT, NCI

LAB/BRANCH
Medicine Branch

SECTION
Tumor Cell Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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

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

Antisense oligos or vectors inhibit mRNA processing, stability or translation without necessarily affecting gene transcription. The idea behind triplex formation is to inhibit gene transcription itself, thereby stopping mRNA production. There are several experimental models for forming triple helices which do not rely on Watson-Crick base pairing. Instead, in one model, triplet formation is based on A-A and G-G hydrogen bonding to the underlying Watson-Crick duplex. Hogan et al have made a triplex sequence indentical to a purine rich stretch of c-myc DNA 115 bp upstream from the P1 transcription start site (270 bp upstream from P2). In vitro, Hogan et al found this sequence, and not its Watson-Crick complement, to inhibit the transcription of the c-myc gene. Postel and co-workers have recently demonstrated that a positive transcription factor, PuF, binds to this very region of the c-myc gene and up-regulates c-myc transcription. Thus, forming a triplex at this site should down-regulate transcription of the gene.

We have synthesized this sequence and its scrambled counterpart in both Po and Ps versions and have examined its effects on the growth and viability of HL60 cells, promyelocytic leukemia cells which overexpress c-myc. Both Po and Ps versions of the triplex oligo inhibit growth, although the Ps version is more effective at lower concentrations, as might be expected due to its increased stability. The scrambled sequence had no effect in either case. In addition, we have demonstrated that triplex oligos will hybridize in vitro under physiological conditions to plasmids containing the appropriate sequences. We have utilized stable transfection of CAT and luciferase constructs to work out the most suitable parameters for triplex action.

Triplex formation should be a means of interfering with activity of specific transcription factors, including steroid receptors, cyclic AMP dependent kinases and retinoids, in intact cells.

Major Findings:

1. Triplex oligos will specifically hybridize in vitro, under physiological conditions, to plasmids containing correct sequences.

2. Utilizing such plasmids containing c-myc regulatory elements upstream from CAT or luciferase genes, we have been able to show triplex inhibition of reporter gene activity in stably transfected cells.

3. Both Ps and Po triplex oligos to a region of c-myc DNA upstream from the P1 transcription start site inhibit the growth of HL60 cells in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06734 01 M

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Analysis of Suramin and Related Growth Factor-Binding Compounds in the Biology of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Renato V. La Rocca	Senior Investigator	MB, COP, DCT, NCI
Others:	Romano Danesi	Visiting Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medicine Branch

SECTION

Molecular Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-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.)*

We have studied the effect of suramin and highly sulfated glycosaminoglycans in the biology of human sarcoma, glioblastoma and prostate cancer cells. With regard to sarcoma cells, we have determined an enhanced inhibition of colony formation and cell proliferation in the presence of suramin, as compared to other tumor types. In all, five different sarcoma cell lines have been tested (four osteogenic and one rhabdomyosarcoma). 144 hour exposure to suramin concentrations below the LD_{50} (in the range of 75-150 μg/ml) result in marked alterations in the degree of heparin-binding growth factor mRNA expression. In particular, c-sis message (which possesses marked homology to the B chain of PDGF) diminishes, while transforming growth factor b message increases compared to control. The degree of both c-myc or c-fos mRNA expression did not change significantly in these cell lines upon exposure to suramin. Brief exposure (i.e., 48 hours) of the G-292 osteogenic sarcoma cell line to a sublethal dose of suramin (50 μg/ml), results in marked morphologic changes with assumption of a more polar fusiform cell shape. Change in the concentration of alkaline phosphatase, a possible marker of osteogenic differentiation, is equivocal in these cells. However, because it is a lysosomal enzyme, alkaline phosphatase may be directly effected by suramin. We have now confirmed that glioblastoma cell lines are variably sensitive to suramin in a manner which correlates to the level of c-sis message expression. In addition, signficiant morphologic changes appear to occur only in the glioblastoma lines containing detectable amounts of c-sis upon exposure to suramin. These cells appear to assume a less differentiated phenotype. The variable sensitivity among human prostate cell lines is independent of a cell line's sensitivity to androgen, bFGF, PDGF or EGF. However, suramin is capable of reverting the stimulatory effect of exogenously administered growth factor, and to a lesser degree, androgen administration on DNA synthesis and cell proliferation. The cellular accumulation of drug determined after 144 hours exposure does appear to correlate with sensitivity. In addition, PSA and acid phosphatase concentrations in the conditioned media of prostate cancer cells exposed to suramin decreases out of proportion to the resulting decline in cell number. The clinical trials with suramin in refractory cancer are ongoing.

PHS 6040 (Rev. 1/84) GPO 914-918

Major Findings:

1. Human pediatric sarcoma cells appear to be more sensitive to suramin than most cell lines derived from adult tumors.

2. Exposure of tumor cell lines to sublethal concentrations of suramin results in marked changes in the level of c-sis and TGF-b mRNA expression.

3. Marked changes in cell morphology upon exposure to relatively low concentrations of suramin occur in the G-292 osteogenic cell line as well as those glioblastoma cell lines containing c-sis and perhaps representing a more differentiated phenotype.

4. Suramin is capable of interfering with the stimulatory effect on human prostate cancer cells of exogenous growth factors and to a lesser degree, testosterone.

PUBLICATIONS

La Rocca RV, Westermark BJ, Rosenblum M, Israel MA. Patterns of proto-oncogene expression in human glioma cell lines. J Neuroscience Res 1989;24:97-106.

La Rocca RV, Stein CA, Myers CE. Suramin - prototype of a new generation of antitumor compounds. Cancer Cells 1990;2:106-115.

La Rocca RV, Meer J, Stein CA. Suramin-induced polyneuropathy. Neurology 1990;40:954-960.

La Rocca RV, Stein CA, Danesi R. Suramin in adrenal cancer: Modulation of steroid hormone production, cytotoxicity in vitro and clinical antitumor effect. J Clin Endocrinology & Metabolism, in press.

La Rocca RV, Cooper MR, Uhrich M. The use of suramin in the treatment of prostate cancer refractory to conventional hormonal manipulation. Urology Clinicas of North America, in press.

La Rocca RV, Stein CA, Danesi R, Myers CE. Suramin: A novel antitumor compound. J of Steroid Biochemistry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03024-21 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Treatment of Extensive Stage Small Cell Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	John C. Phares, MD	Head, Oncology Branch	NNMC
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, Ph.D.	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

COOPERATING UNITS *(if any)*

Radiation Oncology Branch; Biostatistics & Data Management Section; Surgical Oncology Division and Hematopathology Branch, National Naval Medical Center, Bethesda.

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Clinical Investigations

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Although a dose-response curve clearly exists for alkylating agents in the initial chemotherapy of small cell lung cancer, the therapeutic benefit of higher than standard doses of the more recently introduced regimen of etoposide/cisplatin (VP16/PLAT) is uncertain. We randomized at least partially ambulatory patients with extensive stage SCLC and and without major organ dysfunction to receive either VP16 80 mg/m squared + PLAT 27 mg/m squared Days 1-5 q 3 wks or VP16 80 mg/m squared Days 1-3 + PLAT 80 mg/m squared Day 1 q 3 wks for the first 6 wks of therapy. Nonambulatory patients and those with organ dysfunction were assigned standard dose treatment. All patients received the standard dose regimen during wks 7-12. From wks 13-24, patients in complete response (CR) continued standard dose VP16/PLAT, while all other patients received a new 3-drug regimen that led to further improvement in response in only 5 cases. CR's were given prophylactic cranial irradiation. Ninety-eight patients have been entered (77 of whom were randomized). With a median follow-up of 48 mos, preliminary results are:

	N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt
High	36	25%	83%	12 mos	1,600	67,000
Standard	41	22%	80%	11 mos	2,600	162,000
Nonrand	21	5%	71%	6 mos	1,800	90,000

CR rates (p=1.00) and survival (p=0.73) were similar in patients randomized to high and standard dose therapy. There were 2 treatment-related deaths in the high and one in the standard dose arm. We conclude 1) standard dose VP16/PLAT is at least as active as any regimen we have ever utilized for for extensive stage SCLC and produces only modest myelotoxicity, and 2) there is no evidence of superior efficacy when planned drug doses are increased by 67% during the first 6 wks.

GPO 914-918

PROJECT DESCRIPTION

Treatment of Extensive Stage Small Cell Lung Cancer

Professional Staff:

PI:	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	John C. Phares, MD	Head, Oncology Branch	NNMC
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, PhD	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

Collaborating Branches:

Eli Glatstein and Thomas Napier, Radiation Oncology Branch; Seth M. Steinberg, Biostatistics & Data Management Section; Bimal Ghosh, Surgical Oncology Division, National Naval Medical Center, Bethesda; James Cotelingham, Hematopathology Branch, National Naval Medical Center, Bethesda.

Objectives, Rationale, and Background:

This trial has several objectives. We wished to determine in a prospective randomized fashion whether high doses of etoposide (VP16) and cisplatin (PLAT) given during a six-week induction period would produce higher complete response rates and better survival than standard doses of the same drugs in patients with extensive stage small cell lung cancer (SCLC). We also wished to assess the feasibility and value of individualized chemotherapy selection based upon in vitro drug testing of tumor cell lines derived from pre-treatment patient tumor specimens. Objectives of this portion of the study were to determine the frequency with which tumor-containing specimens can be obtained from unselected patients with extensive stage SCLC, the frequency of successful cell culture and drug sensitivity testing, the degree of heterogeneity of drug sensitivity among different cell lines the correlation between in vitro drug sensitivity and clinical response, and the clinical utility of individualized drug selection based upon in vitro data.

The introduction of combination chemotherapy into the management of SCLC has led to four- to five-fold improvement in median survival and five-year disease-free survival in a small fraction of patients. Although median survival is improved to approximately the same degree compared to untreated patients in limited stage and extensive stage disease, survival of two years or more only rarely occurs in patients with extensive disease, defined as tumor extending beyond the hemithorax of origin and the regional lymph nodes. Furthermore, chest irradiation has never been suggested to yield any survival benefit in extensive stage patients. Therefore, virtually all patients with extensive SCLC are suitable subjects for investigational chemotherapy studies.

Methods Employed:

Moderately aggressive chemotherapy which produces leukopenia in the range of 1,000/mcl has been shown to be superior to less intensive treatment that is virtually never associated with leukopenic fever in both randomized and non-randomized studies. However, even more intensive initial (or induction) therapy which is so myelosuppressive that hospitalization of all patients is required has not been demonstrated to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been restricted to cyclophosphamide, doxorubicin, and VP16. VP16/PLAT has been shown to be a highly synergistic combination regimen in treatment of murine leukemia and in early studies appears to be as active as most three- or four-drug combinations in patients with SCLC. VP16/PLAT is also more active than VP16 alone as a salvage regimen in this tumor. PLAT in higher than conventional doses appears to have increased activity in testicular and perhaps ovarian cancer. Although higher than standard doses of VP16/PLAT have been employed in small studies in SCLC, the issue of dose-response with this combination has not been addressed in a prospective randomized trial. We therefore initiated such a study. The first four patients randomized to the high dose regimen received VP16 120mg/m^2 x 5 and PLAT 40mg/m^2 x 5. Two died of infection before Day 21 without recovery from myelosuppression, and the doses of drugs on the high dose arm were subsequently reduced to VP16 80mg/m^2 x 5 and PLAT 27mg/m^2 x 5. Throughout the trial, doses on the standard arm have been VP16 80mg/m^2 x 3 and PLAT 80mg/^{2} x 1. Since a significant minority of extensive stage SCLC patients are not candidates for a very myelosuppressive regimens, such patients (deemed "poor risk") are not randomized but rather assigned to standard dose therapy.

For the past 10 years, the human tumor stem cell assay of Hamburger and Salmon has been most commonly employed for in vitro drug testing of human cancer. In applying this test to fresh tumor specimens from our SCLC patients, however, we found that sufficient tumor colonies for adequate in vitro testing of even a single drug were present only 23% of the time. Clearly, different approaches were needed to apply in vitro drug testing to a large fraction of patients. Since our laboratory has considerable experience in establishing permanent cell lines of SCLC, we decided to utilize cell lines rather than fresh tumors for drug testing. Compared to fresh tumors, cell lines provide tumor cells that are free of contaminating stromal cells and can be subjected to repeated testing. The time from specimen procurement to assay results, however, is delayed.

A modification of the Weisenthal dye exclusion assay was employed for drug testing because the assay is technically simple, does not require a single cell suspension, can be completed in four days, and can be applied to many tumors and most cell lines. Reading the assay, however is labor intensive and subjectiveand can be confounded by cell clumping.

Major Findings:

Ninety-eight patients have been entered. Median follow-up from time of patient entry is approximately 48 months. Twenty-one of the 98 patients were assigned standard dose therapy because of poor performance status, brain, lung or cardiac dysfunction, or refusal to be randomized. The remaining 77 were randomized to receive high or standard dose VP16/PLAT for the first 6 weeks of therapy.

On the high dose arm, 30 (83%) of 36 have responded to therapy, including 9 (25%) complete responders, and actuarial median survival is 12 months. On the standard dose arm, 33 (80%) of 41 patients responded, including 9 (22%) complete responders, and actuarial median survival is 11 months. There is no significant difference between the two groups in complete response rate ($p = 1.00$) or overall survival by the logrank test ($p = 0.73$). As expected, the response rate (5% complete, 71% complete plus partial) and survival (actuarial median 6 months) are inferior in patients judged not suitable for randomization. Among all 98 patients, performance status and number of distant organ systems involved with metastatic disease (0-2 vs. 3-7) are significant predictors of survival ($p < 0.001$ and $p = 0.005$, respectively).

Hematologic toxicity is significantly worse on the high dose induction program (median nadir WBC count 1,600/mcl and platelet count 67,000/mcl) compared with the standard dose induction (median nadirs 2,600 and 162,000, respectively). Among the poor risk nonrandomized patients, median nadir WBC count has been 1,800/mcl and median nadir platelet count, 90,000/mcl. There have been six treatment-related deaths, all due to myelosuppression and infection, two on the high dose arm prior to lowering of the drug doses, one on the standard dose arm and three in poor risk patients assigned standard dose therapy. Although only 41 patients have been treated, the standard dose regimen yields results at least as good as our historical experience in good risk extensive stage SCLC with considerably less hematologic toxicity, suggesting it may have a superior therapeutic index.

A total of 141 pre-treatment staging specimens have been submitted for cell culture from the first 80 patients (1.8/patient). Seventy-eight specimens (55%) contained tumor cells. Twenty-eight cell lines, defined as sufficient in vitro amplification of tumor cell number to allow testing of multiple drugs in duplicate at three concentrations, have been obtained. The largest numbers of positive specimens and cell lines were derived from bone marrow, peripheral lymph nodes, and pleural effusions. Procurement of only five specimens required administration of general anesthesia, but three of these five procedures were performed for diagnostic purposes. Among the 80 patients with a minimum six-month follow-up, at least one staging specimen reached the cell biology laboratory in 79 (99%), and a tumor-containing specimen was procured from 60 (75%) of these previously untreated patients. A cell line was obtained from 26 (33%), or 43% of patients from whom a tumor-containing specimen was available. In addition, tumor-containing specimens have been obtained from 17 of these patients after tumor progression on chemotherapy, and a cell line has been successfully grown from eight.

Actuarial median survival of patients from whom a tumor cell line was successfully grown, patients from whom a tumor-containing specimen was obtained but did not grow in vitro, and patients from whom no tumor-containing specimen could be procured was 8, 11, and 15 months respectively. Patients with no tumor specimen had superior survival by the logrank test ($p < 0.02$). The survival of patients whose tumor specimens were or were not successfully cultured was not significantly different ($p = 0.60$). Thus, whether a patient had sufficient tumor dissemination that a biopsy specimen could be relatively easily obtained was of greater prognostic import than whether a cell line could be established from a positive biopsy specimen.

In vitro drug testing has been completed on tumor cell lines derived from 24 previously untreated SCLC patients. In vitro drug sensitivity of these cell lines correlated extremely well with response to therapy to VP16/PLAT. In 14/15 (93%) lines from patients with complete or partial response at 12-week restaging, two or more drugs were "active." Sensitivity patterns were strikingly different in the six lines from patients who never responded to VP16/PLAT or had progressed by Week 12. In none of these lines were two or more "active" drugs identified. For each of the seven drugs considered individually, lines from responding patients always exhibited a lower mean cell survival at the reference concentration than lines from non-responding patients. Evaluation of these differences with the 2-sample rank yielded p values of less than 0.05 for VP16, doxorubicin, vincristine, and mechlorethamine, and less than 0.10 for methotrexate.

Complete response rates to the first chemotherapy regimen given after VP16/PLAT were compared in patients receiving an "in vitro best regimen" based on in vitro drug testing, or in those receiving vincristine/doxorubicin/cyclophosphamide (VAC) when in vitro drug testing results were not available for whatever reason. Thirty-five patients were treated with VAC after failure to achieve complete response by Week 13, and eight after relapse from complete response induced by VP16/PLAT. In these 43 patients, there were three complete responses (7%). Among the 16 patients who received their "in vitro best regimen," 13 had failed to achieve complete response at Week 13, and three had relapsed. Four patients (25%) attained complete response to their chemotherapy program based on in vitro drug testing ($p = 0.16$, Fisher's exact test).

Significance to Biomedical Research and the Program of the Institute:
Thus far, there is no indication from this study that a high dose regimen of VP16/PLAT (67% higher doses of each drug, 46% higher doses/unit time actually administered) is in any way superior to standard doses of this two-drug regimen. On the other hand, the standard dose program is well tolerated and may be as effective as any other SCLC regimen, based on this data and that of others. Given the low complete response rate to any of the drug programs given to partial or non-responders at Week 13, it is likely that most or all of the survival benefit our patients received from therapy was produced solely by VP16/PLAT.

The interim results of this trial serve to emphasize several problems that arise in implementing a program of individualized chemotherapy selection with our current technology and study design. First, procurement of tumor specimens, establishment of cell lines, and drug testing are extremely labor intensive and time consuming. More efficient assay techniques and better understanding of the relationship between in vitro and in vivo pharmacokinetics would be valuable. Second, drug testing has been possible in only one-third of patients, and improved methods of cell culture are still needed. We believe these interim results justify the more frequent employment of major surgical procedures to procure larger, more rapidly grown tumor specimens in good risk consenting patients, and have already begun such a program in limited stage patients, who would be expected to more frequently be able to tolerate elective general anesthesia. And third, with the time required to establish and perform drug testing on cell lines, treatment based on in vitro testing can often be given only 10 to 12 weeks after a tumor specimen is obtained and may not be relevant to the in vivo drug sensitivity pattern present in residual tumor cells present at that time. Procurement of larger tumor specimens could help to alleviate this problem and allow more rapid drug testing and quicker administration of "individualized" chemotherapy.

Proposed Course:
In a statistical analysis done one month ago, when 77 patients with follow-up had been randomized, 95% confidence limits for differences in 12-month survival ranged from favoring the high dose arm by as much as 15% to favoring the standard dose arm by as much as 9%. We plan to continue accrual to this study until the pre-planned number of 90 patients, which would allow detection of a doubling of complete response rate or a 50% increase in median survival, have been randomized.

Despite these problems and the preliminary nature of our results, we believe several conclusions are justified. First, results of drug sensitivity testing of tumor cell lines are highly correlated with response to initial chemotherapy. Second, preliminary results utilizing in vitro drug testing for individualized selection of chemotherapy regimens suggest modest potential for therapeutic benefit. Third, the close correspondence between in vitro and in vivo response to drugs provides justification for the use of human cancer cell lines in screening for new chemotherapeutic agents. And finally, the availability of multiple SCLC tumor cell lines from patients whose clinical course is well characterized, including some paired lines from patients before and after in vivo chemotherapy, may prove useful in helping to elucidate the basis for drug resistance and other biologic properties of this tumor.

Publications:

1. Ihde DC, Johnson BE, Mulshine JL, Sausville EA, Veach SR, Steinberg SM, Edison M, Lesar M, Minna MD. Randomized trial of high vs. standard dose etoposide and cisplatin in extensive stage small cell lung cancer. Lung Cancer 1988;4(suppl):A103. Presented at Fifth World Conference on Lung Cancer of the International Association for the Study of Lung Cancer, Interlaken, Switzerland, 1988.

2. Gazdar AF, Russell EK, Oie HK, Steinberg S, Ghosh B, Linnoila RI, Minna JD, Ihde DC. Extensive disease small cell lung cancer: A prospective trial of chemotherapy based on in vitro drug sensitivity testing. Adv Biosciences 1988;72:173-176.

3. Ihde DC, Russell E, Oie H, Linnoila RI, Steinberg S, Ghosh B, Cotelingham J, Minna JD, Gazdar AF. In vivo drug sensitivity testing results correlate with chemotherapy response and survival in extensive small cell lung cancer. Proc Am Soc Clin Oncol 1989;8:228. Presented at 25th Annual Meeting of American Society of Clinical Oncology, San Francisco, 1989.

4. Gazdar AF, Tsai CM, Park JG, Ihde DC, Mulshine JL, Carmichael J, Mitchell JB, Minna JD. In vitro assays for predicting clinical response in human lung cancer. In Chapman JD, Peters LJ, Withers HR, eds. Prediction of Tumor Treatment Response. New York: Pergamon Press, 1989;175-186.

5. Stevenson HC, Gazdar AF, Linnoila RI, Russell EK, Oie HK, Steinberg SM, Ihde DC. Lack of relationship between in vitro tumor cell growth and prognosis in extensive stage small cell lung cancer. J Clin Oncol, 1989;7:923-931.

6. Gazdar AF, Steinberg SM Russel EK, Linnoila RI, Oie HK, Ghosh BC, Cotelingham JD, Johnson BE, Minna JD, Ihde DC. Correlation of in vitro drug sensitivity testing results with response to chemotherapy and survival in extensive stage small cell lung cancer: A prospective clinical trail. J Natl Cancer Inst 1990;82:117-124.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06575-15 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Dominant and recessive oncogenes, growth factors, & HIV-like retroviruses in the pathogenesis of lung cancer.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John D. Minna, MD		Branch Chief	NCI-NMOB
Others:	All NCI-NMOB		
M. Nau	Chemist	R. Osborne, MD	Guest Res (EORTC) (until 11/89)
J. Fedorko	Microbiologist	T. Mitsudomi, MD	Fogarty Fellow (with Dr. A. Gazdar NMOB)
I. Chiba, MD	Fogarty Fellow	J. Broers, PhD	Fogarty Fellow (with Dr. I. Linnoila NMOB)
T. Takahashi, MD	Fogarty Fellow	D.L. Buchhagen, PhD	Asst Prof Med NCI-USUHS
D. D'Amico, MD	Guest Res	D. Carbone, MD, PhD	Instructor Med NCI-USUHS/PHS
D. Curiel, MD	Biotech Fellow	J. Viallet, MD	Instructor Med NCI-USUHS
S. Bodner, MD	Biotech Fellow	R. Maneckjee, PhD	Guest Res (Mathers' Fnd)

COOPERATING UNITS *(if any)*

H&W Nash; H. Pass, (Surg Branch, NCI); H. Brauch, M. Lerman, K. Tory, F. Kotler, B. Zbar (Laboratory of Immunobiology, NCI, FCRF); J. Whang-Peng, T. Knudsen, (Med Branch, NCI); S. Steinberg (Biostatistics Branch, COP, DCT, NCI); D. Smith and W. Golembieski (Wayne State Univ); E.C. Holmes, D. Slamon, Lung Cancer Study Group (UCLA); P. Howley, (Lab Virol, DCE, NCI); G Merlo, T Venesio, DS Liscia, APM Cappa, R Callahan, (Oncogenetics Section, NCI & Ospedale S. Giovanni Vecchio, Turin, Italy); Y. Sharoni, E.A. Sausville (Department of Medicine, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University School of Medicine, Washington, D.C.); H. Frucht, R.T. Jensen (Digestive Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases); P. Madigan, J. Mulvihill (Clinical Epidemiology Branch, DCE, NCI); J. De Martini (Colorado State University); Dr. S. Collins (Fred Hutchinson Cancer Center, Seattle, WA); Dr. F. Li, Dana Farber Cancer Center and DCE, NCI)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.5	12.5	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 objective of this work is to identify and characterize the genetic changes (somatic and constitutional) and other deranged mechanisms (such as those involving growth factors, their receptors, signal transduction, or viral infection) leading to the pathogenesis of lung cancer and to use this information to develop new methods to prevent, diagnose and treat this disease. This work has uncovered abnormalities in the dominant and recessive oncogenes ("tumor suppressor" genes). The dominant oncogenes include those of the *myc* and *ras* family, and a complex role for those of the jun family. The recessive oncogenes include several genes in chromosome region 3p, 11p, the rb gene on 13q14, the p53 gene on 17p13 as well as multiple other areas. We have also begun a search for an HIV like retrovirus in the pathogenesis of lung cancer with analogy to the putative retrovirus causing ovine pulmonary carcinomatosis. Growth factor and receptor work has demonstrated the presence of receptors for opioid peptides and nicotine on all classes of lung cancer cells as well as endogenous production of opioids leading to an hypothesis about their role as negative growth regulators, and the role of nicotine in reversing this effect.

Introduction
The Molecular Pathogenesis of lung cancer

Lung cancer cells exhibit a large number of genetic lesions involving mutations activating the dominant cellular proto-oncogenes and inactivating the recessive or "tumor suppressor" genes. The lesions in known and suspected tumor suppressor genes are highlighted by the large number of clonal cytogenetic abnormalities including chromosomal deletions and non-reciprocal translocations. These result in loss of heterozygosity at several loci confirmed by RFLP analysis indicating the involvement of multiple tumor suppressor genes including those on chromosomes 1, 3p, 11p, 13q (*rb* gene), & 17p(p53 gene), as well as other chromosomes. Studies of the *rb* gene indicate it is altered in nearly all cases of small cell lung cancer (SCLC) and at least some non-small cell lung cancers (non-SCLC), while *p53* appears mutant in 40% or more of all lung cancers. We often find absent expression of *rb* protein, in contrast to point or small mutations, leading to the production of mutant p53 proteins. Other candidate recessive oncogenes in lung cancer include a Wilm's related gene at 11p13, the NM23/awd gene, and the DCC gene on 18. Lung cancer cells also exhibit receptors for opioids, produce endogenous opioid peptides, and have their growth inhibited by exogenously added opioids suggesting that this represents a new type of tumor suppression. They also express receptors for nicotine and while nicotine itself has no effect on tumor cell growth, in some cases it can reverse opioid induced growth inhibition suggesting a role in tumor promotion. In addition, lung cancer cells produce autocrine growth factors and *jun* family transcription factors which could aid in tumor promotion. The number of lesions (10-20 per tumor) required for tumors to become clinically evident raises the question of Mendelian inheritence and acquistion of lesions during embryonic development as well as from carcinogen exposure in adult life in lung cancer pathogenesis. Together, our findings suggest that detection of molecular genetic abnormalities in these genes may be applied in studies of prevention, early diagnosis, prognosis, and familial inheritence of cancer.

Dominantly Acting Oncogenes

Studies of mutations of the ras family members.
No mutations were found in small cell lung cancer, while mutations in codon 12 and 61 were found in non-small cell lung cancers (T. Mitsodoni, J. Viallet in collaboration with A. Gazdar; see also Gazdar report).

Studies of expression of myc family members in fresh resected non-small cell lung cancers by in situ hybridization (J. Broers in collaboration with I. Linnoila; see also Linnoila report)

Studies of expression of jun family members

We have cloned the human *jun*-B gene and determined its sequence and transforming and trans-activating activities. *jun*-B is less potent than c-*jun* in transforming and immortalizing primary rat embryo cells in cooperation with activated *ras* (effects enhanced by c-*fos* and TPA); unlike c-*jun*, *jun*-B does not transform Rat-1A cells alone. However, cotransfection of c-*jun* and *jun*-B into primary rat embryo cells with c-Ha-*ras* results in a significant decrease in transformation compared with c-*jun* alone, an effect reversed by TPA. Cotransfection of c-*jun* and *jun*-B with or without c-*fos* into F9 teratocarcinoma cells results in decreased trans-activation of AP-1 compared with either gene alone. Introduction of *jun*-B into primary rat c-*jun*/*ras* transformants or c-*jun* into *jun*-B/*ras* transformants also results in a decrease in trans-activation of the reporter construct. These findings demonstrate that whereas

jun-B and c-*jun* each participate in AP-1 trans-activation and malignant transformation, interactions between them involve negative regulation.

Recessive Oncogenes ("Tumor Suppressor" genes)

Cytogenetic Studies: Cytogenetic studies reveal many, non-random structural and numeric changes in non-small cell lung cancer.

Cytogenetic studies were performed on twenty-seven tumor cell lines and four fresh malignant pleural effusions from thirty patients with non-small cell lung cancer (non-SCLC). Many clonal structural (deletions and non-reciprocal translocations) and numerical abnormalities were found in each specimen. Statstical analysis revealed these changes were non-randomly distributed among the chromosomes. Statistically signficant differences in the number of chromosomal breakpoints occurred for regions: 1q1, 1q3, 3p1, 3p2, 3q1, 3q2, 5q1, 7q1, 13p1, 14p1, 15p1, 16q2, 17q1, 21p1. The breakpoints 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 lung cancer.(M. Nau, H. Oie, A. Gazdar, E. Russel, J. Mulshine, I. Linnoila, J. Minna in collaboration with J.Whang-Peng, T. Knudsen, S. Steinberg)

Search for recessive oncogenes ("Tumor Suppressor" genes) in the 3p chromosome region

A deletion of the short arm of chromosome 3 (3p) is one of the most frequent genetic changes in both small cell and non-small cell lung cancer (see above cytogenetic studies in non-small cell lung cancer). Our group published the first report of cytogenetic deletion of chromosome 3p (In collaboration with our long time collaborators Dr. J. Whang-Peng, and T. Knudsen of the Medicine Branch and Dr. S. Steinberg of the Biostatistics Branch, COP) and the first report of restriction fragment length polymorphism (RFLP) loss (in collaboration with Dr. S. Naylor, San Antonio, TX). We have thus, focused considerable effort on trying to isolate and identify the putative 3p recessive oncogene(s).

Our unpublished cytogenetic studies in both small cell and non-small cell lung cancer indicate that there are at least two and possible three or four different regions on 3p involved in deletions. These include regions: 3p24-25, 3p21, 3p14 as well as regions on 3q. These findings coupled with RFLP analyses suggest that a gene or genes on 3p plays a role in the origin or evolution of lung cancer and in studies by others in the pathogenesis of renal cancer, ovarian cancer, human cervical cancers. breast cancer and other malignancies. Other evidence for a 3p recessive oncogene includes the recent mapping of the von Hippel-Lindau disease (VHL) gene that predisposes to renal cell and other cancers to 3p24-25 where it is linked to RAF1. In addition, there are rare families with inherited translocations involving 3p that are predisoposed to renal cancer.

Scanning with known RFLP probes

We used 10 RFLP probes spanning the length of the short arm of chromosome 3 (3p) to map deletion sites in human lung cancer. Two approaches were used. 1.) When a patient's tumor and normal tissue were available, loci with allelic heterozygosity in the normal tissue were tested for loss of alleles at 3p. 2.) when the corresponding normal tissue was not available, the frequency of heterozygosity at each locus in a panel of tumors was compared to the corresponding published frequencies in nontumor tissue of healthy individuals or patients with lung cancer. In 14 SCLCs with normal DNA for comparison, allele loss was found at all

heterozygous loci, with one exception at a locus near the 3p centromere (D3S4). In the total of 53 SCLCs, which included tumors without paired normal tissue, frquency of heterozygosity was signfiicantly reduced in all 10 3p loci. Three loci, DNF15s2, RAF1, and D3S18, were homozygous in all tumors in the SCLC panel. These loci, which are in regions 3p21 and 3p25, may thus be involved in the origin or evolution of SCLC. We also investigated 24 non-SCLC tumors. In this panel, frequency of heterozygosity was signficantly reduced at seven of the 10 loci tested. Comparison of the results shows that the pattern of allele loss on 3p is different in SCLC and non-SCLC, suggesting a difference in pathogenesis at the genetic level. (In collaboration with H. Brauch, K. Tory, F. Kotler, B. Zbar of the Laboratory of Immunobiology, NCI, FCRF)

Identification of expressed genes on human chromosome 3

In order to identify the hypothesized "recessive oncogene(s)" on 3p three vital pieces of information must be obtained: 1.) there needs to be some evidence of a mutation in DNA (most easily found by a DNA rearrangement or homozygous deletion) This is being pursued by screening with more chromosome 3 specific probes (in collaboration with Dr. D. Smith and W. Golembieski of Wayne State University, MI; and Dr. B. Zbar's group FCRC); 2.) the number and locations of chromosome 3-specific genes that are expressed in the tissues at risk (lung, kidney, ovary, breast). 3.) The functions of these genes and mechanisms by which these genes can be inactivated (deletion, point mutation, lack of expression). We propose to identify those genes on chromosome 3 which are expressed in lung, kidney, ovary, and breast; 2.) to determine if any of the identified genes are specficially altered in lung cancer cells; 3.) to identify their inactivating lesions and from DNA sequence possibly infer function.

Using radiolabelled cDNA prepared from rat lung mRNA as well as subtracted cDNA probes made between human lung cancers that are heterozgous for 3p RFLPs (tracer) and subtracting with 3p deleted SCLCs (drivers) we screened a human chromosome 3 specific cosmid library assembled by our collaborator, Dr. David I. Smith of Wayne State University, Detroit, MI). We have found many cosmids that contained abundantly expressed genes from both the 3p and 3q arms. We are in the process of isolating the cDNAs for these genes which then can be applied in a systematic search for changes in lung cancer of expressed genes. (D. L. Buchhagen, D. Carbone, T. Takahashi, J. Minna in collaboration with D. Smith and W. Golembieski, Wayne State University).

Development of methods to scan for mutations in putative dominant or recessive oncogenes in both tumor and normal samples including methods to look for inherited mutations and mutations acquired during development in normal tissues

A central problem in understanding the pathogenesis of common human solid tumors such as lung, colon, and breast cancer is identifying genes that have suffered mutations. Once such genes are identified it is crucial to have a method for rapidly determining if a given tumor has a mutation in that gene including point mutations. Until recently the only methods to find such subtle mutations were by DNA sequencing or RNAse protection assays. Unfortunately the latter assays only detect 40-50% of all such point mutations as our work with the p53 recessive oncogene discussed below discovered.

To circumvent this problem we have established and adapted a published chemical cleavage method to detect mutations in the p53 gene. Various strategies have been employed in the detection of point mutations in the p53 gene. Sequence analysis, while the most definitive, is extremely labor intensive and thus, not practical for large scale screening. Additionally, difficulty achieving direct sequence analysis of PCR derived products has been noted by ourselves and others. The method of RNase protection has also been employed for the detection of single base differences from normal sequence. While efficient in analyzing larger segments of the coding regions of the p53 gene, the method suffers from the inherent limitation

that even under optimized conditions, less than 50% of single base mutations can be detected. As an alternative strategy, we have recently embloyed chemical mismatch cleavage to screen for mutations in the p53 gene. This method offers the advantages of rapid and efficient analysis of large DNA segments and is thus, ideally suited to large scale screening. Furthermore, the method can theoretically detect all possible single base mutations.

Target DNA to be analyzed is allowed to form a heteroduplex with labeled wild type probe. Both target and probe DNA can be derived by PCR amplification. The heteroduplexes are combined with a chemical coupling reagent; hydroxylamine hydrochloride reacts with unpaired guanidines and osmium tetroxide with unpaired thymidines. Coupled nucleotides are thus susceptible to cleavage by piperidine. The resulting cleavage products can then be analyized by urea-acrylamide gel electrophoresis and autoradiography.

The chemical mismatch cleavage method was evaluated utilizing genomic DNA, and p53 cDNA, derived from SCLC and Non-SCLC tumor samples with known mutations detected by sequence analysis. Evaluation of a p53 cDNA by chemical cleavage correctly detected the single base mismatch in all instances. Additionally, evaluation of 6 genomic DNAs by mismatch cleavage confirmed the presence in the genomic DNA of mutations identified initially in the corresponding tumors p53 cDNA. Thus, this methodology is a powerful tool in screening for single base mutational changes. Large segments of DNA can be analyzed rapidly and with the capacity to detect all differences from normal. We are applying this not only to tumor samples but also to corresponding normal lung, and peripheral blood lymphocyte DNA from cancer patients, including families with multiple lung cancers, and pedigrees with an apparent inherited predisposition for cancer. We are looking for mutations either acquired during development or inherited in a Mendelian fashion. (D. Curiel, DL Buchhagen, I. Chiba, T. Takahashi, J. Minna in collaboration with the Drs. EC Holmes, D. Slamon, Lung Cancer Study Group, and Dr. F. Li, Dana Farber Cancer Center and DCE, NCI).

Test of specific genes expressed in chromosome region 3p for mutations: Retinoic acid receptors (RAR) and Thyroid Hormone receptors.

An attractive hypothesis is that the recessive oncogenes represent a lesion in a nuclear receptor for a differentiating ligand. An obvious candidate is the retinoic acid receptor located at 3p24-25 RARβ. There is a large body of evidence linking retinoic acid to epithelial differentiation, and a deficiency in retinoic acid to lung cancer and squamous metaplasia. Since this region is already homozygous in a large number of patients with lung cancer it is logical to ask if the remaining RARβ has suffered mutations. In fact, many years ago we found that the lung cancer lines were in general, resistant to differentiation by retinoic acid. To answer this question of mutation directly, we have been studying the cDNAs using cDNA/PCR amplification, direct and cloning DNA sequencing as well as chemical cleavage technology for RARβ, and the other retinoic acid receptors RARα, and the recently described RARγ, and RXR for abnormalities in lung cancer. At least some of the lung cancer lines have different forms and novel sequences in their cDNAs for RARβ. It will be of interest to see if these represent true mutations and/or RNA splicing variants. In addition, we are attempting to insert functional RARs into the lung cancer cell lines (T. Mitsudomi, D.L.Buchhagen, T. Takahashi, A. Gazdar, D. Carbone, J. Minna in collaboration with Dr. S. Collins, Fred Hutchinson Cancer Center, Seattle, WA)

Mutations in the p53 Gene are Frequent in All Histologic Types of Lung Cancer Including Primary and Metastatic Lesions.

The p53 gene has been implicated as a tumor suppressor gene and mutations have been reported in lung, colon and breast cancers. Initially we reported on p53 abnormalities in 17/30

(57%) lung cancer cell lines representing all histological types of lung cancer by Southern and Northern blots and RNase protection assays while an additional 17% expressed very low levels of p53 mRNA. Several of these were confirmed by sequencing including fresh non-small cell (NSCLC) and small cell lung cancer (SCLC) samples (Takahashi et al. Science 246: 491, 1989). To extend these results we have analyzed 54 primary resected NSCLC specimens from patients enrolled in LCSG protocols for for loss of heterozygosity of 17p loci and abnormalities in the p53 gene sequence. Loss of heterozygosity for YNZ22 (17p13.3) was found in 10/32 (31%) informative cases. RNAs from all the tumors were analyzed with an RNAse protection assay using probes spanning the p53 open reading frame (ORF) and by sequencing the p53 ORF cDNAs amplified by PCR and subsequent cloning. Mutations changing the coding sequence were found in 20/48 (42%) cases so far analyzed. These mutations were dispersed between amino acids 132 and 283 and involved examples of G to T, A to T, G to C, G to A, C to T, and A to G changes. Of interest, 12 tumors exhibited heterozygosity at 17p in tumor genomic DNA while expressing a mutant p53 mRNA. This suggests that p53 mutations can occur before 17p allele loss consistent with a dominant negative mode of action for mutant p53 proteins and indicates the need to test for p53 mutations rather than relying only on 17p allele loss. Previously, 17p allele loss has been described in 26/26 (100%) of SCLCs (Yokota et al. PNAS 84: 9252, 1987; Mori et al. Cancer Research 49: 5130, 1989; Takahashi et al. Science 246: 491, 1989) suggesting that mutations in p53 may occur in all SCLCs. To study this, we analyzed 12 SCLC tumor cell lines derived from specimens removed from patients enrolled in NCI clinical treatment protocols for abnormalities in the p53 gene sequence by direct sequencing of the p53 ORF cDNAs amplified by PCR. RNase protection abnormalities were found in 5/12 (42%) which were confirmed by DNA sequence changes. However, by direct sequencing of cDNA, mutations changing the coding sequence ultimately were found in all 12 (100%). In no case, was a wild type p53 mRNA expressed. These mutations were dispersed between amino acids 68 and 342 and multiple types of base changes were found. In addition, these studies in NSCLC and SCLC identified mutations in introns 3,6,7,8, and 9 that led to aberrant mRNA splicing. We conclude from these data that p53 mutations: play an important role early in the pathogenesis of many NSCLCs and potentially all SCLCs; can occur prior to 17p allele loss; suggest studies correlating patient survival with p53 mutation; and encourage the search for mutations in preneoplastic tissues as well as for cellular and humoral immune reactions in patients against mutant p53 peptides. (I. Chiba, T Takahashi, M M Nau, D D'Amico, D Curiel, D Buchhagen, D Carbone, J. Minna in collaboration with H Koga, D J Slamon, E C Holmes, and the Lung Cancer Study Group (LCSG).Depts of Surgical Oncology and Medicine, UCLA School of Medicine, Los Angeles, CA 90024).

Identification of intronic point mutations as an alternative mechanism for p53 inactivation in lung cancer and other solid tumors

This study reports the first identification of intronic point mutations as a mechanism for inactivation of the p53 tumor suppressor gene. Abnormally sized p53 mRNAs found in a small cell and a non-small cell lung cancer cell line were characterized by sequence analysis of cDNA/PCR products, the RNase protection assay and immunoprecipitation. These mRNAs were found to represent aberrant splicing leading to the production of abnormal or no p53 protein. Sequence analysis of genomic DNA revealed that a point mutation at the splice acceptor site in the third intron or the splice donor site in the seventh intron accounts for the abnormal mRNA splicing. In one patient the same intronic point mutation was found in the tumor cell line derived from a bone marrow metastasis and in multiple liver metastases but not in normal DNA, indicating that it occurred as a somatic event prior to the development of these metastases. These findings further support the role of inactivation of the p53 gene in the pathogenesis of lung cancer and indicate the role of intronic point mutation in this process. (Takashi Takahashi, Domenico D'Amico, Itsuo Chiba, Dorothy Buchhagen, and John D. Minna)

p53 Mutations and Chromosome 17 Deletions in Primary Breast Cancers

Examination of primary breast tumour samples has revealed frequent loss of 17p heterozygosity in the region of the p53 gene. This coupled with the finding of p53 mutations in lung, colon, and a few cases of breast cancer would suggest that p53 could be playing a role as a recessive oncogene ("tumour suppressor" gene) in these tumours. Mutations in the p53 gene generally have been situated in highly conserved regions of the gene. The presence of p53 mutations has also been inferred indirectly by immunohistochemical detection of p53 protein under the assumption that mutations in p53 result in a mutant protein with a longer half-life compared to the normal protein. However, to date, an analysis involving sequencing the p53 open reading frame (ORF) and thus defining the exact nature and position of mutations has not been reported for a substantial number of primary breast tumours. Until there is evidence that mutations at different sites result in equivalent biological effects, identification of the individual lesions in tumours will be important. Accordingly, a detailed examination of p53 abnormalities in primary breast cancer has been performed. Twenty-eight primary resected breast cancers were studied for: 17p allele loss by RFLP analysis, the presence of mutations in the p53 ORF by RNase protection assays, and sequencing of the ORF by PCR amplification and subcloning of p53 cDNA. Using the YNZ22 (Taq I) and pBHP53 (Bam HI) restriction fragment polymorphisms, loss of heterozygosity at 17p was found in 5/21 (24%) informative cases. RNase protection abnormalities were found in 3/28 and sequencing of cDNA confirmed the mutations in all three. To date 17 of the remaining 25 tumour samples have had sequence analysis of cDNA and mutations were detected in 10 (which are being confirmed by repeat analysis). In all 13 cases, the mutations resulted in change of the amino acid sequence and were located in both conserved and non-conserved regions. The mutations observed comprised point mutations in 11 cases and 2 lesions with apparent splicing abnormalities (deletion of exon 4 in one case and deletion of exon 3 and part of exon 4 with additional point mutations in another case). Of interest, p53 mutations were found in 10 cases which retained heterozygosity for the 17p RFLP markers. We conclude that mutations changing the product of the p53 gene are common in primary breast cancers, that they can involve multiple sites in the p53 ORF, and that these mutations can occur in samples that did not show 17p allele loss. (Richard Osborne, Itsuo Chiba, Takashi Takahashi, Marion Nau, and John Minna in collaboration with: G Merlo, T Venesio, DS Liscia, APM Cappa, R Callahan, Oncogenetics Section, National Cancer Institute, Bethesda, MD, USA. and Ospedale S. Giovanni Vecchio, Turin, Italy)

Alternative splicing of the putative Wilms' gene mRNA in small cell lung cancer cell lines

Chromosome 11p is one of several sites of frequent cytogenic deletion and loss of heterozygosity in lung cancers (Shiraishi *et al*, and Weston *et al*). A putative anti-oncogene involved in Wilms tumors has been localized to one of the regions where loss of heterozygosity is seen on 11p in lung cancers. Recently two sequences for a candidate Wilms gene have been reported (Gessler, M, *et al.* 1990. Nature 343, pp 774-778; Call, K, *et al.* 1990 Cell 60, pp 509-520).These two sequences are very similar but differ from each other by two inframe deletions within the 3' end of the coding region in the region of the zinc fingers. We utilized cDNA polymerase chain reaction (PCR) techniques using primers which span this zinc finger region of the mRNA to look for expression of this candidate Wilms gene in a panel of lung cancer cell lines and corresponding EBV transformed lymphocyte lines. Six of eleven small cell lung cancer (SCLC) cell lines were shown to express levels of this gene sufficient to visualize on ethidium bromide stained gels. None of seven non-small cell lines or five lymphocyte lines showed this same level of expression. In addition, multiple bands were seen and the SCLC cell lines generated two closely spaced fragments, which upon cloning and sequencing were shown to represent the two independently published mRNA forms present in the same cell line. These most likely represent alternative splicing forms. The smaller of the

two deletions is within the fourth consensus zinc finger sequence. The deleted form exhibits better homology to the consensus zinc finger sequence and is more abundant relative to the undeleted form in SCLC with high expression than in lines with low expression. These alternative forms may have distinct activities. (D. Carbone, D. D'Amico, and J. Minna)

Search for drugs and toxins which could inhibit the signal transduction pathways activated in lung cancer with development for use in patient treatment.

The role of opioids and nicotine in the pathogenesis of lung cancer: Opioid and Nicotine Receptors Affect Growth Regulation of Human Lung Cancer Cell Lines

Using specific ligands, we find that lung cancer cell lines of diverse histologic types express multiple, high affinity (Kd = 10^{-9} to 10^{-10} M) membrane receptors for μ, δ, and κ opioid agonists and for nicotine and α-bungarotoxin. These receptors are biologically active since cAMP levels decreased in lung cancer cells after opioid and nicotine application. Nicotine at concentrations (~100 nM) found in the blood of smokers had no effect on *in vitro* lung cancer cell growth, while μ, δ, and κ opioid agonists at low concentrations (1-100 nM) inhibited lung cancer growth *in vitro* . We also found that lung cancer cells expressed various combinations of immunoreactive opioid peptides (β-endorphin, enkephalin, or dynorphin), suggesting the participation of opioids in a negative autocrine loop or tumor suppressing system. Due to the almost universal exposure of patients with lung cancer to nicotine, we tested whether nicotine affected the response of lung cancer cell growth to opioids and found that nicotine at concentrations of 100-200 nM partially or totally reversed opioid induced growth inhibition in 9/14 lung cancer cell lines. These *in vitro* results for lung cancer cells suggest that opioids could function as part of a "tumor suppressor" system and that nicotine can function to circumvent this system in the pathogenesis of lung cancer. (R. Maneckjee and J. Minna)

Signal Transduction Studies with Cholera Toxin: Cholera Toxin Inhibits Signal Transduction by Several Mitogens and the in vitro Growth of Human Small Cell Lung Cancer*.

Cholera toxin (CT) inhibited the in vitro growth of 3 of 4 human small cell lung carcinoma (SCLC) cell lines with a 50% inhibitory concentration of 27 to 242 ng/ml. Loss of surface membrane ruffling and the capacity of [Tyr^4]-bombesin, vasopressin and fetal calf serum to stimulate increases in intracellular free calcium clearly preceded effects on cellular metabolic activity and cell growth. ^{125}I-[Tyr^4]-bombesin binding was unaffected by CT treatment but [Tyr^4]-bombesin stimulated phospholipase C activity was decreased in membranes from CT treated SCLC cells. CT stimulated a rapid but transient increase in intracellular cyclic AMP ([cAMP]$_i$) in SCLC. The effects of CT on susceptible SCLC were not reproduced by elevations of [cAMP]$_i$ induced by forskolin or cyclic AMP analogs. G_{M1} ganglioside, the cellular binding site for CT, was highly expressed in the CT-sensitive but not the CT-resistant SCLC cell lines. In contrast, expression of guanine nucleotide binding protein substrates for ADP-ribosylation by CT was similar. These data demonstrate the existence of a CT sensitive growth inhibitory pathway in SCLC bearing G_{M1} ganglioside. Addition of CT results in decreased responsiveness to several mitogenic stimuli. These results suggest novel therapeutic approaches to human SCLC. (J. Viallet and J. Minna in collaboration with H. Frucht, R.T. Jensen (Digestive Diseases Branch, National Institute of Diabetes and Digestive

and Kidney Diseases); Y. Sharoni, and E.A. Sausville (Department of Medicine, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University School of Medicine, Washington, D.C.)

Search for an HIV like retrovirus and bronchioloalveolar lung cancer

Bronchioloalveolar lung carcinoma (BAC) previously was thought to be a relatively rare category of lung adenocarcinoma but recent studies from our Branch (see reports of Drs. Linnoila and Gazdar) would indicate its frequency is increasing. BAC is an unusual lung tumor in that its association with cigarette smoking is weak, it is frequently diagnosed in young adults (20-40 years old) and it occurs with slightly higher frequency among women. In an effort to determine the etiology of BAC, we have been assaying BAC tumor cell lines and pleural effusions from patients for the presence of retrovirus-like particles and for retroviral reverse transcriptase activity.

Our rationale for searching for evidence of retrovirus infection and/or involvement in BAC stems from the similarity in pathology between this human bronchial carcinoma and jaagsiektse, or ovine pulmonary carcinoma (OPC), found in sheep. Several studies have indicated that a type B/type D retrovirus with a Mg^{++} dependent reverse transcriptase can be recovered from diseased sheep. Innoculation of newborn lambs or goats with this virus results in the development of OPC. In addition, with the recent AIDS/HIV epidemic and a potential increase in the frequency of BAC the question of whether the two could be connected immediately comes forward. While there has not to our knowledge been an increase in the frequency of BAC in persons infected with HIV, it is logical to ask if there could be a separate epidemic of a related retrovirus.

We have approached this in several ways in collaboration with the Clinical Epidemiology Branch DCE, NCI (P. Madigan, J. Mulvihill) and the Vetinary Research Group of Colorado State Univ (Headed by Dr. J. De Martini). Dr. Madigan has begun epidemiology studies (see their annual report). We have given Dr. De Martini a large number of lung cancer cell lines to inject intratracheally into new born lambs to try to directly transmit the disease in Colorado. We have screened our tumor cell lines and some clinical material for reverse transcriptase activity, and hybrdization with known human (HIV, HTLV-1) retroviral probes as well as with Mason Pfzier Monkey Virsus (MPMV) probes, and primers for PCR made from converved DNA pol sequences in known retrovirues. Dr. De Martini has been screening sheep material for activity and is attempting to get us OPC material to work with. In our preliminary investigations, we found evidence of minor peaks of reverse transcriptase activity in some tumor cell lines and material banding at retroviral densities from a malignant BAC pleural effusion from an HIV negative individual. We have assembled a panel of genomic DNAs and some RNAs from several sources for study including: 1.) BAC cell lines that had been established in vitro in this Branch; 2.) other SCLC and non-SCLC cell lines whose clarified supernatant fluids were moderately positive in the reverse transcriptase assay; 3.) lung tissues from sheep that suffered from OPC; and 4.) lung tissues from normal sheep. From Dr. De Martini, we are in the process of obtaining fresh OPC virus to purifey and to use as template for radiolabelled cDNA synthesis. The hot cDNA probe to the OPC virus will then be used to hybridize to Southern and Northern filters for human proviral sequences homologous to OPC. Should these sequences be found, we will molecularly clone them, characterize them by DNA sequencing, and use them to search for a human retrovirus from BAC specimens.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 06579-07 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I.R. Kirsch, MD	Senior Investigator	NCI-NMOB
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	M-H. Stern, MD	Guest Researcher	NCI-NMOB
	S. Lipkowitz	Instructor Med NCI-USUHS	NCI-NMOB

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Acquired Gene Rearrangements

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3.2	

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.)*

Association of a specific chromosomal abnormality with tumor type is well established and may reflect mechanisms of oncogenesis peculiar to that tumor. Alternatively, it may be that these associations reflect the particular differentiated state of the malignant cell, consistent with the model that rearrangements occur only within chromatin in an "active" configuration.

We have found that this second possibility has provided a premise and profitable strategy for the identification of genes important to the growth or development of lymphocytes. Our focus this year has followed from our cloning and characterization of two distinctive translocations associated with clonal proliferations of lymphocytes.

We discovered the gene, SCL, because of its disruption by a translocation in the malignant cells of a patient suffering from a leukemia with the capability of lymphoid, myeloid, or erythroid, differentiation. We have delineated the genomic and cDNA structures corresponding to both the normal and translocated SCL genes. We have found that SCL is a member of a family of genes, each a known or putative transcription factor that plays a fundamental role in the particular developmental systems in which is is expressed. SCL is a transcriptionally complex locus. It is now recognized that chromosomal aberrations disrupt the SCL gene with relative frequency, and in so doing cause a structural or functional abrogation of the gene's normal regulation.

PHS 6040 (Rev. 1/84)

GPO 914-918

A second chromosomal region involved in T-cell clonal proliferation is Xq28. It is associated with clonal but usually "non-malignant" outgrowths of cells in the blood of patients with the disease ataxia-telangiectasia. We have recently identified a transcript from Xq28, conserved cross species, whose complete structure and function is currently being investigated.

PROJECT DESCRIPTION

Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

Professional Staff:

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	V. Bertness	Biol Lab Tech	NCI-NMOB
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	M-H. Stern, MD	Guest Researcher	NCI-NMOB
	S. Lipkowitz	Instructor Med NCI-USUHS	NCI-NMOB

Objectives:

Long Term

1. To define the necessary and/or sufficient features for chromosomal breakage and rejoining in different cell types.

2. To use the occurrence of cell-type specific chromosomal aberrations as an in-road to the exploration of differential gene activation during development.

3. To contribute to the understanding of how gene rearrangements mediated by chromosomal aberrations alter the regulation of the affected loci.

Short Term

To study chromosomal aberrations in hematopoietic cells, particularly:

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 study the genomic activity of loci involved in translocations and inversions of chromosomes 7 and 14 in corresponding cells in which the chromosomal aberration has or has not occurred, and thereby determine if the aberration has caused deregulation or altered expression of these loci. Specifically to to focus on the transcript units found on 1p33, disrupted in t(1;14) (p33;q11.2) translocation and Xq28, disrupted in t(X;14) (q28;q11.2) translocation.

3. To continue our studies on the mechanisms of oncogene deregulation as a result of chromosomal rearrangements in lymphoid malignancies.

Major Findings:

We recently have identified a new human gene, SCL, found initially in a multipotential hematopoietic cell line, DU 528, derived from a patient with a stem cell leukemia. The SCL gene is most highly expressed in immature hematopoietic tissues and cell lines. The protein predicted by the SCL cDNA contains the helix-loop-helix (HLH) DNA binding and dimerization motif encoded by several proto-oncogenes and genes involved in cell-type specific differentiation, including the myc, MyoD, achaete-scute, and Ig enhancer families of genes.

We have characterized 20 independent SCL cDNA clones, obtained from a human bone marrow library, along with the corresponding genomic clones. Analysis of this clones by sequence determination and RNase protection assays demonstrate several different classes of cDNA clones generated by alternate exon usage at the 5′ end. These alternative 5′ exons lie in CpG islands, which are unmethylated in cell lines expressing SCL but are methylated in cell lines not expressing SCL. Preliminary experiments suggest that expression of different forms of SCL message occur in a cell-type specific fashion. Proteins predicted by these alternate 5′ exons would conserve the HLH domain, but have distinct amino terminal ends. In order to verify the predicted SCL reading frame, we raised antisera to SCL specific peptides. This antisera immunoprecipitated two distinct protein bands of 41 and 37 kD using an in vitro translation system.

Based on its restricted spectrum of expression and the presence of the highly conserved HLH motif associated with transcription factors in a variety of developmental systems, we suspect that the scl protein is a transcription factor, possibly with a specific role in hematopoietic differentiation. In this context, it is intriguing that SCL messages which encode proteins that retain the HLH motif while predicting different amino termini are regulated in a cell-type specific fashion. Furthermore, SCL shows a remarkable 84% homology in the HLH domain with another newly described gene, lyl-1, which is also expressed in primitive hematopoetic tissues. It is possible that these two genes form part of an interactive system during hematpoesis analogous to the MyoD, myogenin, myf-5, and Id gene relationship during myogenesis.

Benign chronic T-cell proliferations are frequently found in ataxia-telangiectasia (AT) patients and are associated with a small number of recurrent chromosomal translocations. These translocations involve most frequently the T-cell receptor (TCR) α gene and either chromosome 14 or chromosome X. The recurrent breakpoints on these chromosomes are on bands 14q32.1 or Xq28 where are thought to be located yet uncharacterized oncogenes.

We have characterized at the molecular level a t(X;14) (q28;q12) translocation associated with such a lymphoproliferation. This rearrangement has been present for the last 10 years in over 90% of the lectin-stimulated peripheral lymphocytes from an AT patient. No lymphocytosis or organomegaly are associated with this clonal proliferation. Previous in situ hybridization

experiments have implicated the TCR α locus in the translocation. Using a panel of proves derived from the joining region of the TCR α locus (Jα), rearrangements of this locus were identified and cloned from the patient. One of these rearrangements was identified as the translocation by mapping a DNA segment 5′ of a J α may has been used during the translocation event. No such signal sequence was found on chromosome X in the vicinity of the breakpoint.

Probes derived from the Xq28 region identified a 1.2 Kb transcript on Northern analysis. The expression of this transcript seems to be restricted to tissues and cell lines of lymphoid origin. Sequence analysis of the corresponding cDNA revealed that the transcription unit was disrupted by the translocation. No homology was found with other known genes. This putative gene may participate in the pathogenesis of T-cell proliferations associated with rearrangement of chromosome Xq28.

Publications:

1. Begley CG, Aplan PA, Denning SM, Haynes BT, Waldmann TA, Kirsch IR. A new gene, SCL is expressed during early hematopoiesis and encodes a differentiation-related DNA-binding motif. Proc Natl Acad Sci USA 86:10128-10132, 1989.

2. Bertness V, Felix CA, McBride OW, Morgan R, Smith SD, Sandberg A, Kirsch IR. Characterization of the breakpoint of a t(14;14) (q11.2;q32) from the leukemic cells of a patient with T-cell acute lymphoblastic leukemia. Cancer Genet Cytogenet 44:47-54, 1990.

3. Kirsch IR, Aplan PA, Begley CG, Bertness V, Felix C, Lipkowitz S, Nakahara K, Nussmeier M, Reynolds L, Siebel N. Stern M-H, Tchorz K. Abnormal antigen receptor formation in normal, ataxia-telangiectasia, and malignant lymphocytes. In the Molecular Basis of Immune Disease. Miami Biotechnology Winter Symposia IRL Press/Oxford University Press, pp. 22-24, 1990.

4. Aplan PA, Begley CG, Bertness V, Ezquerra A, Nussmeier MA, Coligan JE, Kirsch IR. The SCL gene is formed from a transcriptionally complex locus. (submitted)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM 06581-07 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetics of Differentiation and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Michael Kuehl, M.D.	Senior Investigator	NCI-NMOB
Others:	Cynthia Timblin, Ph.D.	Staff Fellow	NCI-NMOB
	Francine Foss, M.D.	Ass Prof Med NCFI-USUHS	NCI-NMOB
	Leslie Brents	Res Assoc NCI-USUHS	NCI-NMOB
	Lief Bergsagel, M.D.	Medical Staff Fellow	NCI-NMOB
	Carol Kobrin, Ph.D.	Staff Fellow	NCI-NMOB
	Agnes Cuddihy	Visiting Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION
NCI, COP, DCT, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Our work continues to focus on two approaches to hematopoietic differentiation. First, we demonstrated previously that expression of a c-myc or c-myb transgene reversibly blocks terminal differentiation of a mouse erythroleukemia cell line. We are now using this system to test mutant c-myb transgenes so that we can begin to understand how c-myb affects proliferation and differentiation. Our long term goal remains the same, i.e. to understand the mechanisms which are responsible for the apparent inability of most malignant tumors to differentiate. Second, we have developed a general method for subtractive cloning by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. We have used this novel methodology to identify genes which are expressed in most murine plasmacytomas but rarely in B lymphomas. Thus far, we have identified two classes of genes having this property: 1) two genes are expressed in most plasmacytoma and pre-B lymphoma cell lines; and 2) five genes are expressed in most plasmacytoma cell lines but not in any of the 10 pre-B lymphoma lines examined. Our long term goal for this project is to identify the genes which determine the phenotypes of plasmacytomas and terminally differentiated plasma cells.

Molecular Genetics of Differentiation and Transformation

Overall Objectives:

1. To clarify the cellular and molecular mechanisms which determine and regulate hematopoietic differentiation.

2. To clarify the relationship between differentiation and malignancy.

Species Studied: Mice and humans.

A. Role of c-myc and c-myb oncogenes in hematopoietic differentiation.

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
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	Cynthia Timblin, PhD	Staff Fellow	NCI-NMOB
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Endogenous c-myc and c-myb levels decrease biphasically when murine erythroleukemia (MEL) cells are induced to differentiate with various chemical inducers. By introducing a vector with an inducible metallothionein promoter and either a c-myc or c-myb cDNA coding region into MEL cells we are able to reversibly block differentiation by addition (and subsequent removal) of nontoxic levels of $ZnCl_2$ to the medium. The results are identical for both nuclear oncogenes. First, up-regulation of the transgene restricted to the first day or two of chemical induction has essentially no effect on the fraction of cells which can terminally differentiate when the heavy metal is removed from the medium; in fact, there is little effect if the up-regulation of the transgene continues for a week or more in the presence of a chemical inducer before removing the heavy metal Second, up-regulation of the transgene at any time during the induction process prevents the further accumulation of terminally differentiated cells. Third, the early down-regulation of endogenous c-myc and c-myb mRNAs is unchanged but the late down-regulation of the endogenous c-myc and c-myb mRNAs does not occur when cells are prevented from terminal differentiation by continuous expression of the transgene. This result suggests that the initial down-regulation of the endogenous c-myc and c-myb mRNAs is not required for terminal differentiation, and also that the second phase of down-regulation of these mRNAs is a consequence rather than a cause of terminal differentiation. It also underscores our present ignorance regarding the mechanism(s) by which continuous expression of exogenous c-myc or c-myb block terminal differentiation in this model system.

We have recently constructed and tested three c-myb mutants for their ability to block the terminal differentiation of MEL cells. Deletions of 5' or 3' sequences comparable to the deletions present in avian v-myb do not affect the ability of the c-myb gene to block terminal differentiation of MEL cells. In contrast, an extensive 5'deletion, which removes two of the three 52 amino acid repeats in the "DNA binding" region of the protein, results in expression of a

protein which does not block terminal differentiation of MEL cells. These results suggest that highly conserved regions at the 5' and 3' end of the coding region are not required for this inhibitory activity, but that the binding of c-myb to myb responsive elements (i.e. MRE) in the genome is required. We are just beginning to analyze deletion mutants lacking regions of the protein required for transcription activation or repression of transcription.

We have also shown that transfection of a c-myc cDNA into an IL3-dependent murine myelomonocytic precursor cell line results in cells which have lost the ability to terminally differentiate in differentiation medium containing G-CSF and lacking IL3, although the cells demonstrate an increased ability to survive without IL-3 in this same medium.

Our long term goal is to determine how c-myc and c-myb regulate proliferation and differentiation in normal and malignant hematopoietic cells.

Publications:

1. Kuehl, W.M. The use of an expression vector with a metallothionein inducible promoter to assess the effects of c-myb and c-myc on terminal differentiation of mouse erythroleukemia cells. In: Mechanisms of B Cell Neoplasia. Melchers, F. and Potter, M. (eds.) The Basel Institute Immunology. pp. 267-273. 1989.

2. McClinton, D., Stafford, J., Brents, L., Bender, T.P., and Kuehl, W.M.: Differentiation of mouse erythroleukemia cells is blocked by the late up-regulation of a c-myb transgene. Molec Cell Biol 10: 705-710, 1990.

ZO1 CM 06581-07 NMOB

B. Identification of genes involved in differentiation by subtractive cDNA cloning.

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Cynthia Timblin,Ph	Staff Fellow	NCI-NMOB
	Leif Bergsagel, MD	Medical Staff Fellow	NCI-NMOB
	Carol Kobrin, PhD	Staff Fellow	NCI-NMOB
	Leslie Brents	Res Assoc NCI-USUHS	NCI-NMOB

We have developed a novel and general method for subtractive cloning by incorporating polymerase chain reaction (PCR) technology into the preparation and analysis of subtractive cDNA libraries. Potentially this method can also be applied to subtractive libraries made from genomic DNA. We are continuing attempts to improve this method so that we can "re-subtract" a subtractive insert which contains PCR linkers; this would enable us to use solution hybridization to select subtractive inserts (or probes) which are expressed in a variety of cells but not in other cells.

Using this novel method of subtractive cDNA cloning, we have prepared a mouse plasmacytoma (MPC11) minus mature B lymphoma (A20.2J) subtractive cDNA library. Random selection of 115 clones has identified 16 quantitatively subtractive and 39 qualitatively subtractive clones. From these clones, we have identified 8 potentially interesting genes. One quantitatively subtractive clone identifies an mRNA that is expressed in most plasmacytoma cell lines, but is expressed at an approximately 10-fold lower level in a small fraction of B and pre-B lymphoma

cell lines; preliminary evidence suggests that the expression of this gene is increased by IL-6. From the 31 unrelated qualitatively subtractive clones, we have identified two classes of genes that are expressed in one or none of 8 B lymphomas examined: 1) two genes are expressed in most plasmacytomas and pre-B lymphoma cell lines; and 2) five genes are expressed in most plasmacytoma cell lines, but not in any of the ten pre-B lymphomas examined. The five genes described above represent the first known genes which are expressed in plasmactyomas but not in B lymphoid tumors representing earlier stages of differentiation. As a first step in characterizing these genes, we are presently sequencing all five plasmacytoma specific genes plus the one quantitatively subtractive gene mentioned above. We estimate that we have identified approximately 30% of the different qualitatively subtractive genes in our subtractive library. Thus we are continuing to look for other genes expressed uniquely in plasmacytomas.

Our long term goal is to identify the genes which are critical in determining the plasmacytoma phenotype; this may include some genes which are important in determining the terminally differentiated plasma cell phenotype and perhaps other genes which are required for the malignant transformation to plasmacytoma cells.

Publications:

1. Timblin, C.R., Battey, J., and Kuehl, W.M.: Application of PCR technology to subtractive cDNA cloning: Identification of genes expressed specifically in murine plasmacytoma cells. Nucleic Acids Res 18: 1587-1593, 1990.

2. Timblin, C.R., Bergsagel, P.L., and Kuehl, W.M.: Identification of consensus genes expressed in plasmacytomas but not B lymphomas. Current Topics Microbiol Immunol, In Press.

C. Other publications.

1. Lawler, A.M., Kearney, J.F., Kuehl, M., and Gearhart, P.J.: Early rearrangements of genes encoding murine immunoglobulin kappa chains, unlike genes encoding heavy chains, use variable gene segments dispersed throughout the locus. Proc Natl Acad Sci 86: 6744-6747, 1989.

2. Briskin, M., Damore, M. , Law, R., Lee, G., Kincade, P.W., Sibley, C.H., Kuehl, M., and Wall, R.: LPS-unresponsive mutant pre-B cell lines blocked in NF-kB acitvation. Molec Cell Biol 10: 422-425, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06587-06 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters 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)*

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	Stan Lipkowitz, MD	Instructor of Medicine	NCI-USUHS
	Kenneth Nakahara	Biologist	NCI-NMOB
	Nita Seibel, MD	Guest Researcher	NCI-NMOB
	Marc-Henri Stern	Guest Researcher	NCI-NMOB
	Kathryn Tchorz	SRTP	NCI-NMOB

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Acquired Gene Rearrangements Section

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	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 studied in normal, "premalignant", and malignant tumors and cell lines.

A. Our focus this year has been a study of the mechanism of formation of hybrid interlocus Ig and TCR genes. We have shown that hybrid genes are formed by site specific recombination between variable segments from one locus (for example TCR Y) and joining segments from another (TCR β). We have demonstrated that such events occur in the peripheral T-cell of all normal individuals but are 100 times more frequent in the peripheral T-cell of patients with ataxia-telangiectasia (AT). These hybrid genes 1) affect and alter the repertoire of immune receptor (Ig and TCR) 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.

B. RNA-RNA tissue in situ hybridization. The expression of individual cells within tissue sections from lymph node biopsies and peripheral blood from patients with lymphoid malignancies have been analyzed with immunoglobulin, T cell receptors, and oncogene probes. This technique refines the analysis of such tissue to the point where the unique gene expression of one cell in hundreds of thousands can be identified. Futhermore we have developed a technique of direct RNA sequencing the allows us to utilize tumor specific gene expression as a tumor specific marker providing a potentially powerful and sensitive means of cancer diagnosis and staging.

C. Gene and transcript mapping. We have localized numerous genes of interest to specific regions of human chromosome by in situ hybridization. Refinement of this technique using biotinylated probes is increasing the sensitivity and specificity of our work and allows us to study genotopography in interphase nuclei.

PROJECT DESCRIPTION

Gene Rearrangements as Tumor Specific Markers

Professional Staff:

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	Stan Lipkowitz, MD	Instructor of Medicine	NCI-USUHS
	Kenny Nakahara	Biologist	NCI-NMOB
	Nita Seibel, MD	Guest Researcher	NCI-NMOB
	Marc-Henri Stern, MD	Guest Researcher	NCI-NMOB
	Kathryn Tchorz	SRTP	NCI-NMOB

Objectives: Long Term

1. To develop, master, and refine techniques based on molecular genetics which are of direct current application in the prevention, early diagnosis, classification, and staging of patients with cancer.

2. To demonstrate the usefulness of these techniques in pilot studies.

3. To promote the adoption of these techniques by service oriented laboratories, and supervise the implementation of such techniques in a standardized quality controlled fashion for comprehensive, prospective, best available therapy protocols and epidemiological studies.

4. To use the data generated in the pilot and/or comprehensive analyses as a resource of information and a bank of material for further studies to be carried out in basic research laboratories.

Methods Employed:

As a result of the normal functionally activating recombination events that occur in B and T lymphocytes, the structural configuration of the DNA around the immunoglobulin or T cell receptor loci is irreversibly altered in each differentiated cell. Because of the unique nature of the antigen-receptor molecules being produced, the VJ or VDJ recombinatorial event (and DNA configuration) in one cell making one antibody or T cell receptor will be distinct from a cell making an antibody or T cell receptor with a different antigen-binding capacity. Thus, by the necessity of its role in the immune response, every lymphocyte beyond a certain stage in its development carries within it a unique molecular DNA "fingerprint".

Chromosome In Situ Hybridization and Gene Mapping

Our group was one of those early involved in the localization of genes in the human genome by the technique of chromosome *in situ* hybridization following the technique first developed by Harper and Saunders. Since our first effort localizing the Ig heavy chain locus to 14q32, we have conducted numerous other studies. These studies have included the localization of c-*myc* to 8q24, the localization of the beta globin cluster to 11p15 (in collaboration with Dr. Cynthia Morton) the localization of L-*myc* to 1p32 and of an amplified c-*myc* to an HSR in a small cell lung cancer cell line (in collaboration with Dr. John

Minna and Dr. June Biedler). We have localized the human beta 1-4 galactosyltransferase to 9p13 (within the same chromosome band as is found in the gene defective in the disease galactosemia). In collaboration with Dr. Tak Mak we localized the gene for the TCR alpha chain locus to 14q11.2. In collaboration with Dr. James Battey we localized the gene for human gastrin releasing peptide to 18q21. This was the same site to which we, in collaboration with Dr. Stan Korsmeyer and others, had localized the bcl-2 gene. We have also utilized this technique as part of our molecular genetic analyses of chromosomal aberrations including the human T cell lymphoma line SUP-T1, a human myeloma cell line H929, two patient samples carrying a t(14;14), one patient with a t(1;14) in his leukemic cells, one with a t(X;14) lymphoproliferation, and one with Ewing's sarcoma and a t(11;14;22) complex translocation. We are now mastering the technique of chromosome in situ hybridization using biotinylated probes. Recently this method has increased the sensitivity and specificity of this procedure allowing for more rapid gene mapping and even the mapping of genes or transcripts in interphase nuclei.

Recently a new technique, "polymerase chain reaction" or "PCR", has been developed that in certain conditions allows the identification of genomic rearrangements present in tumor samples at much less than 0.1% and also greatly increases the ability to identify particular mRNAs (or their corresponding cDNAs) of interest.

Major Findings: Hybrid Gene Formation

We studied hybrid antigen receptor genes formed by interlocus recombination between immunoglobulin (Ig) and T-cell receptor (TCR) loci as a model of translocation in lymphocytes. We had previously demonstrated site-specific V-J recombination between different genes of the Ig and TCR families, resulting in chromosomal abnormalities in malignant and non-malignant cell lines. Here, using polymerase chain reaction (PCR), we demonstrated hybrid genes formed by interlocus recombination between TCR γ variable (V) regions and TCR β joining (J) regions in the peripheral blood lymphocytes (PBL) from normal individuals and patients with ataxia-telangiectasia (AT). Sequence analysis of the PCR derived hybrid genes confirmed that site-specific Vγ-Jβ recombination had occurred. 39% of the genomic hybrid genes sequenced maintained a correct open reading frame. By dilution analysis, these hybrid genes were 70 fold more frequent in AT PBL than in normal PBL. We are currently investigating whether this increase is a result of an overactive or aberrant recombinase or rather reflects an increased accessibility of these loci to the recombinase. We also demonstrated expression of these hybrid genes by PCR analysis of RNA from both normal and AT PBL. Sequence analysis of the transcripts showed that, in contrast to the genomic hybrid genes, 86% of the expressed genes maintained a correct open reading frame at the V-J junction that would allow translation into a potentially functional hybrid TCR protein. Another type of hybrid TCR transcript was found among the expressed products where a rearranged TCR γ V-J exon was correctly spliced to a TCR β constant region. This form of hybrid TCR gene may be formed by trans-splicing. These hybrid TCR genes may serve to increase the repertoire of the immune response. In addition, studies of their mechanism of formation and its misregulation in AT may provide insight into the nature of the chromosomal instability syndrome associated with AT, and insight into the formation of the translocations associated with malignant lymphocytes.

Family Studies

Insertions, deletions, amplifications, and point mutations of genes occur in DNA throughout the evolutionary process. When these events occur in germline DNA, they are transmitted vertically from one generation to the next. Differing mutation sites and mutation rates between species and within a given species can be identified by comparison of defined populations by restriction enzyme site patterns for given probes of interest. This study of "restriction fragment length polymorphisms" ("RFLPs") reflecting the structural variability of DNA can be utilized in evolutionary, population, and family studies. Unlike the rearrangements of Ig or TCR genes, these RFLPs need not indicate the programmed rearrangement of a particular locus during the ontogeny of a particular cell type. Molecular genetic technology of DNA analysis can still be applied to this study. In what follows we describe one such example of RFLP analysis in which we have been involved. Its significance to the occurrence of a particular tumor in a particular family remains to be seen. Conceivably it could provide a marker for disease risk in this case. It would also be suggesting a mechanism of tumor formation akin to those now postulated, for example, for Wilm's tumor or retinoblastoma. We investigated a family in which a father and three of his offspring had meningioma with clinical onset at the ages of 35 to 65 years. A fourth offspring died of multiple neoplasms arising at 29 years. No one in the family had any evidence of von Recklinghausen disease. The three siblings with meningioma carried a constitutional Robertsonian translocation, t(14;22) (14qter cen 22qter), in peripheral blood leukocytes. Three other members of the second generation who were beyond the age at which the onset of meningioma is expected had no tumors and had normal karyotypes. In the third generation, whose members are now reaching the age for tumor onset, four carriers of the translocation have been identified; to date they are all asymptomatic except for one woman, who has breast cancer.

Both living members with meningiomas had a polymorphic variant of the c-sis oncogene in peripheral-leukocyte DNA, according to analysis with the Southern blot technique. This variation was also present in one asymptomatic member of the third generation and segregates with the morphologically normal No. 22 chromosome in both the affected and nonaffected members. It is possible that an "active" or mutant gene on the long arm of chromosome 22, possibly even the c-sis oncogene itself may be associated with the development of meningiomas in this kindred. Analysis of the meningioma tumor tissue itself would be of great interest in this regard but is presently not available. Sporadic meningioma is known to often to be associated with monosomy of chromosome 22 or less frequently the absence of the long arm of chromosome 22. The c-sis proto-oncogene has been localized to the tip of the long arm of chromosome 22. In collaboration with Dr. L. Ratner at Washington University, St. Louis, it has been determined that this polymorphism is the result of deletion of alu sequences in the 5th intron of the c-sis gene.

Tumor Genotyping Service

The establishment of a service facility for processing of tissue samples, and DNA and RNA preparation. The capacity to comprehensively screen selected patient populations or tumor samples for the rearrangement and/or expression of particular genes of interest. We are interested in maintaining a supervisory and quality control role over the activities of this laboratory. Envisioned is a NIH wide facility with a board composed of members from different institutes meeting to decide what questions should be addressed and to review the data being generated.

Publications:

1. Felix CA, Poplack DG, Reaman GH, Steinberg SM, Cole DE, Taylor BJ, Begley CG, Kirsch IR. Characterization of immunoglobulin and T-cell receptor gene patterns in B-cell precursor acute lymphoblastic leukemia of childhood. Blood, submitted.

2. Stern M-H, Lipkowitz S, Aurias A, Griscelli C, Thomas G, Kirsch IR. Inversion of chromosome 7 in ataxia-telangiectasia is generated by a rearrangement between T-cell receptor beta and T-cell receptor gamma genes. Blood 74: 2076-2080, 1989.

3. Lipkowitz S, Stern M-H, Kirsch IR. Hybrid T-cell receptor genes formed by interlocus recombination in normal and ataxia-telangiectasia lymphocytes. J Exp Med, in press.

4. Smidt M, Kirsch IR, Bigner SH, Ratner L. Deletion of alu sequences in the 5th c-sis mitron in individuals with meningiomas. J Clin Invest, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06589-06 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Biology, Growth, and Chemosensitivity Testing.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
Others:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Daniel Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Chief	NCI-NMOB

COOPERATING UNITS *(if any)*

Medicine Branch, Surgery Branch, Radiation Oncology Branch, Laboratory of Molecular Biology

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

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

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

E. Mutations of ras genes are a characteristic of a subset of NSCLC, but are not found in SCLC.

GPO 914-918

Objectives:

One of the major objectives of this Branch is to develop newer, more rational therapies for the cancer types representing our major clinical interests. By studying the biology of specific cancers in depth, new ideas for cancer control are generated, tested in vitro and brought to clinical trial. We presume that such an approach is more likely to advance in the therapy of refractory tumors such as colon and non-small cell lung cancer (NSCLC) than the development of new non-targeted cytotoxic agents. Further, a comprehensive knowledge of the biology of a cancer type can aid the physician in interpreting certain clinical phenomena such as hormone secretion, tumor progression, etc. Finally, identification of tumor markers may aid diagnosis, staging, detection of relapse, imaging, sub typing, and monitoring response to therapy.

Three of the currently active clinical protocols for the therapy of SCLC and NSCLC depend on the selection of individualized patients' chemotherapy by in vitro drug sensitivity testing. Thus one of the major objectives of the Branch is to develop methods to 1) amplify tumor cells so that adequate numbers are available for testing; 2) develop and apply rapid, accurate, reproducible testing procedures; 3) demonstrate the clinical relevance of the testing procedures; and 4) utilize in vitro testing for biological and preclinical studies.

Major Findings:

Differentiation in Lung Cancer Tumors and Cell Lines

We have noted previously an increased incidence of adenocarcinoma in our non-SCLC (NSCLC) protocol patients (63% of all NSCLC). We also noted that peripheral adenocarcinomas with some or all of the features of bronchioloalveolar (BAC) carcinomas appeared to be common (about 50% of all adenocarcinomas, or about 30% of all NSCLC tumors).

N-CAM, an important neural adhesion molecule, is expressed concordantly in lung cancer cell lines with neuroendocrine properties. All N-CAM positive lines lack substrate adherence, and grow as floating cell aggregates, a feature characteristic of several neuroendocrine and neural cell lines (Gazdar, Linnoila, Carbone).

We studied lung cancer tumors and cell lines for expression of CEA and the related genes NCA and BGP. Normal lung has abundant NCA, but relatively little CEA or BGP. All three genes are expressed, but discordantly, in lung cancer cell lines. Cell lines expressing neuroendocrine features have a much higher expression of CEA RNA and protein than other lung cancers. Identification of the precise family member expressed in lung cancers may be of diagnostic importance (Kim, Kaye, Gazdar).

In Vitro Drug Sensitivity Testing and Clinical Correlations

The Weisenthal dye exclusion assay is used to test clinical specimens from patients entered onto therapeutic trials for lung cancer. The largest and best studied base currently available is from the Extensive Stage small cell lung cancer protocol, #83 13 (also see report by Dr.D.Ihde). We have extended these studies to permanent cell lines established from patients on this study, suing the MTT dye assay. Even after a mean culture time of 29 months, in vitro testing was predictive for clinical response and survival.

The Role of the Topoisomerase Genes in Human Cancers

We correlated expression of topoisomeraseI and II genes and drug resistance in a panel of 20 lung cancer cell lines. All cell lines expressed both genes, but there were considerable variations between individual lines. Approximately 10% of the lines had rearrangements of one of the genes. In 7/8 lines studied in greater detail, there was an excellent correlation between in vitro chemosensitivity and topoII expression, but not with topoI expression (Giaccone, Gazdar).

The Role of ras Gene Mutations in Lung Cancer

ras genes in primary lung cancers (mainly K-ras at codon 12) have been associated with a subset of adenocarcinomas having a poor prognosis. We investigated 105 lung cancer cell lines, and found codon 12 ras mutations (all K-ras) in about 20% of lung cancer lines. Unlike other studies, the mutations were not limited to adenocarcinoma, but occurred with equal frequency in all forms of adenocarcinoma. There were similar incidences in cell lines initiated from primary or metastatic tumors. No mutations were found in any of 37 small cell lung cancer cell lines. Mutation of K-ras at codon 12 define a subset of non-small cell lung cancer, but not small cell lung cancer. Cell lines having ras mutations did not have different chemosensitivity profiles than cell lines lacking mutations. Thus, ras mutations in NSCLC are not associated with metastases or with increased chemoresistance. (Mitsudomi, Viallet, Minna, Gazdar).

Establishment and characterization of a steroid secreting adrenocortical carcinoma cell line

A unique human cell line has been established from a patient with adrenocortical carcinoma. Mass spectrometry studies indicate that even after 7-10 years in culture the cell line continues to secrete about 35 steroid hormones, representing all of the major pathways of adrenal steroid production (glucocorticoids, mineralocorticoids and sex hormones). These studies indicate that all of the important p450 enzymes involved

in adrenal steroid synthesis are present. The line, which is being patented, should be invaluable for studying steroid hormone synthesis and its regulation (Gazdar, Oie).

Mutations of the p53 gene in Gastric Cancers

We found mutations of the p53 gene in 1/18 gastric tumors (6%) and 3/7 (43%) gastric cell lines. Because the tumors were primary lesions while most of the cell lines were from metastatic lesions, mutations of p53 may be associated with metastases in gastric cancer (Kim, Takahashi, Minna, Gazdar).

Future Studies

We are performing an analysis of the typing of NSCLC worldwide. Sites to be analyzed will include co-operating institutions in North America, Europe and Japan. These studies will be complemented by more limited correlations between histopathology and ultrastructure and immunohistochemistry. By these techniques we will be able to confirm our light microscopic observations regarding the increase in adenocarcinomas, and its BAC subtype.

We will continue to develop and evaluate molecular and biochemical markers for differentiation in all forms of lung cancers, for diagnostic, prognostic and therapeutic applications.

We will continue our current clinical protocols for SCLC and NSCLC based on in vitro selected therapy. Data will be correlated with the patients' responses. Preclinical studies will include testing of phase I and II drugs and correlating in vitro predictions with clinical results.

As amplification and over-expression of the myc gene family are relatively common in lung cancer, especially previously treated SCLC, we will continue to study the relationship between oncogene expression and in vitro chemosensitivity and radioresistance. We will test both lung cancer cell lines as well as rat cell lines transfected with various oncogenes. We will extend our studies with ras genes to tumor samples from three continents, and correlate the data with clinical findings.

Disease oriented panels of cell lines will be used to test potential and actual phase I and phase II agents, both for correlation of in vitro results with clinical response and for the selection of agents to test in future phase I trials in NSCLC.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06594-05 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Molecular Genetic Events in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	John Brennan, MD	Instr Med NCI-USUHS	NCI-NMOB
	Yoshi Osaki, MD	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

Hao-Chia Chen, Ph.D., Section Chief, Endocrinology and Reproduction Research Branch, NICHHD

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Oncopeptides

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.2	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.)*

A. We have recently reported atrial natriuretic peptide mRNA expression and immunoreactivity in tumor and tumor cell lines from small cell lung cancer patients with hyponatremia who did not produce arginine vasopressin (4). High pressure liquid chromoatography (HPLC) analyses of the tumor cell lines and tumors from patients with hyponatremia and mRNA expression of atrial natriuretic peptide have revealed that intracellular and extracellular peptide appears to be the 28 amino acid form of atrial natriuretic peptide, the form that normally circulates in human plasma. These studies are the first to characterize the ectopic production of atrial natriuretic peptide in small cell lung cancer patients and may have identified the third factor (natriuretic factor) that has been hypothesized in the syndrome of inappropriate antidiuretic hormone (SIADH).

B. We reviewed the clinical course of 234 lung cancer patients. In contrast to none of the 123 non-small cell lung cancer (NSCLC) patients, 18 of 111 (16%) small cell lung cancer patients had hyponatremia . Ten of these 18 had tumor cell lines available and 8 expressed ANF mRNA, 5 expressed AVP mRNA, and 3 of 10 cell lines produced both ANF and AVP mRNA. All of the 10 cell lines produced ANF mRNA, AVP mRNA, or both. Studies of 10 tumor cell lines from the 93 SCLC patients without hyponatremia showed 4 produced ANF mRNA and none produced AVP mRNA. From these studies we have observed that all tumor cell lines studied from SCLC patients with hyponatremia produce ANF mRNA or AVP mRNA, or both. Atrial natriuretic peptide may be the previously hypothesized third factor and play an important role in the pathogenesis of hyponatremia in some patients with SIADH.

PROJECT DESCRIPTION

Molecular Genetic Events in Lung Cancer

Professional Staff:

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	John Brennan, MD	Instr Med NCI-USUHS	NCI-NMOB
	Yoshi Osaki, MD	Guest Researcher	NCI-NMOB

Collaborating Branches:

Hao-Chia Chen, Ph.D., Section Chief, Endocrinology and Reproduction Research Branch, NICHHD

Objectives:

1. Study tumor cell lines and tumors from patients with SCLC and hyponatremia for evidence of ectopic AVP or ANF production and correlate this with the patients fluid and electrolyte status.

2. Study tumor cell lines and tumor from patients with small cell lung cancer and hyponatremia that express ANP mRNA to determine the peptide structure and bioactivity.

Major Findings:

Tumors and Tumor Cell Lines from Patients with Small Cell Lung Cancer and SIADH Produce Ectopic Atrial Natiruretic Factor

Atrial Natiurectic Peptide Expression and Peptide Studies

Hyponatremia in patients with small cell lung cancer can be caused by tumor production of arginine vasopressin (AVP) resulting in the syndrome of inappropriate antidiuretic hormone (SIADH). However, AVP peptide has not always been present in analyzed tumor specimens. We therefore examined tumors and tumor cell lines from 5 patients with small cell lung cancer and SIADH for AVP mRNA.

The tumors and tumors cell lines from 5 patients with small cell lung cancer and the syndrome of inappropriate antidiuretic hormone (SIADH) have been studied for production of arginine vasopressin (AVP) mRNA production. Two of 5 patients with SIADH made AVP mRNA by Northern blot analysis and S1 nuclease analysis. Three of the 5 make atrial natriuretic peptide (ANP) by Northern blot analysis and S1 nuclease analysis. Atrial natriuretic peptide expression has also been confirmed by RNase protection assay. High pressure liquid chromoatography analyses of the tumor cell lines and tumors from patients with hyponatremia and mRNA expression of atrial natriuretic facotr have have revealed immunoreactivity that elutes with the 28 amino acid form of atrial natriuretic peptide, the form

that circulates in human plasma. Therefore, it appears that the 28 amino acid form of atrial natiuretic peptide is the intracellular form and is also the form of the peptide that is secreted into the media by small cell lung cancer cells that express atrial natriuretic factor mRNA.

Studies of Patients with Lung Cancer.

In order to extend our observations to a large group of lung cancer patients, we retrospectively reviewed the records of 234 lung cancer patients treated at the NCI-Navy Medical Oncology Branch from November 1977 to July 1988. Eighteen of 111 (16%) SCLC patients had hyponatremia (serum sodium $\leq$ 130 mmol/L), compared to 0/123 (0%) non-small cell lung cancer (NSCLC) patients. Of the 18 SCLC patients with hyponatremia, 10 had tumor cell lines available for RNAse protection assays for ANF mRNA and AVP mRNA. Eight expressed ANF mRNA, 5 expressed AVP mRNA, and 3 of 10 cell lines produced both ANF mRNA and AVP mRNA. All of the 10 cell lines produced ANF mRNA, AVP mRNA, or both. We selected 10 tumor cell lines from the 93 SCLC patients without hyponatremia to serve as controls. Four of the 10 cell lines produced ANF mRNA, and none produced AVP mRNA. We also selected 10 tumor cell lines from the 123 NSCLC patients. One of the 10 cell lines produced ANF mRNA, and none produced AVP mRNA. From these studies we have observed that all tumor cell lines studied from SCLC patients with hyponatremia produce ANF mRNA or AVP mRNA, or both, tumor cell lines from SCLC patients without hyponatremia may produce ANF mRNA, tumor cell lines from NSCLC patients do not commonly produce ANF mRNA or AVP mRNA.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06595-04 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on ona line between the borders.)*

Clinically Relevant Immunohistochemical Markers in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other prolessional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

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

A. Characterization of markers. In a retrospective study a comprehensive group of 113 lung cancers were tested for the immunohistochemical expression of 17 antigens using a sensitive avidin-biotin-peroxidase technique. Logistic regression analysis was used to separate tumors into the proper categories (SCLC and carcinoid tumors versus NSCLC) based on the immunohistochemical markers. As a result 95% of the tumors were correctly predicted using the cell counts and staining intensities of only six markers. The results suggested that 1) individual marker counts are not useful in tumor classification, 2) "specific" NE markers such as serotonin and neuropeptides bombesin, calcitonin, ACTH, vasopression, neurotensin are not useful, 3) the best NE markers are a panel of "general" NE markers (Chromogranin A, Leu 7, NSE) which are present in NE cells throughout the body.

B. Clinicopathologic correlation. This panel of "general" NE markers was applied to the non-SCLC cases on protocol 83-15 in our branch. Although the

those with negative NE markers. Moreover, patients with NE positive tumors developed metastases significantly earlier ($p2 < 0.027$).
The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. Immunohistochemistry provides a highly effective and specific technique to achieve this goal.

PROJECT DESCRIPTION

Clinically Relevant Immunohistochemical Markers in Lung Cancer

Professional Staff:

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

Objectives:

There are four major histological types of lung cancer, namely small cell lung cancer (SCLC) (25%), adenocarcinoma (25%), squamous cell carcinoma (30%) and large cell carcinoma (15%). For a number of biological and clinical reasons, these lung carcinomas may be divided into SCLC and non-SCLC (NSCLC) tumors. SCLC and the rare bronchial carcinoid express many neuroendocrine (NE) features including dense core granules by electron microscopy, high levels of the key amine producing enzyme L-dopa decarboxylase, and the glycolytic isoenzyme neuronspecific enolase (NSE) and hormone or neuropeptide production. SCLC unlike NSCLC is extremely sensitive for chemotherapy and radiation, and there are scattered reports on favorable responses to chemotherapy by "atypical endocrine" tumors of the lung. This knowledge together with the recently established NE markers has prompted us to explore if 1) the expression of neuroendocrine markers in NSCLC is associated with favorable response to chemo- or radiotherapy, and 2) if the degree of expression of neuroendocrine markers in SCLC correlates with clinical outcome. Immunohistochemical technique provides a readily applicable tool for this.

Methods Employed:

1. Tumors. A comprehensive group of 113 primary lung cancers was chosen from the archives of the departments of pathology at the Bethesda Naval Hospital and National Cancer Institute. In addition, tumor material was obtained also from the patients on NCI protocol 83-15. Serial sections from routinely processed paraffin blocks were used.

2. Antibodies. The application of immunologic techniques that use hormone markers has been hampered by the fact that tumors with similar histologic and cytologic features may produce a variety of immunoreative substances, and some tumors may synthesize more than one hormone. Recently, a mouse monoclonal antibody LK2H10 produced against human pheochromocytoma has been shown to be directed against chromogranin A (ChrA) a constituent of secretory granules in most peptide producing endocrine cells. The demonstration of chromogranin in lung tumors serves as a useful marker for a broad spectrum of lung tumors with NE features including SCLC and the rare bronchial carcinoid. Other general immunohistochemical markers for NE differentiation include monoclonal antibody Leu-7 (HNK-1). Leu-7 reactivity was originally identified in subpopulation of lymphocytes called natural killer cells and later noted to be present also in nerves and wide variety of endocrine cells. Antibodies to NSE also react with nerves and cells of the diffuse NE system and its tumors. The advantage of applying such general NE markers in that they provide a more uniform recognition for multiple NE tumors that may in turn synthesize a variety of specific products such as different hormones.

3. Immunohistochemical Staining. Staining was performed using the avidin-biotin peroxidase technique. Appropriate positive and negative controls were included in each assay. Results of the immunostaining were reviewed scoring both for the intensity of the staining and number of positive cells.

Major Findings:

1. Characterization of Markers. We were able to demonstrate that the majoritiy of cells in most SCLC and all carcinoid tumors were positive for the general NE markers and many hormones. Logistic regression analysis was used to separate tumors into the proper categories on the basis of markers and 95% the tumors were correctly classified applying a model created from staining indeces of general NE markers (ChrA, Leu 7, NSE). Evaluation of the expression of multiple markers revealed that 7/77 NSCLC had a staining pattern indistinguishable from SCLC.

We have concluded that 1) Application of the general NE markers produces acceptable classification of lung tumors; 2) Most but not all SCLC and carcinoids express multiple NE markers in a high percentage of tumor cells; 3) Occasional NSCLC show staining patterns indistinguishable from SCLC; 4) Many NSCLC contain a small subpopulation of cells expressing NE markers.

2. Clinicopathologic Correlation. The panel of "general" NE markers (ChrA, Leu7, NSE) was applied to the non-SCLC cases on protocol 83-15 ("Treatment of Non-Small Cell lung Cancer Utilizing In Vitro Drug Sensitivity"). Based on a detailed histopathological evaluation of tumor specimens of the patients already entered in the protocol it appears than in over 80% of the cases such an immunohistochemical analysis on untreated patient specimens

can be performed. Currently we have stained 101 of the 133 cases entered and in 20/98 (20%) non-SCLC at least two out of the three general NE markers were positive. The results of the first 80 cases are summarized in the following table as an example:

GENERAL NE MARKERS IN NSCLC BY HISTOLOGICAL TYPE
(80 CASES ON PROTOCOL 83-15)

(% positive)	Chr A	Leu 7	NSE
Adenocarcinoma	2/45 (4)	10/45 (22)	22/45 (49)
Large cell	6/19 (32)	3/19 (16)	9/19 (47)
Epidermoid	0/11 (0)	2/11 (18)	4/11 (36)
Other	0/2 (0)	0/2 (0)	1/2 (50)
TOTAL NSCLC	8/77 (10)	15/77 (19)	35/77 (45)
Carcinoid	3/3 (100)	3/3 (100)	3/3 (100)

The updated analysis of the response rate to chemotherapy in those 122 non-SCLC patients on protocol 83-15 in correlation with the results of immunohistochemistry revealed a rate 50% (4/8) in the patients whose tumors were positive for at least 2 out of 3 general NE markers versus 16% (6/34) in those with negative NE markers. There was also a strong correlation of the expression of immunohistochemical NE markers with other biochemical markers for NE differentiation, such as L-dopa decarboxylase levels in tumors. While there was no difference in survival between patients whose tumors were NE positive and other non-SCLC, patients with NE positive tumors developed metastases significantly earlier ($p2 < 0.027$).

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. There are at least 150,000 new cases of lung cancer (75% of which are non-SCLC) discovered annually. Our preliminary results support our hypothesis that non-SCLC which express NE features might be more responsive to cytotoxic treatment than other non-SCLC. Immunohistochemistry provides a highly effective and specific manner to screen for these tumors.

An important, practical aspect of this study is that it will provide a valuable archive of large patient material with well characterized clinical data. This enables statistically meaningful correlations allowing systematic evaluation of the biological significance of defined markers.

Proposed Course:

1. The expression of markers will be correlated to the clinical data including performance status, best response, and survival. At the end of the protocol 83-15 we should have accumulated results on 120 patients, if 150 patients are accrued as planned. This will provide a basic correlation.

2. Based on our initial observations we expect that 10-20% of non-small cell lung cancers express neuroendocrine markers. In order to extend the analysis and reach meaningful clinical correlations we have initiated a collaboration with the ECOG (Eastern Cooperative Oncology Group) and LCSG (Lung Cancer Study Group). ECOG and LCSG have full clinical response and survival information on nearly 2.800 treated patients. A large number of these have pretreatment tumor samples available for analysis. We plan to study the expression of general NE markers chromogranin, Leu 7 and NSE, as well as selected other markers, and relate this to tumor type, response to therapy, and survival. At the present time, we have stained 400 cases, and the interim analysis revealed that 12-16% of the cases were positive for at least two out of the three general NE markers, thus confirming our initial findings in independent tumor sets.

Our preliminary analysis of other common immunohistochemical tumor markers in the LCSG cases revealed that patients whose tumors lacked mucin, which is a secretory product of many adenocarcinomas, had an average 5 year recurrence free survival, while the most mucin positive cases had an average recurrence free survival of about one year. Also, patients with elevated tissue staining for carcinoembryonic antigen, CEA, in their tumors had a more favorable outcome.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06596-04 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

COOPERATING UNITS *(if any)*

Eli Glatstein, M.D., Tom Napier, M.D. Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Biological Chemistry Branch

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Oncopeptides and Clinical Investigations

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.6	0.6

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.)*

A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. Twenty-nine patients have been entered onto study and 19 of 26 (73%) patients who have completed therapy have achieved a complete remission. The projected median survival is greater than 2 years and 11 of 29 patients are alive and free of cancer progression with a median potential follow-up of 28 months. One patient has died from combined modality pneumonitis.

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

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

From this studies we conclude that the HTCFA has identified a compound with novel side effects, the maximum tolerated dose is 50 mg per square meter, and achievable plasma levels are much less than that required for in vitro activity.

PROJECT DESCRIPTION

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	John D. Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Eli Glatstein, M.D., Tom Napier, M.D., Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Biological Chemistry Branch

Objectives:

1. Determine the frequency with which adequate tumor tissue can safely be obtained and drug sensitivity data determined.

2. Determine the response rate, toxicity, and survival of limited stage small cell lung cancer patients treated with VP/PLAT, simultaneous twice a day chest radiotherapy, and chemotherapy based on in vitro drug testing or a standard regimen (VAC).

3. Determine the side effects and maximum tolerated dose of dihydrolenperone.

4. Determine the pharmacokinetics of orally administered dihydrolenperone.

5. Determine the activity of dihydrolenperone within the confines of a Phase I trial.

6. Determine the correlation between the in vitro determined activity of dihydrolenperone and the achievable plasma levels of dihydrolenperone.

Methods:

1. Small cell lung cancer patients undergo staging

2. Limited stage patients undergo surgical biopsy of tumor tissue

3. Induction with 12 weeks of VP-16/Plat with concomitant 150 RAD twice a day radiothearpy to 4500 RAD over 19 days.

4. Patients with in vitro drug sensitivity data receive an additional 12 weeks of the in vitro best regimen, patients with no in vitro data receive 12 weeks of a standard vincristine, doxorubicin, and cyclophosphamide regimen.

5. Patients are followed for survival and toxicity.

6. Small cell lung cancer patients failing conventional combination chemotherapy and non-small cell lung cancer patients for whom no curative therapies are available are identified for Phase I drug trial.

7. Patients are treated orally twice a day for 28 days with dihydrolenperone and observed for toxicity.

8. Patients with tumor tissue available have in vitro testing with DHLP

Major Findings:

The Limited Stage Small Cell Lung Cancer Trial Administering VP 16 and Cisplatin Plus B.I.D. Chest Radiotherapy has a Decreased Rate of Pulmonary Toxicity and the Preliminary Survival Data shows a Prolognation of Median Survival

Between 7/86 and 4/90, 29 patients (pts) with LTD stage SCLC were entered onto a combined modality study. 21 were male, 8 female, 3 were PS 0, 24 PS 1, and 2 PS 2. The median age was 57 (range 34-71). Pts were treated with VP 80 mg/m^2 d1,2,3,27,28,29, PLAT 80 mg/m^2 d1,27 with concurrent chest RT 150 cGY bid Mon-Fri d5-24. Patients then received 2 additional cycles of VP/PLAT followed by 4 cycles of either CAV or individualized chemotherapy based on in-vitro drug sensitivity testing. 26 pts have completed therapy and are evaluable for response. 19 had a CR and 7 a PR, overall 100%. Sixteen of 29 patients are still alive and 11 of those 16 have no evidence of tumor progression. The median potential follow-up is now 28 mo (range 1-48). The actuarial survival is 90% at 1 year and 60% at 2 years . One patient died of combined modality pneumonitis with no evidence of cancer at post mortem examination. This regimen is well tolerated, associated with minimal toxicity, and the pt survival data are encouraging.

Phase I Trial of Dihydrolenpoerone, A Novel Compound Active Against Lung Cancer Identified by the Human Tumor Colony Forming Assay

The phase I and pharmacokinetic study of dihydrolenperone was approved to enter patients in January of 1986. Thirty-two lung cancer patients have been entered to date and have completed 27 courses of dihydrolenperone. The initial dose was 10mg/m^2 orally twice a day for 28 days. The dosage has been escalated to 20mg/m^2, 30mg/m^2, 40mg/m^2, 50mg/m^2, 60mg/m^2 b.i.d. and has recently dropped back to 50mg/m^2 because of excessive toxicity. The prominent side effects have been somnolence and hypotension in all patients. Somnolence was the dose limiting toxicity. Six patients developed grade 3, 10 grade 2, and 15 grade 1 somnolence. The somnolence was reversible when the drug was discontinued. Four patients developed grade 2 and 14 developed grade 1 hypotension. The hypotension observed in the first two patients treated with 20mg/m^2 was 70/50 when the patients were standing. This prompted us to alter the loading schedule with dihydrolenperone so the dosage was increased 10mg/m^2 twice daily until the target dosage is reached. The hypotension with this schedule has been more tolerable. There was no significant hematologic, renal or hepatic toxicity.

Twenty-two different patients have completed at least one course of therapy and are available for analysis of response to therapy. Ten patients had progressive disease, 11 had stable disease, and 1 had a minor response.

The pharmacokinetic studies by Robert Parker and John Strong of the Biologic Chemistry Branch using HPLC have demonstrated that peak absorption occurs approximately 3-5 hours after the oral dosage with the peak achievable plasma levels of 50-200 ng/ml. The in vitro studies using an MTT assay showed dihydrolenperone inhibited 50% of the growth of lung cancer cell lines at concentrations of 25-165 ug/ml, more than 100 fold greater than the peak achievable plasma levels. We have completed the phase 1 trial and if the final plasma drug levels show that the achievable peak plasma levels are more than 100 fold less than the amount required for in vitro activity, the drug will be not be used for a phase II trial in lung cancer.

Publications:

1. Feld R, Abeloff M, Drings P, Gregor A, Jett JR, Johnson B, Maasilta P, Saijo N, Sorenson S, and van Zandwijk H. Consensus report on toxicity and supportive care for patients with small cell lung cancer held at the 3rd International Association for the Study of Lung Cancer Workshop on Small Cell Lung Cancer. Lung Cancer 1989; 5:146-151

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4. Johnson BE, Grayson J, Makuch RW, Linnoila I, Anderson M, Cohen MH, Glatstein E, Minna JD, Ihde DC. Ten year survival of patients with small cell lung cancer treated with combination chemotherapy with or without irradiation. J Clin Oncol 1990, 8:396-401

5. Dearing MP, Steinberg S, Phelps R, Ihde DC, Johnson BE. Effect of stage migration on prognostic factors in small cell lung cancer patients. J Clin Oncol 1990. 8:1042-1049

6. Ihde DC, Grayson J, Woods E, Gazdar AF, Edison M, Lesar M, Linnoila RI, Minna JD, Glatstein E, Johnson BE. Twice daily irradiation as an adjuvant to etoposide/cisplatin therapy of limited stage small cell lung cancer. In Adjuvant Therapy of Cancer, VI. Salmon SE, Editor. W.B. Suanders, Philadelphia. 1990; In Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06597-04 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Non Small Cell Lung Cancer Therapy Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Research Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB

COOPERATING UNITS *(if any)*
Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (J. Nesbitt); Radiation Oncology Branch, Surgery Branch, (R. Deming); Clinical Oncology Program

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Biotherapy

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

A primary objective of this Branch is to improve the state-of-the-art in the therapy of lung cancer. In the past, this Branch had focused this effort in the study of small cell lung cancer. With the advances both in the therapy of the small cell patients as well as in the study of small cell lung cancer biology, we decided to generalize the Branch effort to include the systemic evaluation of non-small cell lung cancer. The vehicle for this pilot study of the feasibility and value of using in vitro criteria to select therapy for patients with metastatic non-small cell lung cancer.

PROJECT DESCRIPTION

Non Small Cell Lung Cancer Therapy Project

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Research Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB

Collaborating Branches:

Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (J. Nesbitt); Radiation Oncology Branch, Surgery Branch, (K. Salem); Clinical Oncology Program

Objectives:

1. To improve therapy of non small cell lung cancer by selecting chemotherapy on the basis of in vitro analyses, both of drug sensitivity and neuroendocrine markers. To use that protocol as a vehicle for the in-depth study of small cell lung cancer biology.

2. Pilot study to evaluate if patients treated on the basis of their tumor cells' in vitro response to a panel of chemotherapeutic agents have more effective tumor cytoreduction than conventionally treated control patients or historic controls.

3. To determine if non-small cell lung cancer patients with tumors expressing neuroendocrine features characteristic if small cell lung cancer experience natural history more typical of small cell lung cancer.

4. To evaluate our ability to prospectively establish clinical specimens as long-term cell lines.

   a. Optimizing our ability to grow specimens, especially in developing serum-free media systems.

   b. Use the computerized clinical and laboratory data bases to correlate

Methods Employed:

1. Clinical trial

2. In vitro drug sensitivity analysis

3. Immunohistochemistry

4. Biochemical Marker analysis

5. Cell culture

Major Findings:

Since this study opened in April, 1984, over 100 patients have been accrued to this study. We performed an interim analysis on the study.

As a function of protocol design, all patients had tumor tissue come to the laboratory. In several instances, the tumor tissue non-viable due to immersion in formaldehyde, but excellent cooperation between surgeons and pathologists resulted in a better than 95% yield. In order to obtain tissue from as many patients as possible, we frequently obtain tissue from patients undergoing potential curative thoracic resection. We treat only those patients with metastatic disease that is measurable or can be evaluated. Of the 35 patients who already received chemotherapy on this protocol, tumor tissue arrived in the lab was of sufficient size and condition to do at least limited in vitro drug sensitivity analysis in 29%. Some patients have relapsed and died without any chemotherapy (5 patients) and many more are still followed without any evidence of recurrent disease (39 patients). We have established continuous cell lines on 23% of the patients we have evaluated. We project that the frequency of successfully performing in vitro analysis with our current approach may increase to 40% of the total prospective cases. Further refinements of this approach will be necessary to permit this approach to be more generally applicable and we will outline some of the research directions we are pursuing to accomplish this.

These cell lines are a very useful recourse in conducting further experiments to improve the frequency of successful drug sensitivity analysis. First, the initial cell lines derived in the course of non-small cell protocol are being used in validation of another technique of drug sensitivity analysis, the semi-automatic colorimetric assay. This work will be discussed elsewhere in this document, but there are two areas in which the work with this assay impacts on the non-small cell clinical trial. First, this assay requires significantly less operator time to perform, has a more objective mode of analysis, and ultimately may require a smaller number of tumor cells for analysis. Due to the efficacy of this technique, we might also be able to achieve the goal of testing combinations instead of single agents in vitro. For these reasons, we are motivated to substitute this assay for the dye exclusion assay, after we determine the degree of comparability between the two assays. Second, we have used this assay to examine the growth factor requirement of small cell lung cancer to optimize a serum-free media system for those cells. We are now ready to extend this approach to non-small cell tumors as it is apparent from our low rate of successfully generating tumor cell lines that our current media systems are suboptimal. Both of these adjustments, a more efficient in vitro assay and a more effective media system, have the potential of improving the biggest shortcoming of this approach, this is, increasing the percentage of cases that we can successfully test for drug sensitivity in vitro.

As discussed previously, the number of patients actually receiving the combinations of drugs selected by the assay as being most active (based on single agent activity) is small (8 patients). This number will increase since we plan to accrue another 50 patients and as more of the patients, who underwent potentially curative thoracic resection, develop recurrent disease. Nevertheless, the results of the in vitro analysis suggested their tumors would be minimally responsive. The eight predicted most active combinations resulted in only a half log of cell kill in vitro.

70% of the single agents tested with these 8 tumors were resistant by our arbitrary scale (resulting in less than 50% tumor cell kill). None of these patients had an objective tumor response, but their median survival was five months. The survival rate was equivalent to the patients who received empiric etoposide/cisplatin. Since we are still dealing with small numbers of patients, we have not evaluated the two groups for the equivalence of prognostic features, so it is too early to conclude anything about the utility of the in vitro drug selection to see if a trend emerges. This study, which is really a pilot effort, will not definitely answer the questions regarding the clinical value of in vitro drug sensitivity analysis, but it will provide a departure point for constructing subsequent clinical trials to further resolve such issues.

One of the most provocative directions explored in this study is the prospective evaluation of the fate of the subset of non-small cell lung cancer without biochemical features of small cell cancer. We have prospectively analyzed cell tissues obtained in this study for the expression of four biochemical features generally felt to be characteristic of small cell. Based on the previous retrospective work in characterizing these biochemical markers, expected this phenomenon would be present in about 15% of clear cut non-small cell lung cancers. Our hypothesis was that the patients with these tumors would respond to their treatments in a fashion similar to small cell lung cancer patients (i.e. a higher response rate). We were able to do at least one biochemical parameter on 71 of 81 adequate tumors (88%). 11% of these specimens had elevated levels of expression of at least one biochemical marker. Seven non-small cell lung cancer parts with neuroendocrine biochemical features were treated with a combination chemotherapy used extensively in the Branch for small cell lung cancer (cytoxan, methotrexate, CCNU, vincristine, adriamycin, procarbazine). The response rate has been 43% for those seven "neuroendocrine" patients versus 11% for the remaining 28 non-small cell lung cancer patients treated to date on this study with corresponding median survival rate of 9 months versus 6 months. Considerable work has been done with the in vitro characterization of these neuroendocrine non-small cell lung cancer cell lines, especially in regard to their in vitro drug sensitivity. This will be discussed elsewhere in this document.

Significance to Biomedical Research and the Program of the Institute:

Lung cancer is the leading cause of cancer mortality in our society. Non-small cell lung cancer which comprises 75% of all lung cancer is universally fatal once it has metastasized. Despite intensive clinical research, no major improvement has occurred in the treatment of disseminated non-small cell lung

cancer. To address this the NCI-Navy Medical Oncology Branch has attempted to integrate a systemic effort to study the biology of this cancer in conjugation with an attempt to optimize the best available treatment. This entails testing a patient's tumor tissue in the laboratory for its response to standard chemo therapy agents. Based on the in vitro result, a combination is constructed that represents the most cytotoxic single agents for a particular patient's tumor.

This approach potentially has general merit in attempting to specifically tailor available treatments to the unique biology of a patient's tumor. This approach also insures tumor tissue comes to our laboratory and is potentially available to be established as a continuous cell line. Over 30 cell lines have already been established in the course of this study and these lung cancer lines comprise an excellent model system for a variety of laboratory investigations.

Proposed Course:

Further accrual of patients to the ongoing protocol will continue. A successor protocol is being developed to replace this study. The new trial would evaluate a non-cisplatin-based combination for non-small cell lung cancer patients with advanced diseased. The laboratory basis for this drug combination was recently reported at the annual cancer meeting.

Independent validation of the enhanced initial responsiveness to chemotherapy of patients whose tumor empresses neuroendocrine differentiation is required to corroborate the preliminary clinical trial outcome. To accomplish this, collaborations have been developed with two cooperative groups to analyze for the expression of neuroendocrine features from tumor specimens obtained from patients already treated with chemotherapy. The goal would be examine if the correlation over neuroendocrine expression with enhanced responsiveness to chemotherapy. Further associated biological studies will also proceed.

Publications:

1. Gazdar AF, Tsai C-M, Park J-G, Ihde DC, Mulshine JL, Carmichael J, Mitchell J, Minna JD. In vitro assays for predicting clinical response in human lung cancer. In: Peters L, Chapman D, eds. Prediction of Tumor Treatment Response. New York: Permagon Press, in press.

2. Linnoila RI, Mulshine JL, Steinberg SM, Funa K, Matthews MJ, Cotelingham. Neuroendocrine differentiation in endocrine and non-endocrine lung carcinomas. Am J Clin Path 1988;90:641-652.

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9. Fridman R, Giaccone G, Kanomoto T, Martin FR, Gazdar AF, Mulshine JL. Lamin stimulates the attachment, the tumorgenicity and the drug resistance of small cell lung cancer cells. in press, Proc Natl Acad Sci, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06598-04 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Res Assoc Prof NCI-USUHS	NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	Barnett Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
	Ingalill Avis, RN	Biologist	NCI-NMOB
	Anthony M. Treston PhD	Guest Researcher	NCI-NMOB
	Francis Scott PhD	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Johns Hopkins (B. Eipper, M. Tockman)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Biotherapy

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The efforts of this Branch has been central to the recognition of gastrin releasing peptide as an autocrine growth factor for small cell lung cancer. Dr. Cuttitta developed a monoclonal antibody (2A11) to the active portion of that peptide and demonstrated that the immunoglobulin could block the mitogenic effect of GRP in vitro and in vivo. We have recently, in collaboration with Hybritech, Inc. (San Diego, CA), initiated a clinical trial to test whether one can control autocrine mediated malignant proliferation of small cell lung cancer using a monoclonal antibody. Our Branch has a long standing interest in the role of growth factors in cancer, so that information from 2A11 antibody clinical trial could be a foundation from subsequent anti-growth factor trials.

The phase I portion of the 2A11 antibody trial identified 250mg/m^2 on the monoclonal as the optimal dose. The Phase II portion of the 2A11 evaluation has recently started. We have previously reported the diagnostic application of lung associated monoclonal antibodies derived at this Branch for use in the early detection of lung cancer. We have patented the method for this approach with collaboration from John Hopkins and in conjunction with the Lung Cancer Study group will proceed to rapidly follow up on this critical area. We have followed up on this work with several publications including a report characterizing the fine binding affinity of one of the antibodies used for early lung cancer detection. The proposed follow up trial should start this year.

PROJECT DESCRIPTION

Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Res Assoc Prof NCI-USUHS	NCI-NMOB
	Daniel C. Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	Barnett Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
	Ingalill Avis, RN	Biologist	NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch, (E. Glatstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Johns Hopkins Medical School (B. Eipper, M. Tockman)

Objectives:

1. To study the pharmakinetics of monoclonal antibody delivery, attempting to maximize delivery of antibody to tumor involve sites.

2. To study methods of radiolabeled monoclonal antibody imaging as a staging tool in evaluating patients with cancer.

3. To determine if monoclonal antibody can be used to block growth factor stimulated tumor proliferation.

4. To study tumor cells to identify other growth factors which are potential targets for immunomolecular regulation.

5. To determine the utility of monoclonal antibodies as differentiation markers which have potential utility for the early detection of lung cancer.

Methods Employed:

1. Radionuclide Imaging

2. Immunohistochemistry/immunocytochemistry

3. Radioimmunoassay

4. Radioautography

Major Findings:

A. This Branch was involved in an early monoclonal antibody therapy trials in cutaneous T-cell lymphoma to determine if the monoclonal antibody could mediate cytoreduction by enhancing immune clearance of malignant T-cell. This trial failed to demonstrate significant therapeutic benefit, but did provide a vehicle for successful diagnostic imaging studies. The initial diagnostic imaging studies were developed by Paul Bunn, M.D., and continued by Dr. Mulshine. The intravenous delivery of In^{111} labeled T101 has resulted in the highest percent of tumor targeting achieved as of the time of its reporting. This localization efficiency was further therapeutic improved after regional delivery vial the lympahtics of subcutaneously delivered In^{111} labeled T101. Further studies included a comparison of quality imaging In^{131} T101 versus ^{111} T101. In this study, the In^{111} conjugate was clearly superior. This work now proceeds to further analysis of specificity of targeting by using an isotopically matched In^{111} T101-control antibody in sequential scanning studies with In^{111} T101. Information generated in the course of these studies include enhanced understanding of the pharmacology of antibody targeting, the immunogenicity of administered mouse immunoglobulin, and the efficiency if regional delivery techniques. These studies collectively provide the basis for proceeding with the radiolabeled T101 therapy trial which is discussed separately. Efforts have been productive, both in terms of published manuscripts and in developing useful collaborations with other investigators at the Clinical Center engaged in clinical research with monoclonal antibodies.

B. Small cell lung cancer has been extensively studied both at this Branch and elsewhere as a model of a neuroendocrine tumor. Small cell lung cancer has already been reported to produce over 25 different peptide hormone products. Recently, workers from our lab sequenced the gene for GRP from small cell lung cancer. A family of previously unknown peptides synthesized from open reading frames found on the GRP gene. Hetero anti-sera were developed to the three GRP gene associated peptides (G-Gap peptides). By several assays, immunologic evidence of expression of these three distinct products was documented in both small cell tumors and in fetal tissues. These facts suggest that despite the already known numbers of peptide products of small cell lung cancer, there may be a considerably larger number of small cell tumor products. With the rapid development of many areas of biotechnology, the techniques may now be available to begin systematically evaluating the total peptide synthetic capabilities of small cell.

C. We are interested in elucidating and additional new peptide products of small cell, we propose to focus on those peptide products that possess mitogenic capabilities. To facilitate this, we have invested considerable time in validating a semi-automatic colorimetric assay for evaluation of growth factor effects. The parameters to evaluation for such an application are considerably different than the conditions for the assay as reported by Carmichael and others from our Branch. The advantage of this assay is that it provides the exceedingly efficient assay to monitor for growth stimulatory effects, which will be essential when screening large numbers of purified fractions generated in typical HPLC purification efforts.

D. Using the semi-automatic colorimetric assay, we have already demonstrated the mitogenic effect of insulin-like growth factor-I (IGF-I) on small cell lung cancer cell lines. We have further demonstrated that this effect can be blocked by a monoclonal antibody specific for the anti-IGF-I receptor.

We have studied the biology of IGF-I in small cell and it appears to be an attractive candidate to target for a therapy approach similar to the anti-GRP monoclonal antibody trial. In thinking about GRP and a candidate for immunotherapy, the limited role this molecule plays in normal adult physiology potentially permits one to completely block this peptide effect without lethal consequences. The situation with IGF-I may not be similar as this molecule plays a more obvious role in normal adult physiology. Although that might not prevent us from exploring the same type of anti-autocrine factor strategy we employed in the anti-GRP trial, it did provoke us to consider approaches.

E. Many investigators have suggested that cancer can be thought of as a reexpression of normal embryonic and fetal developmental processes. An extrapolation is that autocrine type stimulation may be an important developmental mechanism. If so, such autocrine proliferation should be controlled through some signaling mechanism to allow for the uniform development of a fetus. In cancer, autocrine proliferation proceeds unabated either because of the regulatory signal. We tested to see if the stimulation of small cell lung cancer mediated be IGF-I could be inhibited by glucagon, a normal antagonist of IGF-I activity. Of interest, at a concentration of 10µg/ml, glucagon inhibits the growth enhancement of exogenous effect of IGF-I in other cell lines. In addition, we are attempting to define the mechanism mediating the inhibitory effect in the cell lines responsive to glucagon.

F. Work in early lung cancer detection using on immunocytochemical analysis continues and has been described in detail in the literature. The prospective confirmatory trial is due to begin in 1990.

Significance to Biomedical Research and the Program of the Institute:

These studies have two goals: First to complete the ongoing trial which represent a first effort to establish the clinical utility of monoclonal antibody based imaging and treatment approaches; Second, we have design ongoing in vitro analysis in conjunction with the clinical trials as well as other laboratory investigations to develop second generation biological trials which lend to more effective therapeutic control of malignant proliferation.

Proposed Course:

The Phase I component of the anti-GRP trial is complete and will be extended to Phase II at the 250mg/m^2 dose levels. Clinical trials with the other antibodies will also continue with the goal of moving to radionuclide conjugate therapy using monoclonal antibodies in cutaneous T-cell lymphoma and lung cancer. Further work will continue to develop a feasible approach to block IGF-I stimulation of lung cancer. Dr. Cuttitta is generating antibodies against synthetic peptides from various portions of prepro IGF-I and the IGF-I receptor. Using either an available reagent or Branch derived product, we will

do further in vivo work to block IGF-I stimulation. This work may lead us to a clinical trial in a similar fashion to the anti-GRP monoclonal antibody trial. Due to the frequent requirement of neuropeptides for alpha terminal amidation we have initiated a series of experiments to characterize the processing enzyme responsible for the event. Two of presentations at the national meetings summarize that work. The alpha amidation processing step may comprise a very critical regulatory even in the maturation of multiple lung cancer autocrine growth factors.

Publications:

1. Nakanishi Y, Cuttitta F, Kasprzyk PG, Avis I, Steinbergt SM, Gazdar AF, Mulshine JL. Growth factor effects on small cell lung cancer cells using a colorimetric assay: Can a transferrin-like factor mediate autocrine growth. Exper Cell Biol 1988;56:74-85.

2. Nakanishi Y, Mulshine J, Kasprzyk P, Natale RB, Maneckjee R, Avis I, Treston AM, Gazdar AF, Minna JD, Cuttitta F. Insulin-like growth factor I can mediate autocrine proliferation of human small cell lung cancer cell lines in vitro. J Clin Invest 1988;82:354-359.

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6. Tockman MS, Gupta PK, Myers JD, Frost JK, Baylin SB, Gold EB, Mulshine JL. Sensitve and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: A new approach to early lung cancer detection. J Clin Oncol 1988;6:1685-1693.

7. Mulshine JL, Avis I, Treston AM, Moblely C, Kasprzyk PG, Carrasquillo JA, Larson SM, Nakanishi Y, Merchant B, Minna JD, Cuttitta F. Clinical use of a monoclonal antibody to bombesin-like peptide in patients with lung cancer. Proceedings of the First International Symposium of Bombesin-Like Peptides. NY Acad Sci 1988;547.

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10. Cuttitta F, Fedorko J, Gu J, Lebacq-Verheyden AM, Linnoila RI, Battey JF. Gastrin releasing peptide gene associated petides (GGAPs) are ex ressed in normal human fetal lung and small cell lung cancer: A novel peptide family found in man. J Clin Endocrinol Metab 1988;67:576-582.

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14. Mulshine J, Avis I, Treston AM, Kasprzyk PG, Nakanishi Y, Mobley C, Carrasquillo JA, Larson SM, Merchant B, Cuttitta F. In vivo diagnosis and therapy of human tumors with monoclonal antibodies selection of antibodies and preliminary clinical studies in small cell carcinoma of the lung. Nucl Med Biol 1989;16:159-162.

15. Minna JD, Cuttitta F, Battey J, Mulshine J, Linnoila I, Gazdar AF, Trepel J, Sausville EA. Autocrine growth factors including gastrin releasing peptide (bombesin) as targets in the pathogenesis and treatment of lung cancer. In: DeVita V, Hellman S, Rosenberg S, eds. Important Advances in Oncology. Philadelphia: JB Lippincott Co, 1988;55-64.

16. Nakanishi Y, Cuttitta F, Kasprzyk PG, Treston AM, Avis I, Minna JD, Kleinman HK, Mulshine JL. The effects of growth factors on the in vitro growth of small cell lung cancer as determined in a colorimetric assay. In: Rosen S, Mulshine JL, Cuttitta F, Abrams PG, eds. Tumor Biology. New York: Marcel Dekker, 1988;59-84.

17. Mulshine JL, Kasprzyk PG, Nakanishi Y, Avis I, Seifter E, Cuttitta F. Considerations in the development of clinical trials employing monoclonal antibodies. Proceedings First International Workshop Small Cell Lung Cancer Antigens, Lung Cancer 1988;4:81-83.

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19. Mulshine JL, Tockman M, Smart C. Considerations in the developement of lung cancer screening tools. J Natl Cancer Institute 1989;81:900-906.

20 Kyogashima M, Mulshine J, Linnoila R, Jensen S, Magnani J, Nudelman E, Hakomori S, Ginsberg. Antibody 624H12, which detects lung cancer at early stages recognizes a sugar sequence in glycosphinogolipid difucosylneolactonorhexaosylceramide. Arch Biochem Biophys 1989;275:309-314.

21. Mulshine JL. Sputum cytology review: Editorial. Primary care and cancer. in press, 1990.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 07250-04 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
New Drug Discovery Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Investigator	NCI-NMOB
	Daniel Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*
Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The primary goal of this group is to identify new agents of potential clinical use in treating solid tumors. A major effort over the past 2 years has been the use of an in vitro assay which may be helpful as a preclinical screening model for antitumor agents. The model has been used to predict the clinical activity of 7 chemotherapeutic agents against 11 human colorectal carcinoma cell lines which have been developed in this branch. Using the model, we have shown that leucovorin enhances the in vitro cytotoxicity of the fluoropyridines versus our panel of colorectal cell lines. A study was also performed to detect possible synergy between etoposide and cisplatin in a panel of 8 human bronchogenic carcinoma cell lines. Extensive analysis revealed no in vitro synergy, a finding at variance with standard feeling. Schedule dependent drug interaction has been documented between methotrexate and 5-fluorouracil. Persantine has ben shown to enhance the cytotoxicity of 10-EDAM in human lung cancer cell lines. Clinical trails are planned to explore this.

At present, we are involved in several trials of new experimental therapeutic agents: a radiolabeled monoclonal antibody (^{90}yttrium-T101) in mycosis fungoides and chronic lymphocytic leukemia; 4-ipomeanol in lung cancer and a Phase I trial of hepsulfam.

PROJECT DESCRIPTION

New Drug Discovery Project

Professional Staff:

PI:	Barnett S. Kramer, MD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Investigator	NCI-NMOB
	Daniel Ihde, MD	Prof Med NCI-USUHS	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)

Objectives:

1. Identification of new compounds for the treatment of solid tumors.

2. Preclinical testing of combinations of drugs to detect synergy.

3. Validation of in vitro chemosensitivity test.

4. Testing new compounds in the clinic for lung and colon cancers.

Methods Employed:

1. In vitro chemosensitivity: MTT assay (a tetrazolium-based colorimetric test for cell viability.

2. Phase I trials of new drugs in cancer (for example, ipomeanol in lung cancer; sulfamic acid).

Major Findings:

1. 5-FU was the only one of 7 drugs tested which we predict would be effective in some of our colorectal cell lines.

2. Leucovorin enhanced the cytotoxicity of 5-FU and of FUDR in 10 of 11 colorectal cell lines tested.

3. The ipomeanol study has opened (2 patients treated to date); the ^{90}yttrium-T101 study has also opened (9 patients treated to date). Thirteen patients have been treated with sulfamic acid.

Significance to Biomedical Research and the Program of the Institute:

New drug development is a major charge of the National Cancer Institute. The preclinical screening program of the NCI is based upon the MTT assay. It is important to pursue innovative therapies, such as treatment with radiolabeled monoclonal antibodies directed against malignant cells (e.g. ^{90}yttrium-T101).

Publications:

1. Park J-G, Kramer BS, Lai S-L, et al. Chemosensitivity patterns and expression of human multidrug resistance (MDRI) gene by human gastric and colorectal cell lines. J Natl Cancer Inst, in press.

2. Tsai C-M, Gazdar AF, Perng R-P, and Kramer BS. Schedule dependent in vitro combination effects of methotrexate and 5-fluorouracil in human tumor cell lines. Proc ASCO 1990.

3. Dearing MP, Englee-Miller MJ, Kramer BS, et al. Enhanced cell of human lung cancer cell lines by 10-Ethyl-10-deazouminopterin (10-EDAM) when given with dipyridamole (DPM). Proc ASCO 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 07255-02 NMOB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Birrer, MD, PhD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	Powel Brown, MD	Clinical Associate	NCI-NMOB
	Eva Szabo, MD	Clinical Associate	NCI-NMOB
	Dennis Sanders, MD	Clinical Associate	NCI-NMOB
	Lisa Preis	Research Associate	NCI-NMOB

COOPERATING UNITS *(if any)*

University of California San Diego (Dr. Michael Karin)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION
NCI, COP, DCT, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Recent developments in molecular biology has led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes in various tumor systems. These genes, called oncogenes, are involved in various aspects in the regulation of cell growth. It is now critical to understand the precise mechanism by which these genes function so molecular agents ultimately can be derived to alter or repress their effects.

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

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

GPO 914-918

PROJECT DESCRIPTION AND RESULTS

Biologic Properties of Nuclear Oncogenes and Attempts to Block Their Effects

PI:	Michael J. Birrer, MD, PhD	Asst Prof Med NCI-USUHS	NCI-NMOB
Others:	Powel Brown, MD	Clinical Associate	NCI-NMOB
	Eva Szabo, MD	Clinical Associate	NCI-NMOB
	Dennis Sanders, MD	Clinical Associate	NCI-NMOB
	Lisa Preis	Research Associate	NCI-NMOB

Project #1 The L-myc Proteins and Their Biologic Activities:

The protein products of the L-myc gene have been further characterized. The larger molecular weight species possibly arise from alternative translational initiation sites (including a non-AUG initiation site) and post-translational phosphorylation. These various proteins have been shown to cotransform rat embryo cells with an activated ras gene and are presently being examined for differences in their biologic activities. (Birrer in collaboration with Minna)

Project #2 The Transforming Activity of the c-jun Proto-oncogene:

The transforming activity for the c-jun proto-oncogene was established by demonstrating the cotransforming activity of this gene in combination with an activated ras gene in rat embryo cells. Further, it was shown that c-jun can transform an immortalized rat fibroblast cell line Rat-1a as a single gene. This demonstrates that no mutational activating event is required for c-jun to transform mammalian cells. (Birrer, in collaboration and Minna)

To elucidate the mechanism of transforming activity of c-jun we have undertaken a mutation/deletion study of the gene. Presently, the transforming activity of the gene maps to two highly conserved regions in the gene, one of which contains the DNA binding domain. Preliminary experiments map these transforming domains to those required for transactivation. Further, in isolating these mutants, some non-transforming ones were found to inhibit the transforming activity of the full length gene, hence displaying a "dominant-negative" phenotype. We are presently characterizing these for their biologic and biochemical properties. (Brown, Szabo, and Birrer)

Project #3 Identification of AP-1 Regulated Genes

In an attempt to further elucidate the mechanisms involved in the biologic activities of c-jun we will identify downstream genes regulated by AP-1. We will use 2 approaches: 1) isolation of gene whose transcription is up-regulated by c-jun by cDNA subtractive hybridization. We will subtract a normal cell mRNA from one transformed by c-jun. 2) isolation of genes with AP-1 sites by identification of genomic clones through binding of Jun/Fos protein complex in an in vitro assay (Sanders and Birrer).

Publications:

1. Birrer MJ, Raveh L, Dosaka H, Segal S. A transfected L-myc gene can substitute for c-myc in blocking murine erythroleukemia differentiation. Mol Cell Bio 1989;9:2734-2737.

2. Schutte J, Minna JD, Birrer MJ. Deregulated expression of human c-jun transforms primary rat embryo cells in cooperation with an activated c-Ha-ras gene and transforms Rat-1a cells as a single gene. Proc Natl Acad Sci USA 1989;86:2257-2261.

3. Birrer MJ, Minna JD. Genetic Changes in the pathogenesis of lung cancer. Ann Rev Med 1989;40:305-317.

4. Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vinocour M, Levit M, Pass H, Gazdar AF, Minna JD. p53: A frequent target for genetic abnormalities in lung cancer. Science 1989;246:491-494.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07256-02 NMOB

PERIOD COVERED

October, 1989 to September 30, 1990

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

Mechanisms of Oncogene Action in Tumorigenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederic J. Kaye, MD	Asst Prof Med-NCI/USUHS	NCI-NMOB
Others:	Albert Lin, MD	Medical Staff Fellow	NCI-NMOB
	Greg Otterson, MD	Medical Staff Fellow	NCI-NMOB
	Eiji Shimizu, MD	Guest Researcher	NCI-NMOB
	Jean Gerster, MA	Research Assoc, Biologist	NCI-NMOB
	Robert Kratzke, MD	Medical Staff Fellow	NCI-NMOB
	Paul S. Lin, MD	Howard Hughes Med Scholar	NCI-NMOB

COOPERATING UNITS *(if any)*

Duke University Medical Center, Durham, NC (J. Horowitz)
Dana-Farber Cancer Institute, Boston, MA (D. Livingston)

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Genetics, Molecular Biology, and Immunology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

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

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

We have undertaken a study to identify the critical genetic events in the pathogenesis of human cancer. We have currently focused our research efforts on studying the mechanism and implication of inactivation of the retinoblastoma (Rb) gene in human cancer. Our recent findings are as follows: 1) essentially all small cell lung cancer tumors have absent or aberrant Rb protein products; 2) we have identified and characterized a series Rb mutants (defective in phosphorylation and oncoprotein binding); 3) we are investigating the possibility of these Rb mutants to function as transforming genes (dominant negative effect); 4) we have successfully transfected a wild-type or mutant Rb gene in a SCLC cell line to study its biological effect; and 5) we are using our Rb open reading frame reagents to identify putative cellular proteins that normally interact with the Rb protein and presumably modulate its growth inhibitory effect.

In addition, we continue to maintain a research effort studying mechanisms of L-myc gene activation and plan to purify and characterize several specific L-myc DNA binding proteins which we have previously characterized.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

Mechanisms of Oncogene Action in Tumorigenesis

Role of the Retinoblastoma Gene in the Pathogenesis of Human Cancer

PI:	Frederic J. Kaye, MD	Asst Prof Med-NCI/USUHS	NCI-NMOB
Others:	Albert Lin, MD	Medical Staff Fellow	NCI-NMOB
	Greg Otterson, MD	Medical Staff Fellow	NCI-NMOB
	Eiji Shimizu, MD	Guest Researcher	NCI-NMOB
	Jean Gerster, MA	Research Assoc, Biologist	NCI-NMOB
	Robert Kratzke, MD	Medical Staff Fellow	NCI-NMOB
	Paul S. Lin, MD	Howard Hughes Med Scholar	NCI-NMOB

We have demonstrated inactivation of the Rb gene in eventually all SCLC tumors.

We now wish to address two critical questions:

A. Can we revert tumorigenicity in SCLC by reintroducing the Rb gene and can we use this information to implement preventive or therapeutic strategies?

B. What is the role of the Rb gene in normal cellular physiology and how does its inactivation result in tumorigenesis?

Question A: Transfection of the Rb gene into SCLC cell lines.

We have successfully transfected either a wild-type or mutant Rb gene in a SCLC cell line lacking endogenous Rb expression. Analysis of parameters of tumorigenesis are in progress (Kaye, Gerster, Kratzke in collaboration with S. Segal).

Question B: Identification and characterization of mutant Rb proteins in SCLC.

Although 40% of SCLC tumors express a normal sized mRNA, we have now shown that these transcripts are defective and result in absent or mutant Rb protein.

Therefore, in excess of 90% of SCLC tumors studied to date have evidence for Rb inactivation. In collaboration with J. Horowitz and R. Weinberg (Boston, MA), we have identified several cell lines with mutant proteins and have characterized the molecular defects that generated these mutants. In addition we have identified a SCLC line with an Rb protein defective in phosphorylation. This is of great interest since Rb phosphorylation is believed to regulate cell cycle events. We have characterized this mutant protein and found a missense mutation changing a single amino acid. This analysis will help define functional domains of the Rb protein. We have now generated a series of in vitro mutants in the region to further identify phosphorylation domains and/or tertiary structure of the Rb protein.

We also have studied the potential transforming effect of these mutant proteins in rat embryo fibroblast system to determine if they might have a direct effect on growth regulation similar to that observed with mutant p53 proteins.

A key experiment is to identify cellular proteins which interact with Rb to modulate its growth inhibitory effect and these studies are ongoing in collaboration with W. Kaelin and D. Livingston, Boston, MA. (Kaye, Kratzke, Gerster, Lin)

Publications:

1. Horowitz J, Park S, Bogenmann E, Cheng J, Yandell D, Kaye F, Minna J, Dryja T, Weinberg R. Frequent inactivation of the retinoblastoma anti-oncogene is restricted to a subset of human tumor cells. Proc Natl Acad Sci USA 1990;87:2775-2779.

2. Harbour W, Lai S, Gazdar A, Minna J, Kaye F. Expression in lung cancer of a transcribed sequence at the DNF 15S2 locus at chromosome 3p21. Anticancer Res 1990;10:23-28.

3. Kaye F, Kratzke R, Gerster J, Horowitz J. Mutation of a single amino acid of the retinoblastoma protein blocks phosphorylation and oncoprotein binding. Proc Natl Acad Sci USA 1990, in press.

4. Kaye F, Barksdale S, Harbour JW, Minna JD. Oncogenes in lung cancer. In: Sluyser M., ed. Molecular Biology of Cancer Genes. Ellis Horwood Series in Biomedicine. VCH Publishers UK; Ltd (Cambridge, UK). 1990

5. Kaye F. Molecular biology of lung cancer. In: Cossman J., eds. Molecular Genetics and the Diagnosis of Cancer. Elsevier Science Publishing company (New York). June 1990.

6. Kaye F, Kratzke R, Gerster J, Lin P. Recessive oncogenes in lung cancer. Ann Rev Resp Dis 1990, in press.

7. Lin P, Kaye F. Inactivation of the retinoblastoma gene by gene inversion in a small cell lung cancer sample. First meeting on The Molecualr Basis of Human Cancer, Frederick, MD 1990.

8. Gerster J, Kratzke R, Segal S, Kaye F. Stable transfection of a mutated or wildtype retinoblastoma gene in a small cell lung cancer tumor. Sixth Annual Meeting on Oncogenes, Frederick, MD 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CM 07257-02 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shoshana Segal, PhD	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Matia Bar-Ner, PhD	Fogarty Visiting Fellow	NCI-NMOB
	Lora Messing	Research Assoc NCI-USUHS	NCI-NMOB

COOPERATING UNITS *(if any)*

Hematology, Oncology Section, University of Pennsylvania
Experimental Immunology Branch, NCI

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Differentiation

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

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

Based on these finds and the published reports on the homology between C, N, and L-myc protooncogenes, we investigated the ability of the related L-myc gene to substitute for c-myc in blocking MEL differentiation. Our results clearly indicated that constitutive high levels of transfected L-myc mRNA block inducer in our laboratory with N-myc transfectants. These studies strongly suggest that down regulation of c-myc expression in these cell lines is a necessary event for mutants of the c-myc gene for mapping the regions(s) responsible for its apparent critical role in MEL and F9 teratocarcinoma cell differentiation. In addition, we are developing a new approach for identifying proteins that interact with the c-myc protein during differentiation.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

Molecular Biology of Erythroleukemia and F9 Teratocarcinoma Cell Differentiation

PI:	Shoshana Segal, PhD.	Assoc Prof Med NCI-USUHS	NCI-NMOB
Others:	Matia Bar-Ner, Ph.D.	Fogarty Visiting Fellow	NCI-NMOB
	Lora Messing	Research Assoc NCI-USUHS	NCI-NMOB

Collaborating Branches

Hematology, Oncology Section, University of Pennsylvania
Experimental Immunology Branch, NCI

Objectives

1. To study the role of C, N, and L-myc protooncogenes in growth and differentiation of MEL and F9 teratocarcinoma cells.

2. To identify and map regions on the c-myc gene essential for differentiation.

3. To study mechanisms and genes involved in hematopoietic and F9 teratocarcinoma cell differentiation.

A. Members of the Myc Family Block Chemically Induced Differentiation of MEL and F9 Teratocarcinoma Cells.

MEL and F9 teratocarcinoma cells express high levels of the c-myc protooncogene, however, shortly after the addition of inducer (NMBA, DMSO, RA) a sharp decline in c-myc mRNA occurs which is followed by a cessation of cell growth and terminal differentiation. We transfected both cell lines with a plasmid containing the c-myc gene driven by the Molony LTR. All clones obtained from the MEL cell line expressed constitutive high levels of the transgene and were blocked in their ability to differentiate in response to chemical inducers. F9 derived clones expressed high levels of the exogenous c-myc gene, but the mRNA was down regulated in a similar fashion to the endogenous gene causing only a partial block to differentiation. To further support these findings we introduced, by stable transformation, into MEL cells a related myc family gene L-myc. A number of studies have shown greater than 90% sequence homology between C, N, and L-myc in several discrete areas of the gene. Although MEL cells do not express normally L-myc, all of the clones expressing high constitutive levels of the transfected gene fail to differentiate in response to the chemical inducer HMBA. Similar results were obtained recently in our lab with MEL/N-myc transfectants.

B. Identification of Regions in Human c-myc that are Involved in Cellular Differentiation. (In collaboration with W. Lee, University of Pennsylvania.

The involvement of c-myc in normal and neoplastic growth makes it important to understand its function(s) and the structural basis of some of its properties.

Studies by Lee et al. have identified three areas that are essential for rat embryo cells cotransforming activity. The mapping of these areas was accomplished by the use of a large number of c-myc deletion/insertion mutants. We undertook a similar approach for identifying regions involved in differentiation.

MEL cells were transfected with deletion mutants spanning the normal c-myc coding regions (exons 2 and 3). Clones expressing the mutated c-myc gene were isolated and analyzed for HMBA induced differentiation. Results obtained from independent transfectants indicate that sequences at the 5' and 3' ends of the coding region are necessary for activity; however, short deletions at the 5' end are tolerated.

The helix loop helix (HLH) as well as the leucine zipper motifs located at the 3' end of the gene are essential for inhibition of differentiation. A large deletion in the center of the coding region (a.a 145-262) has an intermediate effect of differentiation.

C. The Role of the jun Family of Genes in Differentiation of F9 Teratocarcinoma Cells. (In collaboration with J. Schuette and J. Minna).

We have analyzed F9 teratocarcinoma cells for the expression of the different jun family members and discovered that jun-B and c-jun were not expressed in the stem cells, but low levels of expression were detected following induction with RA. jun-D, on the other hand, was expressed in high levels in the induced and induced cells.

F9 cells were transfected with c-jun, jun-B, c-fos, and a combination of c-jun+c-fos, jun-b+c-fos, c-jun+jun-B, and c-jun+jun-B+c-fos.

Single cell clones expressing the different transfected genes were isolated and are being analyzed for the transactivating activity of c-jun and jun-B on endogenous genes such as collagen Type IV Laminin, Retinoic Acid and α ß and γ receptors and other genes involved in the differentiation process of these cells.

D. Identification of Proteins Interacting with the myc Gene Products During Differentiation of MEL cells. (In collaboration with D. Segal, Experimental Immunology Branch, NCI).

A construct containing a single chain FV fragment from an anti DNP antibody was ligated in frame to coding region of the human c-myc gene. We plan to transfect the hybrid gene into MEL cells, obtain clones expressing the transfected gene which do not differentiate following induction with HMBA. We will make protein extracts and mix them with DNP-sepharose beads. Since the fusion protein contains anti-DNP binding activity, c-myc and its associated proteins will bind to the beads. More detailed analysis of the proteins will be done after elution from the beads.

Publications

1. Schutte J, Viallet J, Nau M, Segal S, Fedorko J, Minna J. jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. Cell 1989, 59:987-997.

2. Bar-Ner M, Messing L, Segal S. Regions of human c-myc involved in inhibition of murine erythroleukemia differentiation. In preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07258-02 NMOB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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, M.D.	Asst Prof Med NCI-USUHS	NCI-NMOB
	W. Michael Kuehl, M.D.	Senior Investigator	NCI-NMOB
Others:	James Lynch, M.D.	Med Staff Fellow	NCI-NMOB
	Ross Turner, MS	Res Assoc NCI-USUHS	NCI-NMOB
	Dat Nguyen, M.D.	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

Robert Gallo, LTCB, DCE, NCI, NIH
Edward Sausville, MB, DCT, NCI, NIH

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Differentiation

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	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 helper T-cells, 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. In addition, 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.

Thus far, we have identified reverse transcriptase-like activity in cultured cells and in cocultivations of MF cells and permissive cell lines, and further characterization of this activity is underway. 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. We are currently exploring the theraputic role of DDI in this disease.

PROJECT DESCRIPTION

Etiology of Cutaneous T-cell Lymphomas

PI:	Francine Foss, MD	Asst Prof Med NCI-USUHS	NCI-NMOB
	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	James Lynch, MD	Med Staff Fellow	NCI-NMOB
	Ross Turner, MS	Res Assoc NCI-USUHS	NCI-NMOB
	Dat Nguyen, MD	Guest Researcher	NCI-NMOB

Collaborating Branches:

Robert Gallo, LTCB, DCE, NCI, NIH

Objectives:

1. To determine the origin of the malignant cells in Mycosis Fungoides by attempting to establish cell lines from various sites.

2. To study the biology of the sezary cell lines with respect to growth factor production and response, oncogenic alterations, and sensitivity to 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.

4. To define the possible role of retroviruses in the etiology of the disease.

Major Findings:

Cell Culture Experiments:

Previous efforts to establish long-term cultures of sezary cells have yielded only one cell lines, 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 T-cell mitogens. Kinetic studies reveal that the cells in the circulating compartment are largely non-proliferating, in contrast to those in lymph node and skin. We have attempted to establish cell lines from blood, bone marrow and lymph node from patients with Mycosis Fungoides and have successfully maintained cells from bone marrow in four patients and from lymph node in two. These cell lines are of two types, one being characteristic of T-cells and one bearing markers of cells of monocytoid origin. Further characterization is underway.

Retroviruses as Etiologic Agents

In collaboration with Dr. P. Browning and Dr. R. Gallo, we have been able to identify reverse transcriptase activity in cultured cells from two CTCL patients. Both of these lines represent populations of cells which appear to be of monocytoid origin. Further analysis of these cells indicates that the reverse transcriptase activity can be isolated on sucrose gradients. However, the cells proliferate very slowly, and obtaining large volumes of cell supernatant for definitive virus isolation has been difficult. Two strategies have been evolved to address this problem in our lab. First, we have cocultivated the cells from these and other MF patients with permissive lines, such as HUT 78 and A3.01 in attempt to passage virus. Several of these cocultivations have reproducibly developed reverse transcriptase activity two to four weeks after cocultivation. DNA and RNA from these cocultivations and from fresh patient tissue has been screened for retroviral reverse transcriptase-like sequences using PCR.

Our second strategy to enhance growth ability of MF patient cells entails attempts to immortalize the cells by transfecting them with viral immortalization genes mediated via retroviral vectors. Infections using c-myc, ras, SV40 large T antigen, and E1A antigen are underway, in collaboration with M. Birrer.

Our PCR-directed analysis of DNA from MF patients yielded a DNA sequence which is part of a reverse-transcriptase containing gene not homologous to any known viruses or endogenous retroviruses. Southern blot analysis demonstrates that this sequence is present in all mammalian DNA, and we are pursuing the hypothesis that this is a novel human retrovirus.

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 amidst 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.

Genotyping and Karyotypic Analysis of MF tissues

We have extensively genotyped the periperal blood and lymph node from at least 40 MF patients with respect to rearrangements of the TCR and IG loci. We are attempting to correlate patterns of detected rearrangements with clinical features and prognosis.

Development of New Therapies for MF

Over the past year we have attempted to evolve new therapies in the lab which could be directly applied to patient care. We have used MTT testing to determine sensitivity of MF cells and of the MF cell line Hut78 and other T-lymphoid cell lines to a variety of chemotheraputic 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 demonstrated synergy in-vitro.

In addition to studies of cytotoxic therapies, we have attempted to delineate the role of growth factor therapy in these patients by studying the in-vitro effects of growth factors on cell viability. We have identified a possible role for IL-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.

Proposed Course

A large part of our effort will be to continue to explore the possible retroviral etiology of MF. We hope to propagate patient cells in culture using transfected immortalization genes and to study their DNA and RNA for retroviral-like sequences. We hope to perform electron microscopic analysis of patient cells shortly after placing them in culture to look for retroviral particles. Our cocultivated specimens with demonstrated reverse transcriptase activity will be further evaluated by electron microscopy and by genetic analysis for presence of retroviruses.

The retroviral-like sequence we derived from an MF patient will be used to screen northern blots from both patients and normals to look for message, and a genomic library will be screened to obtain a full length sequence of this gene.

Characterization of early stage skin lesions will continue. We will attempt to perform in-situ hybridization using a specific TCR-B rearrangement in attempt to correlate the morphologic features of the skin infiltrate with clonal genetic alteration.

We also hope that, by sequencing TCR VDJ regions from many patients, we can answer the question of whether there is selective V-region utilization in the malignant cells of MF patients.

In-vitro drug and growth factor sensitivity studies will continue. We will attempt to apply these results to the design of new clinical trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07259-01 NMOB

PERIOD COVERED
October 1,1989 to September 30,1990.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular pathology of pre-malignant lung

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	Jos Broers, PhD	Visiting Fellow	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Branch Chief	NCI-NMOB

COOPERATING UNITS *(if any)*
Surgery Branch,NCI (Harvey Pass,MD), Anatomic Pathology, NCI,NIH (Bill Travis, MD), and Anatomic Pathology, Naval Hospital

LAB/BRANCH
NCI-Navy medical Oncology Branch

SECTION
Human Tumor Biology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

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

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

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

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

GPO 914-918

PROJECT DESCRIPTION

Molecular pathology of pre-malignant lung

Professional Staff:

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
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	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Branch Chief	NCI-NMOB

Collaborating Branches:

Surgery Branch, NCI (Harvey Pass,MD), Anatomic Pathology, NCI,NIH (Bill Travis, MD), and Anatomic Pathology, Naval Hospital

Objectives:

The malignant transformation of the human bronchial epithelium is an end point of the deregulation of multiple events involving the growth control which are produced by exposure to carcinogens and possible inherited predisposition to lung cancer. Our goal is to define the molecular events that occur in the bronchopulmonary epithelium in the premalignant state.

Bronchial carcinogenesis is a complex process associated with genetic abnormalities in both dominant and recessive ("tumor suppressor") oncogenes.

Dominant, classic cellular oncogenes can cause cellular transformation in selected model systems through inappropriate activation which reflects a positive deregulation of their function following a change in only one of the maternal or paternal alleles.
The dominant oncogenes implicated in the pathogenesis of lung cancer include myc family oncogenes, ras, raf, and neu. Activation can happen through several mechanisms:1) Amplification of protooncogenes has been reported in up to 21% of NSCLC in which about 90% of the amplified genes were of the myc, ras, or erbB/neu family. 2) Point mutations are characteristic of ras family genes in NSCLC. Other mechanisms include 3) gene rearrangement producing chimeric or truncated genes, and 4) rearrangement of a gene in a region outside the transcribed sequence.

In contrast to the dominant oncogenes the antioncogenes or tumor suppressor genes posses a normalizing or negative regulatory role on growth and their inactivation through deletions or mutations produce the malignant phenotype. Two genetic lesions are required for their effect; one for inactivation of the maternal allele and the other for the paternal allele, and thus they have been also called recessive oncogenes.. Lung cancer cells demonstrate numerous specific chromosomal deletions suggesting that anti-oncogenes are important in the pathogenesis of lung cancer. In addition to structural and numerical cytogenetic changes, comparison of tumor and normal tissue DNAs by means of restriction fragment length polymorphism (RFLP) probes revealed loss of heterozygosity in chromosome regions 3p,13q, and 17p. The known recessive oncogenes implicated in the pathogenesis of lung cancer include retinoblastoma (rb) gene an the p53 nuclear protein.

The incidence of lung adenocarcinoma is increasing in the U.S.A. The progenitor cells for adenocarcinoma include type II pneumocytes and Clara cells which are the metabolically active progenitor cells of peripheral airways. The pre-malignant changes of this are not understood. The characterization of genes specific for peripheral airway cell differentiation SP-A(the major surfactant associated protein) and Clara specific protein prompted us to investigate the expression of PAC differentiation genes and oncogenes in the progenitor cells for lung cancer in non-neoplastic lung.

Methods Employed:

1.Tissues. A frozen tissue bank has been established composed of resection specimens of lung carcinomas and corresponding non-neoplastic lung that reveals pre-neoplastic changes. The study will initially concentrate on NSCLC of all types, with specific interest in adenocarcinomas since tissue samples for SCLC are more difficult to collect(patients are not undergoing resections of their SCLC tumors). A great emphasis was placed on appropriate collection of specimens to preserve tissue RNA and morphology. The collection and cataloging is an ongoing project to obtain a series of progressive premalignant changes occurring randomly in resected non-neoplastic lungs. In addition to frozen tissue blocks, RNA and DNA will be prepared from all specimens.

2.Methods. RNA-RNA in situ tissue hybridization with S 35 labelled probes of c-myc(2nd exon 420bp),L-myc(3rd exon, 580 bp) and N-myc (3rd exon 660bp) were used. Immunohistochemical staining using p53 antibodies and Ki67 proliferation cell antibody was performed using the avidin biotin peroxidase technique.

Major Findings:

c-myc oncogene was overexpressed in 8 out of 17 tumors, 2 of which also expressed L-myc, while 15 out of 17 lungs showed low levels of c-myc both in airway epithelium and alveoli. N-myc levels both in tumors and lung tissue remained undetectable. In situ hybridization results were confirmed by Northern blot analysis. In contrast to c-myc, Ki67 was intensely positive only in basal cells of bronchial epithelium. In c-myc positive tumors a subset of tumor cells were positive for the proliferation antigen Ki67. The expression of PAC differentiation genes SP-A and Clara cell protein was focal in 4 tumors and restricted to type II cells in alveoli nad bronchiolar cells of the lung respectively. By Immunohistochemistry 5 tumors were positive for p53 staining signifying the possible presence of mutated form of this tumor suppressor gene. Furthermore, positive staining was also detected in the basal cells of bronchial epithelium. We conclude that 1) overexpression of the myc-protooncogenes is common in NSCLC; 2) c-myc expression is not restricted to a single cell type in non-neoplastic lung; 3) c-myc and Ki67 expression may characterize different aspects of proliferation and 4) PAC differentiation is restricted to subpopulations of bronchopulmonary cells.

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the identification of the expression of multiple oncogenes, tumor suppressor genes, growth factors and receptors simultaneously at the cellular level in the same surgically removed specimens. This enables to establish the order of genetic events and their correlation to premalignant changes and tumor histology. Similar techniques can be used to identify oncogene expression in clinical cytology specimens and as possible adjunct in the early detection of lung cancer. The results will help to define the early versus late genetic events in the development of lung cancer.

Proposed Course:

1. The expression of growth factors and receptors, oncogenes and tumor suppressor genes will be correlated with individual cell types of lung in malignant and non-neoplastic lung obtained through routine surgical removal of NSCLCs and the surrounding lung tissue.

2. Specimens from patients at risk for getting a lung cancer will be collected and analyzed for the expression of growth factors and receptors, oncogenes and tumor suppressor genes and results will be correlated with the clinical outcome.

3. Cell culture and animal models will be used to define the relative growth /transformation potential of the bronchopulmonary cells which express selected oncogene/differentiation profiles.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06813-08 PB

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carol J. Thiele-Galetto	Senior Investigator	PB, NCI
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	Carlo Gaetano	Fogarty Fellow	PB, NCI
	Leonard Wexler	Clinical Associate	PB, NCI

COOPERATING UNITS *(if any)*

University of California, San Francisco (M. Israel), National Cancer Institute, Surgery Branch (S. Rosenberg)

LAB/BRANCH

Pediatric Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, Maryland 20891

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Summary:

We use human pediatric tumors as a model system to study the molecular events associated with the development of malignant tumors during childhood. Since neuroblastoma (NB) can be induced to differentiate in vitro with retinoic acid, it is a particularly useful system in which to study the molecular mechanisms regulating growth and differentiation. Our current focus is to identify additional chemicals and biologic response modifiers that control cell growth and/or induce differentiation and use recombinant DNA technology to identify the molecular mechanisms and clone the genes important for the regulation of these processes in pediatric peripheral neuroectodermal tumors. During the past year a clinical protocol utilizing trans-retinoic acid to treat children with pediatric malignancies, evolved in part from in vitro studies demonstrating its efficacy. Furthermore, the inclusion of interferon-gamma pre-treatment of children with neuroblastoma in a TIL(tumor-infiltrating lymphocytes) therapy clinical protocol developed from in vitro studies analyzing the expression of Class I Major Histocompatibility Complex antigens on neuroblastoma tumors. Ultimately our goal is to develop new strategies and novel therapeutics based on understanding the specific alterations in these pediatric malignancies.

Accomplishments and Results:

1. Evaluation of neuroblastoma cell differentiation:

Previously, we have characterized NB cell lines that are growth arrested by retinoic acid (RA) and using molecular genetic analysis we have determined a pattern of proto-oncogene, growth associated gene and differentiation gene expression. Using this model we have compared the phenotypic changes associated with other growth controlling or differentiation inducing agents in neuroblastoma. While RA induces a neuronal phenotype and decreases expression of genes associated with a neuroendocrine phenotype, cAMP induces rapid increases in the expression of genes associated with a neuroendocrine phenotype. When the combination of RA and cAMP are used a neuronal phenotype predominates and the expression of an adrenal-specific anonymous gene is extinguished. This provides a model to investigate lineage-specific issues in the development of chromaffin and neuronal cells. Current studies are focused on evaluating TNFalpha, vasointestinal peptide (VIP) and interferon gamma (IFN gamma) and expanding our analysis to cell lines derived from other peripheral neuroectodermal tumors such as Ewing's sarcoma and peripheral neuroepithelioma.

2. Evaluation of neuroblastoma cell growth

An understanding of the mechanisms controlling cell proliferation is critical to delineating mechanisms of dysregulation in cancer. Furthermore control of cell growth is, usually, a prerequisite for terminal differentiation. We have initiated studies to examine the regulation of the human homologs of the recently described yeast cell-cycle genes (cdc2 and cyclin A and B)in human tumor cells. We have found that the expression of $p34^{cdc2}$ is not down-regulated when tumor cells are growth arrested by nutrient deprivation in contrast to normal cell lines. However, when retinoic acid is used to control cell growth and induce differentiation in NB cells a 25-fold decrease in $p34^{cdc2}$ levels is detected, suggesting that such treatment restores normal growth regulation to these tumor cells. Similar results have been obtained in the promyelocytic tumor cell line HL60. We have observed the decreased expression of $p34^{cdc2}$ only in cell lines that are growth arrested and differentiated suggesting that regulation of cdc2 may be an important link between the ability of a cell to continue to proliferate and its ability to differentiate. $p34^{cdc2}$ expression is regulated post-transcriptionally since protein levels are undetected despite cdc2 mRNA levels being comparable to untreated cells. Current studies are underway to study the regulation of the $p34^{cdc2}$ protein kinase activity since its known substrates include the tumor suppressor genes RB and p53.

3. Evaluation of resistance to retinoic acid:
Since retinoic acid (RA) has been approved for clinical trials we have instituted a study to evaluate the inability of some NB cell lines to be growth arrested and differentiated by RA as well as the in vitro development of resistance to RA. We have found that many cell lines that are resistant to RA express IGF-II mRNA. IGF-II has been shown to be constitutively expressed in some NB cell lines and function as an autocrine growth factor. We have developed an in vitro model in which a NB cell line that initially is growth arrested by RA rapidly develops resistance and RA-resistant cell lines have been isolated. In this model, RA appears to rapidly increase IGF-II expression and resistant cell lines express high levels of IGF-II mRNA.

4. Evaluation of suppressor genes in pediatric peripheral neuroectodermal tumors (PNET):
Numerous genetic alterations have been described in PNET including chromosome 1p deletions, loss of heterozygosity on chromosomes 11, 14, and amplification of MYCN in NB and t(11:22) and amplification of MYC in Ewing's sarcoma (ES) and peripheral neuroepithelioma (NE). Although ES and NE are distinct clinical entities, cytogenetic, molecular genetic and biochemical analyses suggest that these tumors may have a common origin. We have initiated a study to determine if PNETs have complementing genetic alterations by making somatic cell hybrids between these NB, NE and ES tumor cell lines and evaluating tumorigenicity. As a prerequisite for such an analysis drug resistant cell lines are required. We have established the following drug resistant cell lines which will serve as hybridization partners in subsequent analysis: $G418^r$ KCNR (NB-MYCNamplified), AS (NB), SY5Y (NB), TC32 (NE) and $Hygromycin^r$ TC106 (ES) and TC32 (NE). Studies are planned to evaluate NB x NE, NE x ES and NB x ES hybrids for suppression of tumorigenicity.

Despite the numerous cytogenetic alterations in NB, RA is capable of restoring growth control in many cell lines. We have initiated studies using subtractive cDNA cloning to isolate genes expressed when RA induces growth control in NB cells and to analyze if these genes are capable of suppressing growth and tumorigenicity when transfected into other NB cell lines. Currently we are evaluating expression vector cloning strategies and optimizing transfection techniques.

5. Class I Major Histocompatibility Antigen Expression:
NB tumors and cell lines express low levels of Class I MHC antigens and our studies indicate that this may be related to their developmental stage since Class I MHC is regulated during adrenal medullary development. Although some studies indicate amplification of MYCN may specifically down regulate Class I expression, our studies indicated that transfection of MYCN into PNET expressing high levels of Class I failed to alter its expression. We are

studying the ability of IFN gamma to alter Class I expression in low Class I expressing NB as well as alter tumor cell growth and induce differentiation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CM 06830-20 PB

PERIOD COVERED

October 1, 1989 - September 30, 1990

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

Infectious Complications of Malignancy and HIV Infection in Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip A. Pizzo Chief, Pediatric Branch	Head, Infectious Disease Section,	PB, NCI
Others:	Thomas J. Walsh	Medical Officer	PB, NCI
	Karina Butler	Senior Staff Fellow	PB, NCI
	Emile (Pim) Brouwers	Visiting Scientist	PB, NCI
	Robert Husson	Medical Officer	PB, NCI

Continued on next page

COOPERATING UNITS *(if any)*

Medicine Branch, Surgery Branch, NCI; Diagnostic Microbiology, Department of Transfusion Medicine, CC; Bethesda Naval Hospital; Duke University; Medical Illness Counseling Center

LAB/BRANCH

Pediatric Branch

SECTION

Infectious Disease

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

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

Our studies are devoted to developing methods to define cancer patients who are at risk for developing serious infection, to improving the ability to diagnose these infections early, to treat them effectively, and ultimately to prevent them. We are developing new therapeutic approaches based on the ability of new antibiotics particularly the beta-lactams and the quinolones. We have shown that certain beta-lactams used as single agents can replace the need for combination antibiotic therapy. Our studies are also defining the appropriate antibiotic therapy for documented infections, particularly the role of oral antibiotic therapy; the necessary duration of empiric therapy for patients with unexplained fevers and the choice of empiric antifungal therapy.

We have developed a unique model for studying the pathophysiology, natural history, treatment and prevention of invasive candidiasis in the neutropenic host. This model permits the testing of new antifungal agents as well as immunoregulatory agents. To prevent infections we are evaluating the role of passive immunization with a pooled immunoglobulin preparation that contains activity against the enterobacteriaceae as well as the pseudomonads. We are also studying other immunoregulatory agents that may serve as adjuncts to the treatment of infection, including interleukin 1 and 2, GM-CSF and M-CSF.

We have developed a program to evaluate the benefits of antiretroviral agents in children with HIV infection. To date, these have focused on studies with dideoxynucleosides. Studies with immunoregulatory agents and with biologicals (e.g., rCD4) are also underway.

PHS 6040 (Rev. 1/84)

GPO 914-918

Professional Personnel (Continued):

Tore Abrahamsen	Special Volunteer (Norwegian Cancer Society)	PB, NCI
Alison Freifeld	Medical Officer	PB, NCI
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Patricia O'Connor	Nurse Specialist (Research)	PB, NCI
Susan Sandelli	Nurse Specialist (Research)	PB, NCI

Accomplishments and Results:

A. Diagnosis, Management and Prevention of Infectious Complications in Cancer Patients

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

2. In a randomized trial, we have demonstrated that it is appropriate to continue empiric antibiotic therapy for a limited (i.e., 2 week) course for patients who have defervesced following the initiation of antibiotics but who remain febrile.

   In a follow-up study, we are comparing the use of a new class of oral antibiotics, the quinolines ,for patients who have defervesced on parenteral therapy, have no defined site of infection, and remain persistently granulocytopenic. This study has considerable importance, since it can serve to re-define the role of inpatient versus outpatient therapy 65 patients have been randomized.

3. To decrease the frequency of infectious complications associated with indwelling intravenous catheters of the Hickman-Broviac type, we have performed a randomized trial to compare the Hickman-type catheter to a subcutaneously implanted catheter (Port-a-Cath). Since the subcutaneous catheter requires less manipulation, our hypothesis is that it will have a low incidence of infection. However, if infected, it is possible that these infections will not be capable of eradication unless the catheter is removed. One hundred patients were randomized, 48 to a Hickman catheter and 52 to a Port-a-Cath. Overall, 10,592 days of catheter placement were evaluated in the Hickman group and 14,634 catheter days for the Port-a-Cath group. Analysis to date shows no difference in the frequency of infectious or non-infectious complications.

4. In order to help predict which patients will eventually require modifications of their empirical antibiotics, we analyzed initial characteristics of febrile neutropenic patients receiving different empirical regimens (single agent and combination therapy). Certain characteristics were more often associated with the eventual need for antibiotic modifications, although both regimens were equally effective. Knowledge of these characteristics predictive of the need for modification may be helpful in the management of persistently neutropenic patients.

5. To reduce the incidence of infection in patients who have protracted neutropenia, we evaluated, in a double-blind randomized trial, the value of passive immunization with a pooled intravenous immunoglobulin (IVIG). We also assessed the utility of this immunoglobulin in attenuating the types of infections which occur. Of 65 evaluable episodes, 33 were randomized to IVIG and 32 to placebo. No benefit from the IVIG was observed.

6. In preparation for *in vivo* administration of elutriated monocytes to patients with progressive infection, a variety of studies are underway. We have demonstrated that these cells have good phagocytic, chemotactic and microbicidal activity. We are studying their production of cytokines such as IL1, TNF and GM-CSF, in order to obviate potential toxicities and maximize efficacy. Optimal culture and storage conditions are being determined, and potential for *in vitro* activation of these cells explored.

   Preliminary data has been obtained from two patients with aplastic anemia and a rapidly progressive aspergillus infection. One patient was given nine transfusions with elutriated monocytes. Preliminary results indicate little toxicity, trafficking of the cells to the site of infection, and stabilization of the infectious process. A protocol to evaluate this modality further has been submitted to the Institutional Review Board.

7. In an attempt to reduce the duration of neutropenia associated with cytotoxic chemotherapy, we have initiated a prospective randomized trial in children with sarcomas whereby, following chemotherapy, they are randomized to receive or not receive rGM-CSF. The goal of this study is to determine whether the cytokine will reduce the incidence and severity of the fever of infection usually associated with neutropenia. Should this prove effective, it may permit altering chemotherapy schedules in a manner that might better optimize their antitumor efficacy. To date, 17 patients have been enrolled in this trial.

B. Invasive Mycoses: Preclinical and Clinical Studies

Invasive fungal infections are significant and increasing problems of morbidity and mortality in cancer patients and those with AIDS. Accordingly, we investigated the antifungal activity, pharmacokinetics, and immunomodulatory properties of several of these most promising agents for potential use in our high risk patient populations.

1. We developed a system consisting of three animal models of experimental disseminated candidiasis for targeting antifungal chemotherapy to three specific clinical patterns: acute, subacute, and chronic disseminated candidiasis; and for three antifungal regimens: preventive, early, and delayed treatment.

2. We demonstrated that three potent antifungal triazole compounds (itraconazole, fluconazole, and SCH-39304) were most effective when administered as preventive or early antifungal chemotherapy and have the clinical potential for use in early empirical antifungal therapy.

3. We demonstrated that the new antifungal triazoles (itraconazole, fluconazole, and SCH-39304) were as effective as amphotericin B plus flucytosine in early treatment of experimental disseminated candidiasis but that amphotericin B plus flucytosine was more effective against chronic (hepatosplenic) candidiasis.

4. These experimental antifungal studies provided the scientific rationale for design of a multicenter clinical trial to test the concept of early empirical antifungal therapy with fluconazole and for the first phase I trial of a systemic antifungal agent (fluconazole) in children. This study will commence shortly.

5. We identified the microbiological basis for amphotericin B resistance in *Trichosporon beigelii* (an emerging and often fatal systemic fungal pathogen in cancer patients). We found *T. beigelii* was inhibited but not killed by amphotericin B at safely achievable plasma concentrations. The minimum fungicidal concentration was markedly greater than the minimum inhibitory concentrations, thus suggesting the need for other antifungal compounds to treat this infection in neutropenic patients.

6. We have demonstrated the superior efficacy of cilofungin (LY-121019) when administered by continuous infusion compared to intermittent infusion against disseminated candidiasis in persistently granulocytopenic rabbits, representing for the first time an experimental rationale for continuous infusion of a systemic antifungal compound.

7. We have shown using in vitro timed kill assays that cilofungin is a highly fungicidal compound against *Candida albicans*, that combinations with other antifungal compounds do not appreciably augment its activity, and that the compound is fungicidal against *Torulopsis glabrata* at higher concentrations.

8. We demonstrated that cilofungin (LY-121019) is excreted via the biliary tract, has a short plasma half-life following first order kinetics with single dose administration but with continuous or frequent intermittent infusion, we demonstrated the non-linear saturable pharmacokinetics of cilofungin (LY-121019), thus accounting for the heretofore unexplained basis of accumulation of this promising compound in human volunteers.

9. We have demonstrated that fluconazole and SCH-39304 have long plasma half-lives and extensive tissue penetration into multiple tissue sites, including the CSF, brain, choroid, and vitreous fluid, this permitting the administration of these compounds to patients with CNS fungal infections. Fluconazole has now been approved for use in CNS cryptococcosis and SCH-39304 is being studied in HIV-infected patients with CNS mycoses.

10. We demonstrated that a new antifungal triazole (BAYR-3783) is converted into active metabolites, one of which has an exceedingly long plasma half-life with penetration into the central nervous system.

11. We demonstrated that Candida cholecystitis, an increasingly reported manifestation of invasive candidiasis, may be effectively treated by drainage and IV amphotericin B and that the biliary concentrations of amphotericin B exceed those of plasma by two to eight-fold.

12. We studied the effects of amphotericin B, 5-fluorocytosine, ketoconazole, fluconazole, cilofungin, and SCH-39304 on the granulocyte function and found that within the usual therapeutic concentrations the new and established antifungal compounds either did not affect or enhanced chemotaxis, phagocytosis, intracellular killing, and superoxide generation.

13. We developed a new method of studying continuous infusion pharmacokinetics in rabbits by utilizing a double silastic central venous catheter technique and a portable mini pump, permitting the study of the pharmacokinetics of continuous infusion of antimicrobial compounds as well as systemic immunomodulators.

14. In collaboration with the Antifungal Therapy Committee of the Medical Society of the Americas, we documented a substantial increase in the usage of systemic antifungal agents worldwide and within the NIH Clinical Center, consistent with the increased recognition and frequency of invasive mycoses in compromised patients.

15. We demonstrated that the depth, duration and recovery from granulocytopenia are important determinants in the clearance of experimental disseminated candidiasis; we further showed that recovery from granulocytopenia is not a sufficient condition for clearance of tissue candidiasis if profound granulocytopenia was present during the time of infection. These studies served as the foundations for developing a rational approach to the use of G-CSF for the prevention and treatment of disseminated candidiasis in granulocytopenic hosts.

16. Recombinant human G-CSF was found to be most effective in the prevention rather than the treatment of disseminated candidiasis in granulocytopenic rabbits. G-CSF was able to shorten duration but not depth of neutropenia. G-CSF induced increased superoxide activity which was associated with enhanced bactericidal but not fungicidal activity both in vitro and ex vivo.

17. We demonstrated the origin of cryptococcal antigenemia in invasive trichosporonosis as arising from cell wall and matrix of Trichosporon beigelii. We further identified key morphological, microscopic, and biochemical markers of clinical isolates of trichosporon that distinguished invasive versus non-invasive strains.

18. Following extensive pre-clinical investigate, we completed a multi-center trial demonstrating the expression of antigenemia due to Candida cytoplasmic enolase (a 48 k DAg) as a new marker of invasive candidiasis in cancer patients.

19. We found that empirical amphotericin B was not effective n 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.

20. During a study of catheter-associated fungemia, we found that the onset of fungemia occurred earlier than previously reported and was associated with high mortality if amphotericin B was not initiated within 48 hours of onset of fungemia.

C. Pediatric AIDS

1. We have continued our Phase I-II studies of children with symptomatic HIF infection. Since beginning this project in December, 1986, we have evaluated approximately 170 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. In addition, a third arm in this protocol randomizes patients to receive oral dideoxyinosine (ddI). 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, 11 patients have been randomized.

3. Our prior studies with AZT demonstrated that the dose-limiting toxicity was myelosuppression related to both dosage and duration. Consequently, we have evaluated two schedules to spare AZT-induced myelosuppression. The first alternates AZT with ddC, and the second combines it with rH-GM-CSF or more recently, with rH-G-CSF. In the study with ddC, we first evaluated this newer dideoxynucleoside in a limited phase I trial, studying four dosage levels (0.015, 0.020, 0.030, and 0.0404 mg/kg/q/6h) administered over an 8 week period. Fifteen patients were treated. We observed decreases in P24 antigen in 5/9, increments in DC4 counts in 8/15 during the 8-week trial of ddC as a single agent. We also treated 13 of these 15 patients with an alternating schedule of ddC and AZT and found this to be non-toxic and tolerable during a minimum follow-up of 18 months.

4. We also initiated a protocol to evaluate the combination of AZT with colony stimulating factor in order to overcome the myelosuppression of AZT. One patient was treated with rGM-CSF plus AZT, and although the leukocyte count rose, more than 99% were eosinophils. More recently, we have begun to treat patient with rG-CSF and AZT. The preliminary results are encouraging.

5. 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, 78 children have been enrolled at several dosage levels (20, 40, 60, 90, 120 mg/m2/every 8 hours. This protocol enrolls both children who have received no prior anti-retroviral therapy as well as children who have become refractory or intolerant to AZT. We have completed the 6 month follow-up on the first 43 class P2 symptomatic HIV-infected children, (27 previously untreated children and 16 prior AZT recipients) and have evaluated doses of 60, 120, 180, 360, and 540 mg/m2/day. ddI was rapidly absorbed after oral administration, however, there was significant variability in its bioavailability. Pancreatitis occurred in two patients, one at each of the two highest dose levels. Median CD4 cell count increased from 218/mm3 at baseline to 327/mm3 at 24 weeks (P=-0.001). Patients with baseline CD4 cell counts greater than 100/mm3 were significantly more likely to show an increase in this parameter. Median p24 antigen declined from baseline to 24 weeks (p=0.005), and there was a significant correlation between ddI plasma concentration and decline in p24 antigen level. A significant correlation was also found between ddI plasma concentration and improvement in cognitive function. Improvements in clinical and immunological parameters occurred in previously untreated patients and in prior AZT recipients. Dideoxyinosine was well tolerated and shows promising antiretroviral activity in HIV-infected children. The correlation between response and plasma ddI concentration indicates that bioavailability is an essential consideration for optimizing ddI activity in the treatment of HIV infection.

6. As part of our efforts to evaluate new antiretroviral agents in children, we initiated a Phase I study of recombinant soluble CD4 (rCD4) administered steroid by continuous infusion. To date, 10 patients have been enrolled. In addition to evaluating the safety, toxicity and antiviral activity of rCD4 as a single agent, we are evaluating the combination therapy of rCD4 and ddI in this study, by administering oral ddI, in addition to intravenous rCD4, to patients on this study after an initial 12 weeks of rCD4 alone. No significant toxicity has been observed among patients receiving CD4, or rCD4 in combination with ddI.

7. PMN from HIV-infected children were demonstrated to have significant impairment in their bactericidal capacity against S. aureus. In vitro incubation of defective PMN with GM-CSF corrected the bactericidal impairment. These findings may help explain the increased incidence of bacterial infections in this population, and suggest a potential therapeutic role for GM-CSF.

8. To better understand the humoral deficiency of HIV+ children, we measured IgG subclasses and correlated their levels with the frequency of bacterial infections. No association was found between low levels of specific IgG subclasses and increased susceptibility to bacterial infections. We concluded that other functional parameters than quantities of antibodies are more important in humoral deficiency of these patients.

9. Because T helper cells are the critically involved immune cells in HIV infection, we investigated their function in a group of HIV+ children and compared it to that of HIV-adults and healthy control children. Different patterns of unresponsiveness of T helper cells to recall and allogeneic antigens as well as 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.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06840-15 PB

PERIOD COVERED
October 1, 1989, to September 30, 1990

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, Leukemia Biology Section	PB, NCI
Others:			
	F. Balis	Senior Investigator	PB, NCI
	P. Adamson	Medical Staff Fellow	PB, NCI
	S. Berg	Medical Staff Fellow	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).

LAB/BRANCH
Pediatric Branch

SECTION
Leukemia Biology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

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

Clinical research into the biology and treatment of acute leukemia is pursued with particular emphasis on acute lymphoblastic leukemia (ALL) of childhood. Major issues being addressed include: 1) development of therapeutic strategies aimed at improving overall prognosis of children with ALL, 2) investigation into the mechanisms of treatment failure with particular emphasis on evaluation of pharmacologic approaches to leukemic therapy, 3) characterization of adverse sequelae of antileukemic therapy and design of treatment regimens which avoid them, and 4) studies of the biology of ALL aimed at improving our basic understanding of the biology of this disease, identifying new diagnostic and prognostic tests and providing insight into the biologic basis for treatment failure.

An earlier ALL treatment protocol demonstrated that high-dose, protracted systemic methotrexate infusions could substitute for cranial radiation as central nervous system (CNS) preventive therapy for the majority of patients with ALL. Analysis of data from this study also identified a patient group at particular risk for CNS relapse. A new, high risk protocol has been devised in an attempt to improve the prognosis for these and other poor risk patients. The results to date indicate that this therapy is highly effective in preventing both systemic and central nervous system relapses while avoiding the use of cranial radiation. In patients in the average risk category, a comparison of two forms of CNS preventive therapy (intrathecal vs high dose methotrexate) is under way. A major, multi-institutional pharmacologic monitoring protocol is in progress which is studying the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL. Detailed analysis of the immunologic and molecular phenotype of acute lymphoblastic leukemia has led to the concept of a hierarchy of differentiation for both T cell and pre-B cell ALL. Studies are in progress to determine the relationship of molecular phenotype to prognosis. Evaluation of the P53 gene, a candidate tumor suppressor gene, suggests this gene may play a role in the pathogenesis of this disease.

Objectives

1. To develop effective treatment strategies which will improve the event-free survival of children with ALL, with particular emphasis on a) the development of alternative CNS preventive therapy and b) improvement of treatment for patients with poor risk features.

2. To characterize the long-term adverse sequelae of antileukemic therapy and design treatment regimens that avoid them.

3. To study the clinical pharmacology of antileukemic agents with the objective of optimizing ALL treatment through: a) exploration of the pharmacologic basis of treatment failure in ALL, b) development of new treatment strategies, with currently available antileukemic agents, which are based on sound pharmacologic rationale, and c) identification of promising new antileukemic agents.

4. To conduct studies of the biology of ALL in an attempt to increase our basic understanding of this disease and to identify biological characteristics which will provide avenues for new therapeutic approaches.

Methods and Major Findings:

A. Treatment Studies of Acute Lymphoblastic Leukemia

1. NCI 77/02/CCG 191 Treatment Protocol

A randomized protocol investigating the efficacy of high dose intravenous methotrexate infusions as CNS preventive therapy. Patients received either cranial radiation plus intrathecal methotrexate or high dose 24-hour intravenous methotrexate infusions. One-hundred-eighty-one (181) average and high risk patients were randomized on this study. The overall remission rate was 98%. The continuous complete remission rate is approximately 67% at three years for the entire study group. With a median duration on study of 86 months, there is no significant difference in the CNS relapse rate for either treatment group. Longitudinal evaluation of neuropsychological function has demonstrated a striking decrease in IQ test scores and impaired academic achievement in children treated with cranial radiation and intrathecal chemotherapy. No such changes have been observed in children treated with high dose methotrexate. The results of this study not only demonstrated that alternative CNS preventive therapy is feasible and as efficacious as cranial radiation and IT MTX, but also served to focus attention on the importance of avoiding neurotoxic regimens using cranial radiation. This study led to the development our two current clinical trials discussed below.

2. NCI 83-P/CCG 134P

The major aim of this pilot protocol is to demonstrate that high risk patients can be effectively treated on a regimen that uses CNS preventive therapy devoid of cranial radiation. To date, 107 patients have been entered on study; 96% achieved complete remission. With a median duration on study of 3.7 years, the event free

survival (at 24 months) is approximately 65%. The occurrence of isolated CNS relapse in only three patients, to date, suggests that effective CNS preventive therapy can be achieved without the use of cranial radiation in high risk patients.

3. NCI 84-A/CCG 144

This protocol randomizes average risk patients in one of two forms of CNS preventive therapy - either high dose methotrexate infusions or intrathecal methotrexate alone. One hundred sixty-six patients have been randomized on study. With a median potential duration on study of 46 months, 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.

B. Pharmacologic Approaches to Leukemic Therapy: Relationship to Treatment Failure

A detailed study of the bioavailability of the major orally administered antileukemic agents is being undertaken in an attempt to examine the reasons for treatment failure in children with ALL.

1. Prospective Evaluation of Oral 6-MP and MTX Bioavailability.

This study is attempting to correlate the results of prospective periodic pharmacokinetic bioavailability studies of 6-MP and methotrexate with relapse rate and remission duration in a multi-institutional setting. Approximately 100 patients have been entered to date.The bioavailability and pharmacokinetics of oral 6-MP and MTX are studied on four separate occasions during the course of maintenance therapy in children with average and good risk ALL. Erythrocytes are periodically examined for MTX and 6-MP nucleotide content. To date, clinical information regarding disease status and toxicity in this group is still too incomplete for meaningful analysis. However, we have begun to analyze the "population" pharmacokinetics of these two agents. We have confirmed the wide inter-patient variability in plasma MTX and 6-MP concentrations following oral administration under standardized conditions, and have defined the "normal" range of the area under the plasma concentration-time curve (AUC) for both drugs. We are also able to evaluate the intra-patient variability in drug bioavailability; preliminary analysis reveals much greater variability with 6-MP than with MTX. This variability within the same patient may limit the application of therapeutic drug monitoring of 6-MP therapy. The absorption of these two agents does not appear to decline over the course of maintenance therapy, and the degree of absorption of one agent does not correlate with how well or poorly the other drug is absorbed. When patient accrual is complete and sufficient follow-up is available, the final pharmacokinetic analysis and clinical correlations will be made.

2. Chronopharmacology of Maintenance Therapy.

A retrospective study recently cited a five-fold greater risk of relapse in children with ALL who took their oral maintenance therapy on a morning rather than an evening schedule. A possible explanation was that the difference in the efficacy of

the morning and evening schedules reflected a difference in total drug exposure that resulted from circadian periodicity in the disposition of 6-MP and MTX. We have investigated the chronopharmacokinetics of both 6-MP and MTX in children with ALL to determine if there is a pharmacokinetic basis for this observation. Children were fasted and given either 6-MP or MTX at both 8:00 AM and 8:00 PM. No significant differences were noted in the plasma concentrations of either 6-MP or MTX on the two administration schedules. Thus, we are unable to confirm that diurnal variation in the absorption or elimination of 6-MP and MTX plays a role in the response to maintenance therapy with these drugs.

3. Alternate Dosing Methods.

We have investigated alternate routes of administration for both 6-MP and MTX as possible methods of optimizing maintenance chemotherapy with these agents.

Subcutaneous MTX.

We have studied subcutaneous MTX as a parenteral alternative to oral administration. The subcutaneous route has several potential advantages including slow release of the drug leading to more prolonged drug exposure, ease of administration, and more complete and less variable absorption than oral administration. Two dose levels (7.5 and 40 mg/m^2) were studied, and each child was monitored twice, after an oral dose and after the same dose administered subcutaneously. The subcutaneous dose was well tolerated and well absorbed at both dose levels studied. In contrast, the oral dose produced comparable plasma concentrations at the lower dose, but total drug exposure (AUC) at the higher dose was only one third that achieved with the subcutaneous dose, presumably a result of saturation of the mechanism responsible for MTX absorption in the gastrointestinal tract. Subcutaneous administration appears to be a viable alternative in patients with poor gastrointestinal absorption at lower doses and in patients receiving doses greater than 30 mg/m^2.

4. Alternative Maintenance Agents.

We are actively studying nonclassical antifolates which may be alternatives to MTX, such as trimetrexate and piritrexim. A phase I trial of trimetrexate on a once weekly for 3 weeks schedule has recently been completed and a phase I trial of oral piritrexim is ongoing (see *Clinical Pharmacology Project Report*).

C. Drug Resistance.

In order to overcome drug resistance we must also define the mechanisms through which leukemic cells become resistant to the antileukemic agents. In collaboration with laboratories in the Medicine Branch we have screened lymphoblasts from our patients on a molecular level for the presence of multidrug resistance caused by overexpression of *mdr*-1/P-170 and glutathione S-transferase (GST). In addition, mechanisms specific to individual agents, such as the overexpression of dihydrofolate reductase (DHFR), the target enzyme of MTX have also been evaluated in leukemic cells.

Multi-Drug Resistance.

Lymphoblasts from 28 patients were studied for evidence of *mdr*-1/P-170, the gene encoding for the plasma membrane glycoprotein associated with multidrug resistance, using RNase protection, RNA *in situ* hybridization and immuno-histochemistry. Overexpression without gene amplification was identified in the cells of three relapsed patients and from one patient at diagnosis (this patient failed to achieve a complete remission with induction therapy). In situ hybridization, immuno-histochemistry, and drug uptake studies demonstrate that this overexpression is heterogeneous. It appears from these studies that overexpression of *mdr*-1/P-170 is one mechanism of drug resistance in ALL.

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, performed in collaboration with the NCI/Navy Medical Oncology Branch, have been aimed at determining whether there is a correlation between molecular genotype in ALL and a variety of biologic and clinical features known to have a prognostic import (e.g. cytogenetics, initial white blood cell count, FAB morphologic classification, etc.) as well as with treatment outcome. Lymphoblasts obtained at diagnosis from patients treated on our "front line" ALL treatment protocols have been prospectively studied with cytogenetics, immunophenotyping (using FACS analysis and a panel of monoclonal antibodies), and molecular characterization. An analysis of this data suggests that genotypically less mature leukemias may manifest a more difficult course, and that genotype heterogeneity may be of clinical relevance. In a current study 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 is being evaluated.

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. 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.

E. Interleukin-2 and Acute Lymphoblastic Leukemia

IL-2 as therapy for Acute Lymphoblastic Leukemia

In vitro studies with IL-2 have demonstrated its ability to induce phenotypic and functional maturation in a subset of acute leukemic lymphoblasts. This observation led to the development of a Phase I trial in patients with hematologic malignancies. IL-2 was administered intravenously by continuous infusion for five days on a weekly, for three successive week, schedule. The starting dose of 10^5 units/m^2/day was escalated according to standard phase I guidelines. While the aim of this ongoing study was to determine the maximally tolerated dose of IL-2 on this continuous infusion schedule, biological studies were also performed to evaluate the effects of IL-2 *in vivo*. This study has been completed and has defined a dose of $3x10^6$ units/m2/day as a safe dose for Phase II trials. As part of this trial, serum levels of a soluble form of the P55 subunit of the Il-2 receptor complex (sTAC) were measured at regular intervals in patients undergoing Il-2 therapy. Predictable patterns of sTAC release were observed in accordance with the dose and schedule of IL-2. The studies indicate that sTAC analysis is a useful clinical tool for monitoring patients on treatment regimens with IL-2. As a result of this study, a Phase II study of IL-2 is being pursued on a national level by the Children's Cancer Study Group.

F. New Agent Studies in Relapsed Patients

1. Phase I and Phase II Trials.

The major focus of our studies for relapsed patients with ALL is on phase I and phase II trials of investigational agents. Emphasis is placed on those new drugs, examined in our laboratory, for which there exists a significant pharmacologic rationale for their use in the treatment of leukemia. Within the past year we have carried out and completed two phase I trials, including piritrexim and Interleukin-2. For a detailed listing and discussion of these new agent studies the reader is referred to the *Clinical Pharmacology Project Report.*

2. New Intrathecal Agents.

In recent years, we have focused attention on the development of new pharmacologic approaches to the treatment of CNS leukemia. Although numerous

drugs are available for systemic administration to treat ALL, the number of agents suitable for intrathecal use is limited; no new intrathecal agents have been identified in over 25 years. In contrast to the successful treatment of systemic leukemia which is predicated on the use of combination chemotherapy, the extremely limited number of intrathecal agents restricts clinicians to the use of only one or two agents (e.g. MTX and Ara-C) which belong to the same drug class (antimetabolites). It is conceivable that if effective new intrathecal agents could be identified the development of combination intrathecal chemotherapy regimens could have the same impact on the control of CNS leukemia as combination chemotherapy has had on control of bone marrow disease. In addition, since CNS preventive therapy with cranial radiation is associated with adverse CNS sequelae, new intrathecal agents are also needed for CNS preventive therapy. Thus, the identification of effective new intrathecal agents has become an appropriate and important priority. Four new intrathecal approaches developed in our nonhuman primate model are currently undergoing clinical study including intrathecal diaziquone (AZQ), intrathecal 6-mercaptopurine, intrathecal mafosfamide and continuous intraventricular methotrexate infusions. These studies are detailed in the *Clinical Pharmacology Project Report.*

G. Memory and Learning Sequelae in Long-Term Survivors of Acute Lymphoblastic Leukemia

A systematic study of verbal and nonverbal memory and learning was undertaken in long-term survivors of acute lymphoblastic leukemia to assess the incidence and pattern of impairments and to determine the relationship between these deficits and computed tomography (CT) brain scan abnormalities. Twenty-three children who had received cranial irradiation (2,400 cGy) and intrathecal chemotherapy as central nervous system (CNS) preventive therapy and who were off all therapy for at least 4 years were evaluated. On the basis of their CT brain scan findings, patients were divided into three groups: those with intracerebral calcifications (n = 5), those with cortical atrophy (n = 8), and those with normal CT findings (n = 10). Significant deficits in verbal memory ($p < 0.025$) and verbal learning (p 0.05) were observed that were associated with the presence and type of CT brain scan abnormalities; the greatest impairments were observed in patients with calcifications. No significant differences between CT scan groups were found for nonverbal memory and learning. Previous evaluation of attentional processing in these patients using reaction time tests had revealed the presence of deficits primarily in the ability to sustain attention. Combining those data with findings from the present study showed that memory impairments, particularly those in short-term memory, were primarily attributable to an underlying attentional defect that affect the encoding stage of memory processing.

Publications

Balis FM, and Poplack DG: Central nervous system pharmacology of antileukemic drugs. Am J Pediatr Hematol/Oncol 1989;11(1):74-86 .

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06880-13 PB

PERIOD COVERED

October 1, 1989, to September 30, 1990

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)*

PI:	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
Others:			
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	P. Adamson	Medical Staff Fellow	PB, NCI
	S. Berg	Medical Staff Fellow	PB, NCI
	S. Blaney	Walter Reed Army Medical Center	

COOPERATING UNITS *(if any)*

Medicine Branch, NCI (C. Allegra); Childrens Cancer Study Group (J. Holcenberg); St. Jude Children's Cancer Research Hospital (R. Heideman).

LAB/BRANCH

Pediatric Branch

SECTION

Leukemia Biology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

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

The clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies is studied with emphasis on the role of pharmacologic monitoring and on both pre-clinical and clinical pharmacologic studies of Phase I agents. The clinical pharmacology of orally administered antileukemic agents has been evaluated and the limited bioavailability and variable drug levels of 6-MP achieved following oral administration has been documented. Studies are underway to determine the extent to which this phenomenon is the cause of treatment failure. Preclinical and clinical pharmacokinetic studies of a variety of new agents including Piritrexim, All-trans retinoic acid and Thiotepa plus GM-CSF are in progress. A major effort of this project is to study experimental approaches to the treatment of CNS malignancy. A unique primate model is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities of various CNS treatments; and to evaluate and screen newer CNS treatment modalities and drug schedules. Information gained from the studies with this model is then applied to the design of clinical treatment protocols. Protocols evaluating strategies such as prolonged intravenous 6-MP infusions and intravenous Thiotepa for brain tumors are under way. Clinical studies of intrathecal AZQ, intrathecal 6-MP, and intrathecal mafosfamide, all approaches developed in this model, are in progress. A clinical study evaluating continuous intra-CSF drug infusion via a unique indwelling drug delivery device also is under way. As part of the Pediatric Branch AIDS research effort, the Leukemia Biology Section is studying the clinical pharmacology of antiretroviral agents in children. The study of these agents is a natural extension of our work on the clinical pharmacology of anticancer drugs, since most of the antiretroviral agents are nucleoside analogs, similar to the antimetabolites used in the treatment of ALL. The CNS pharmacology of antiretroviral therapies is being systematically evaluated in our non-human primate model, to determine which agents may be most effective against the CNS HIV infection. We have also participated in the design of clinical trials of antiretroviral agents in children and performed detailed pharmacokinetic studies in the children treated on these trials.

GPO 914-918

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 explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.

3. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.

4. To study the pre-clinical and clinical pharmacology of new anti-retroviral agents undergoing Phase I testing in children.

Methods Employed and Major Findings:

A. Clinical Pharmacology of Antineoplastic Agents

1. Clinical Studies on Thiotepa

We have evaluated the clinical pharmacology of Thiotepa in children with malignancy. Thiotepa is an active alkylating agent with a steeper dose-response curve than cyclophosphamide. Our studies have demonstrated that substantial amounts of both thiotepa and its metabolite Tepa are present in the CNS following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent than intrathecal injection. As a result of these studies, a Phase I study of intravenous thiotepa in pediatric patients was undertaken and completed. The results suggested that systemically administered thiotepa may be a valuable agent for the treatment of CNS malignancies. In addition, this study demonstrated that thiotepa can be safely administered to pediatric patients at significantly higher doses (the MTD was 65mg/m^2) than those used conventionally in adults. *In vitro* studies of the activity of thiotepa and tepa against human CNS tumors have been performed using medulloblastoma and glioma cell lines. Both thiotepa and tepa show significant *in vitro* activity against these CNS tumor cell lines at drug concentrations achievable in patients at the dose recommended in our phase I trial.

Based on these findings the following studies have been pursued:

Phase II Trial of Intravenous Thiotepa in Pediatric Brain Tumors

A collaborative study of intravenous thiotepa at a dose of 65 mg/m^2 for pediatric patients with brain tumors is in progress.

Phase II Trial of Thiotepa in Pediatric Solid Tumors

A Phase II study of thiotepa in pediatric solid tumors is being pursued together with the Children's Cancer Study Group.

Phase I trial of the Combination of Thiotepa and GM-CSF

High dose thiotepa, in combination with other agents, is being used as preparative therapy for autologous bone marrow transplantation for the treatment of brain tumors. Although promising clinical responses have been observed, the inability to repeatedly perform autologous bone marrow transplantation limits this therapeutic approach. Granulocyte-macrophage colony stimulating factor (GM-CSF) is one of several cloned hematopoietic growth factors which have been demonstrated to significantly modify the degree and duration of chemotherapy induced neutropenia. We have initiated a phase I protocol designed to evaluate the feasibility of administering escalating intravenous doses of thiotepa in conjunction with GM-CSF. In this study, patients will receive intravenous thiotepa on an every three week schedule starting at the MTD defined in our previous phase I study. GM-CSF is administered subcutaneously during the post-chemotherapy period. The thiotepa dose will be escalated (in 30% increments) with the aim of determining the highest dose of thiotepa which can be safelv administered with adjunctive GM-CSF therapy. If effective, subsequent studies evaluating this approach in patients with CNS and other pediatric malignancies will be initiated.

2. Preclinical Studies of Cyclopentenyl Cytosine (CPE-C)

Cyclopentenylcytosine (CPE-C), a synthetic cytidine analogue is currently undergoing extensive preclinical testing and has been demonstrated to have significant antitumor activity. It is active in vivo against the P388 and L1210 murine leukemias and against human lung, melanoma and breast cancer xenografts grown in athymic mice. In addition, cytarabine resistant murine leukemia lines are collaterally sensitive to CPE-C. The plasma and cerebrospinal fluid (CSF) pharmacokinetics of cyclopentenyl cytosine (CPE-C) have been studied following i.v. bolus and continuous i.v. infusion in rhesus monkeys. Following an i.v. bolus dose of 100 mg/m^2 plasma elimination of CPE-C was biexponential with a mean $t_{1/2\alpha}$ of 8.8 min, a mean t1/2B 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 cytarabine (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-U 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 total drug exposure than in rodents and dogs that clear CPE-C at a much lower rate by renal excretion. The significant interspecies differences in the disposition of CPE-C discovered in this study are important and should be considered in the selection of a starting dose and schedule for human trials. These findings also suggest that a pharmacologically directed dose escalation scheme should be used in the planned phase I studies.

3. Phase I Studies

In addition to studies of Thiotepa we have performed Phase I studies on other agents including Piritrexim, a new nonclassical antifolate that is available in an oral formulation, and interleukin-2, which is discussed in the Childhood Leukemia Project Report. A phase I trial of All-trans retinoic acid has recently been initiated.

4. New Intrathecal Agents

Based on our work in the non-human primate model three new intrathecally administered agents are being investigated in clinical trials, intrathecal AZQ,intrathecal 6-MP, and intrathecal mafosfamide. An additional, novel approach being studied involves the continuous intraventricular administration of methotrexate using a portable, computerized delivery pump. We are also evaluating two additional strategies in our pre-clinical models which have potential application relevant to the treatment of meningeal malignancy, these include intrathecal 5-Fluorouracil and the use of carboxypeptidase as a rescue from intrathecal methotrexate overdose.

Intrathecal AZQ (Diaziquone).

AZQ is a lipophilic alkylating agent designed for enhanced penetration of the blood-brain barrier. In preclinical studies, we demonstrated that following intravenous infusion, significant levels of AZQ were achieved in CSF . However, in subsequent clinical phase II studies evaluating parenteral AZQ for treatment of brain tumors, the systemic administration of this compound was found to be associated with severe, cumulative and dose-limiting hematologic toxicity. Because of the considerable preclinical data indicating that AZQ is active against a variety of CNS tumors as well as leukemias, we evaluated the possibility of administering AZQ intrathecally. Initially we studied the CSF pharmacokinetics of AZQ following intraventricular injection in our sub-human primates and found that ventricular and lumbar CSF drug exposure (AUC) were 20- and four- fold higher, respectively, than the CSF AUC achieved with intravenous administration of 80 times the intraventricular dose . The feasibility and safety of intraventricular AZQ was also confirmed in the model. As the result of these studies, we developed a phase I/II trial of intrathecal AZQ which is currently in progress. Two dose schedules of AZQ are being evaluated in patients with refractory meningeal neoplasia, including standard bolus intrathecal administration of 1 mg twice weekly or a CxT schedule (0.5 mg intraventricularly every 6 hours x 3 doses). The CxT approach is designed to take advantage of the greater antitumor activity that we noted with this agent *in vitro* following prolonged drug exposure. To date, a total of 38 patients with refractory meningeal malignancy have been entered onto this protocol. Complete

responses have been achieved in 15 patients, ranging from one to nine months in duration. No significant neurologic or systemic toxicity has been observed. These promising results in a group of heavily pretreated patients suggests a future role for intrathecal AZQ in the treatment of CNS leukemia and other meningeal malignancies.

Intrathecal 6-Mercaptopurine.

We have examined the feasibility of administering 6-MP by the intrathecal route. In initial studies in the nonhuman primate model we demonstrated that 6-MP could be safely administered by the intraventricular route. CSF 6-MP concentrations were found to decline biexponentially with t1/2's of 40 minutes and 2.8 hours. In addition, our results indicated that concentrations of 6-MP found to be cytocidal *in vitro* against a variety of human tumor cell lines could be readily achieved in CSF at doses that are well tolerated. As an extension of these studies we recently initiated a clinical phase I trial of intrathecal 6-MP in patients with refractory meningeal malignancy. Both bolus administration (at a dose of 10 mg) and a CxT schedule (1 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 possibility of administering 4-hydroperoxycyclophosphamide or mafosfamide intrathecally. In our nonhuman primate model intrathecal injection of these compounds 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.

5. 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 are currently studying in our Rhesus monkey model. In previous studies in our laboratory, pharmacokinetic modeling with cytarabine demonstrated the potential pharmacokinetic advantages of continuous intrathecal administration in maintaining a minimal cytotoxic concentration in the CSF for a prolonged period with a much lower total dose. In addition, the chemical arachnoiditis frequently associated with intrathecal therapy has been linked with the high peak CSF concentrations following bolus injection. This can be avoided when the drug is given by low-dose continuous infusion. The Rhesus monkey model was adapted to enable us to perform these studies. A new technique was developed in which a cannula is inserted into the lateral ventricle and then attached to a subcutaneously implanted catheter with a reservoir which is attached to a portable infusion pump containing the drug to be studied. In preliminary studies we have found that with continuous infusion of MTX, ventricular CSF MTX concentrations are maintained at 1 µmol/L for two- to three-fold longer than with the bolus dose, despite the fact that only one tenth of the total bolus dose was administered by infusion. Thus, these studies directly demonstrate the clear pharmacokinetic advantage for continuous intrathecal infusion. A clinical protocol evaluating this approach has recently been initiated. 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.

6. Intrathecal 5-Fluorouracil: A Potential Treatment for Meningeal Malignancy

We are exploring the feasibility of intrathecal treatment with 5-Fluorouracil (5-FU). Were intrathecal 5-FU feasible it might have a clinical role in treating meningeal spread of a variety of disorders including leukemia, breast cancer, and lung cancer. The pharmacokinetics and clinical toxicity of 5-FU administered intraventricularly have been studied in a previously described nonhuman primate model. Three rhesus monkeys received a 10 mg intraventricular bolus dose of 5-FU via a fourth ventricular Ommaya reservoir. Ventricular (V) CSF was sampled before the dose and at 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose. Lumbar (L) CSF was also obtained from a percutaneous lumbar catheter at 2, 4, 6, and 8 hours post-dose in one animal. The mean V-CSF 5-FU concentration at 0.5 hr was 14,000 µmol/l and declined monoexponentially with a half-life of 50 minutes. V-CSF 5-FU concentration remained above 1 µmol/L (a cytotoxic concentration *in vitro*) for 10-12 hours, while the L-CSF concentration at 8 hours post-dose was 10 µmol/L. Total V-CSF drug exposure as measured by the mean area under the V-CSF concentration-time curve (AUC) was 18,000 µmol/L•hr, and the V-CSF clearance of 5-FU was 0.07 ml/min (CSF bulk flow rate=0.04 ml/min), suggesting that 5-FU is cleared from the CSF by other mechanisms in addition to bulk flow. Despite the high dose (equivalent to a 100 mg IT dose in man), there was no evidence of systemic or neurologic toxicity following a single bolus administration of 5-FU in these animals. Chronic administration of this dose on a weekly basis was found to produce neurologic toxicity in 2 of 8 animals. Evaluation of a lower, potentially less toxic dose schedule is underway. Intrathecal administration of 5-

FU may yet prove to be a feasible method of achieving cytocidal levels of this highly active antitumor agent in the CSF.

7. Rescue of Experimental Intrathecal Methotrexate Overdose with Carboxypeptidase - G_2

MTX is the most commonly used intrathecal (IT) agent for the treatment of meningeal malignancy. The frequency of overdose is low (probably 6 to 12 cases per year), the prognosis of patients administered an overdose is grave. The carboxypeptidase G class of enzymes rapidly hydrolyze methotrexate (MTX) into the inactive metabolites 4-deoxy-4-amino-N^{10}-methylpteroic acid and glutamate. We evaluated the use of carboxypeptidase-G_2 ($CPDG_2$) as a potential intrathecal (IT) rescue agent for massive IT MTX overdose. The cerebrospinal fluid (CSF) pharmacokinetics of MTX with and without $CPDG_2$ rescue was studied in adult rhesus monkeys (*Macaca mulatta*) using a nontoxic IT 5 mg dose (equivalent to 50 mg in humans). Without $CPDG_2$ rescue, peak CSF MTX concentration was 2904±340 μM. Within 5 minutes of administration of 30 U IT $CPDG_2$, CSF MTX concentrations decreased greater than 400-fold to 6.55±6.7 μM. Subsequently, groups of three monkeys received either 25 mg IT MTX (equivalent to 250 mg in humans) followed by 150 U IT $CPDG_2$ or 50 mg IT MTX (equivalent to 500 mg in humans) followed by 300 U IT $CPDG_2$. All animals survived without neurotoxicity. These studies suggest that $CPDG_2$ may prove to be an important addition to currently recommended approaches for the management of IT MTX overdose.

B. In Vitro System for Evaluating Cytotoxicity of Unstable Drugs

Conventional *in vitro* methods for determining the activity of anticancer agents do not address the problem posed by chemically or metabolically unstable drugs. With a prolonged duration of drug exposure, unstable drug's cytotoxic potential may be significantly underestimated. To circumvent this problem, an *in vitro* system was developed in which tumor cells can be exposed to constant concentrations of unstable drugs for prolonged periods. This model consists of 2 chambers separated by a permeable membrane. Tumor cells are grown in the static "cell" chamber (CC), and drug in the CC is continuously replenished across the membrane from a flow-through "infusion" chamber (IC) resulting in a uniform concentration of drug in CC culture medium. The Molt-4 lymphoblastic cell line was successfully cultured in and cloned from the CC. Infusion of fazarabine (FZB), a chemically unstable drug (tissue culture decay $T_{1/2}$ =10 h) through the IC, yielded a FZB concentration in the CC that was 50% of stock drug solution by 8 h and this was maintained for >24 h. The addition of a loading dose of FZB to the CC with concomitant infusion of FZB through the IC resulted in a constant, predictable FZB concentration in the CC that was maintained for longer than the 22 h doubling time of Molt-4 in culture. This new *in vitro* model 1) provides a method for assessing cytotoxicity following prolonged exposure of cells in culture to unstable drugs, and also 2) permits the comparison of clinically relevant schedules of drug administration *in vitro* (e.g. bolus vs. continuous infusion).

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 Leukemia Biology Section is studying the clinical pharmacology of antiretroviral agents in children. The purpose of this project is to investigate the clinical pharmacology of both new and clinically available anti-AIDS drugs in children. Specifically we are studying 1) the pharmacokinetics of antiretroviral agents in order to determine the optimal route and schedule of administration and to establish correlations between pharmacokinetic parameters and both treatment response and toxicity; and 2) the central nervous system pharmacology of existing and proposed AIDS therapies in order to predict the potential clinical efficacy against AIDS dementia complex. An additional aim that relates to the clinical pharmacology of our new agent studies is to 3) study the clinical pharmacology of the nonclassical antifolates, TTX and piritrexim, which are now proposed for the treatment of Pneumocystis carinii pneumonia in patients with AIDS.

1. Pharmacokinetics of Antiretroviral Agents in Children

Studies with AZT

We have characterized the pharmacokinetics of AZT in 37 children with symptomatic HIV infection treated in the Pediatric Branch. These children were being treated on one of two phase I protocols utilizing either an intermittent (every 6 hour) or continuous infusion schedule of AZT. With intravenous bolus dosing the elimination of AZT in children was rapid and biexponential with half-lives of 14 and 90 minutes and a total clearance of 680 ml/min/m^2. The major pathway of elimination appears to be the metabolic transformation of AZT to its 5'-glucuronide conjugate (GAZT). The renal clearance of 170 ml/min/m^2 suggested that the drug is both filtered and secreted by the renal tubule. There was considerable interpatient variation in the rate of drug elimination and there was no evidence of dose-dependency in the rate of AZT elimination. Oral bioavailability of AZT was also determined to be 68%. A simulation of the dose and schedule of AZT (180 mg/m^2 every 6 hour) proposed for children revealed that, with intermittent IV bolus dosing of AZT, plasma concentrations of AZT remain above the target level of 1 µmol/L for less than half of the dosing interval, and that the steady state trough concentrations are less than 0.2 µmol/L suggesting that this dose and schedule may be inadequate given the presumed importance of sustained continuous exposure to virostatic concentrations of AZT. For this reason we pursued an alternative approach utilizing the continuous infusion of AZT via a portable infusion pump. We studied 21 children treated on this schedule at one of four dose levels. Plasma concentrations of AZT were maintained above 1 µmol/L even at the lowest dose level, demonstrating a clear pharmacokinetic advantage for this schedule over

intermittent administration. This point was illustrated by a pharmacokinetic simulation which demonstrated that, using this intermittent schedule, a dose of 1,000 mg/m^2 every six hours would be required to maintain a minimum plasma concentration of 1 µmol/L. Pharmacokinetic parameters obtained on the continuous infusion schedule were similar to those obtained on the bolus AZT study. Drug clearance was age-related, especially when normalized to body weight with younger patients demonstrating more rapid clearance. The difference was less striking when clearance is normalized to body surface area, suggesting that dose should be calculated based on surface area rather than weight in future studies. In this Phase I study neutropenia was the dose-limiting toxicity; the degree of neutropenia appeared to be related to the plasma concentration of AZT. Patients who dropped below an ANC of 500/mm^3 during the first six weeks of therapy had significantly higher plasma AZT concentrations (mean 3.6 µmol/L) than those who remained above 500/mm^3 (mean 2.6 µmol/L).We have suggested that 3.0 µmol/L should be considered a toxic level on the continuous infusion schedule, pending more extensive studies. The identification of this toxic level along with the significant interpatient variability noted indicates a potentially important role for therapeutic drug monitoring in AZT therapy. Since the results of the clinical trial of continuous infusion AZT suggest efficacy for this schedule (see AIDS Project Report) , two new approaches are currently being investigated to provide continuous exposure to AZT-- the continuous infusion of AZT subcutaneously (see below) and the development of an oral sustained-release formulation.

2. Pharmacokinetics of Subcutaneous Azidothymidine in Rhesus Monkeys

The pharmacokinetics of subcutaneous bolus and continuous infusion azidothymidine (AZT) was studied in rhesus monkeys. Three animals received 100 mg/m^2 as a bolus injection both intravenously and subcutaneously, with the order of administration randomly determined. Two animals received a continuous subcutaneous infusion of 25 mg/m^2 per h for 12 or 24 h. AZT was measured in plasma by a reverse-phase high-pressure liquid chromatographic assay. Following intravenous bolus administration, AZT elimination was rapid, with a mean half-life of 1.2 h and a mean clearance of 318 ml/min per m^2 (range, 200 to 441 ml/min per m^2). The bolus subcutaneous dose was rapidly (time to peak concentration, 15 to 30 min) and nearly completely (fraction absorbed, 92%) absorbed without evidence of local tissue toxicity. With continuous subcutaneous infusion of AZT, the steady state was attained within 4 h and steady-state concentrations in plasma in the two animals exceeded 3.0 mmol/liter. No local tissue toxicity was observed at the infusion site. The subcutaneous route may be a practical alternative to intravenous administration of AZT and deserves further clinical study.

3. Pharmacokinetics of Dideoxycytidine in Children with HIV Disease

Dideoxycytidine (ddC) a dideoxynucleoside which, like AZT, inhibits reverse transcriptase, was evaluated in a Phase I study of children with symptomatic HIV infection (see AIDS Project report). Patients on this pilot study were evaluated at 4 dose levels of ddC (0.015, 0.02, 0.03 and 0.04 mg/kg) administered orally on a q 6 h schedule. Pharmacokinetic studies were performed and the results compared to

adults receiving doses of ddC ranging from 0.03 to 0.5 mg/kg. The pharmacokinetic studies of ddC in the children in this study were limited by the low doses of the drug administered and the resultant low plasma concentrations achieved. Nevertheless, the pharmacokinetic parameters derived were found to be similar to those reported in adults. The total body clearance of ddC was 156 ml/min/m^2 and the half-life 0.8 hrs in children compared to 227 ml/min/m^2 and 1.2 hours in adults. The mean bioavailability of ddC in children was 54% with a broad range of from 29-100% compared to that in adults of 88% (range 72-121%). The data derived in this pilot pediatric study will be useful for planning subsequent Phase II studies incorporating ddC treatment for children with HIV disease.

4. Pharmacokinetics of Dideoxyinosine in HIV Infected Children

Dideoxyinosine (ddI) demonstrates potent *in vitro* activity against HIV and is reported to have a wider therapeutic index than AZT. Preliminary studies of this drug in adults were associated with little hematologic toxicity and demonstrated promising antiretroviral activity. To determine the safety and tolerance of ddI in children, a Phase I/II trial of ddI was undertaken by the Pediatric Branch in symptomatic HIV infected pediatric patients. In this study, 5 dose levels of ddI (60, 120, 180, 360, and 540 mg/m^2/day) were evaluated. 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. Twenty-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/L at the 20 mg/m^2 dose to 22 μM/L at 180 mg/m^2; the mean AUCs ranged from 3.4 μM•h/L to 32 μM•h/L. The half life of ddI following the intravenous dose was 0.8±0.4 hours and the total body clearance was 490±190 ml/m^2. Thirty-four 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 21% 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 that attention must be focused on 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 the desirable plasma concentration. Studies are under way to develop an optimal dose sampling methodology to facilitate this goal.

5. Central Nervous System Pharmacology of Antiretroviral Agents

Using our Rhesus monkey model we have systematically studied the CSF penetration of the antiretroviral agents and define those physicochemical properties that influence the degree of CNS penetration. Initially, the pyrimidine dideoxynucleosides AZT and ddC were studied in collaboration with the Clinical Pharmacology Branch. CSF penetration, as measured by the ratio of CSF to plasma drug concentration, was 21% for AZT and only 3% for dideoxycytidine. In contrast, when injected intraventricularly, no difference was noted in the CSF drug concentrations of these two agents, indicating that the difference in penetration was not due to a difference in the rate of clearance from the CSF. To determine the portion of the molecule that was responsible for this marked difference in penetration, we subsequently evaluated the penetration of dideoxythymidine, which had a CSF to plasma ratio of 30%, and azidodideoxycytidine which had a CSF to plasma ratio of 1%. These studies clearly indicate that the base (cytosine vs. thymine), rather than the 3'-substitution (azido group vs. none) on the sugar, determines the extent of CNS penetration. Of interest, the plasma protein binding and octanol/buffer partition coefficients of each of these compounds was also determined. None of the compounds was significantly protein bound. The azido group on the sugar resulted in a significant increase in the lipid solubility, but there was no apparent relationship between CSF/plasma ratios and lipid solubility. It appears, therefore, that a carrier-mediated process is primarily responsible for CNS entry of this class of drugs. As part of the phase I trial of continuous infusion AZT in children we measured simultaneous CSF and plasma steady state AZT concentrations in 21 children and found a CSF to plasma ratio of 24% - confirming the predictive ability of the Rhesus monkey model in studying antiretroviral agents. This degree of penetration correlates with improvements in neurologic status of the patients treated on this trial.

We are also studying a series of halogenated dideoxynucleotides to determine the degree to which these agents, which were developed to optimize CNS penetration, enter the cerebrospinal fluid.

6. Studies of Absorption of Anti-retroviral Compounds in an Animal Model

A sustained release oral preparation of the currently available dideoxynucleosides would have the advantage of providing more prolonged exposure to drug over the dosing interval than current oral formulations. However, sustained-release formulations are only of use with drugs that can be absorbed along the entire length of small intestine. Drugs absorbed at a specific site via a carrier are absorbed poorly from a sustained-release preparation. Therefore, prior to developing a sustained-release formulation, we evaluated the mechanism of absorption of AZT and ddI in an ex vivo rat intestinal loop model. These studies reveal that both drugs are passively absorbed, equally in all segments of the small intestine and would, therefore, be amenable to formulation in a sustained-release form.

7. Treatment of P. Carinii Pneumonia with Nonclassical Antifolates

The nonclassical antifolate, TTX, originally designed and tested as an anticancer drug, has been shown to be efficacious in the treatment of Pneumocystis carinii pneumonia (PCP). We previously performed a phase I trial and pharmacokinetic study of TTX in children with refractory cancer, including development of a specific assay for the drug and identification of metabolic pathways . The bioavailability of oral TTX was studied in patients with AIDS and found to be 44% . Oral absorption did not appear to be saturable, as has been previously described for naturally occurring folates and MTX, suggesting that TTX is absorbed by a different mechanism. Plasma TTX concentrations nearly equivalent to those achieved with an intravenous dose were attained by administering an oral dose that was two-fold higher. We are currently measuring 24 hour drug concentrations in a large number of AIDS patients in an attempt to correlate this level with toxic reactions and response.

Currently, we are performing a phase I trial and pharmacokinetic study of another nonclassical antifolate, piritrexim. This study, which is in progress, may also have application for the treatment of PCP. Piritrexim is already available in an oral formulation.

8. CNS Penetration of Antifungal Agents

The need for effective agents to treat central nervous system fungal disease has become especially evident with the advent of HIV disease. The incidence of CNS fungal infections is significant in the HIV infected population. For example, the incidence of cryptococcal meningitis in HIV infected patients has been reported to be as high as 8%. Although amphotericin B is effective in treating fungal meningitis in other patient populations, it has not been as effective in patients with AIDS. In addition, the use of fluocytosine, which is frequently combined with amphotericin-B to treat cryptococcal meningitis, is frequently precluded by the hematologic toxicity of this compound in patients with AIDS.

We have used our nonhuman primate model to evaluate new antifungal compounds that have promising properties for treatment of CNS fungal infections. SCH 39304, (SCH) a new antifungal triazole compound, has potent activity against cryptococcus neoformans, candida spp. and aspergillis spp. To investigate the potential application of SCH for treatment of fungal infections of the central nervous system, we characterized the cerebrospinal fluid penetration and pharmacokinetics of SCH 39304 in adult rhesus monkeys with Ommaya reservoirs. SCH concentrations in both CSF and plasma were determined over a 72 hour period in each of three animals receiving a single dose of SCH (2.0 mg/kg, po). The mean CSF:plasma area under the curve (AUC) ratio was 0.71; the maximum concentration of CSF was 1.34 mg/ml; the maximum concentration of plasma was 1.96 mg/ml . The mean plasma half life was 44.1 hr and the mean CSF half life was 41.1 hr. Thus, this study demonstrated that SCH 39304 effectively penetrates into the cerebrospinal fluid. Moreover, the concentrations in the cerebrospinal fluid met or exceeded the MIC's of SCH 39304 against most strains of cryptococcus neoformans and candida throughout a 48 hour period following a single dose. In addition to penetrating well into the central nervous system, the long plasma and CSF half lives of this compound may permit alternate day or even once weekly dosing for maintenance or

remission of CNS cryptococcus in AIDS. This agent has significant promise for the treatment of CNS fungal infection in patients with HIV disease.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06890-11 PB

PERIOD COVERED

October 1, 1989, to September 30, 1990

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

Lymphoma Biology and Epstein Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ian Magrath	Head, Lymphoma Biology Section	PB,NCI
Others:	Melissa Adde	Nurse Specialist (Research)	PB,NCI
	Abdulla Al-Nasser	Visiting Associate	PB,NCI
	Kishor Bhatia	Visiting Associate	PB,NCI
	Carolyn Felix	Biotechnology Fellow	PB,NCI
	Walter Goldschmidts	Biotechnology Fellow	PB,NCI

Continued on next page

COOPERATING UNITS *(if any)*

Department of Pathology, New York University (R. Dalla-Favera)

LAB/BRANCH

Pediatric Branch

SECTION

Lymphoma Biology Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS: 10.0	PROFESSIONAL: 8.0	OTHER: 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 major goal of this project is to elucidate the molecular consequences of the non-random chromosomal translocations (particularly 8;14 translocations) associated with the small non-cleaved (undifferentiated) lymphomas (SNCL) with a view to understanding the immediate causes of neoplastic behavior in these tumors and the determinants of geographic and clinical heterogeneity. Encompassed within this goal are our studies directed towards elucidating the nature of the association of Epstein-Barr virus (EBV) with the SNCL, and stemming from it, our investigations into the possibility that the molecular abnormalities can be used as a target for a tumor-specific treatment approach. Clinical studies parallel and complement these biological investigations as well as examining important chemotherapeutic issues including the use of dose intensity analyses to dissect the importance of various components and the duration of combination chemotherapy regimens, and to study prospectively the possibility and value of increasing dose intensity through use of colony stimulating factors.

PROFESSIONAL PERSONAL (CONTINUED)

Marina Guttierez	Guest Researcher	PB,NCI
Vinay Jain	Visiting Associate	PB,NCI
Mary McManaway	Biotechnology Fellow	PB,NCI
Ligita Novikovs	Biologist	PB,NCI

OBJECTIVES AND FIELDS OF RESEARCH

Our goals include 1) to understand as much as possible the molecular pathogenesis of the SNCL, and, ultimately, to use such information to develop novel therapeutic approaches, and 2) to improve the management and treatment results of patients with childhood non-Hodgkin's lymphomas. Our ongoing areas of research fall into several overlapping categories:

1) *Molecular analysis of chromosomal breakpoint locations.*

2) *Use of the polymerase chain reaction (PCR) to detect translocations and viral associations.*

3 *Involvement of other genetic lesions in SNCL*

4) *Determination of the pathogenetic role of EBV in SNCL.*

5) *Development of oligonucleotides directed specifically against SNCL cells.*

6) *Therapeutic Studies.*

7) *Collaboration with Cancer Centers in Less Developed Countries*

SIGNIFICANCE

Biological Studies

The SNCL, although relatively rare tumors, have provided a crucially important model which has influenced all branches of oncology. It is probably safe to say that the cause and nature of neoplastic growth is better understood in the SNCL than in any other tumor. Our establishment of a library of cell lines derived from SNCL has been crucial to our ability to study the biology of these tumors and has benefited not only ourselves, but many other investigators.

Our studies are comprehensive, in that they encompass epidemiology, molecular pathogenesis, clinical correlates of molecular findings and therapeutic trials. The molecular categorization, in terms of the chromosomal breakpoint locations of these tumors, provides not only a new and considerably more precise epidemiological and diagnostic tool, but is also generating important leads to the understanding of the genesis of the chromosomal translocations, the mechanisms whereby the c-*myc* gene is deregulated, and the possible role of EBV in pathogenesis. Further, this new knowledge could lead to completely novel, tumor-specific treatment approaches as exemplified by our work with anti-intron 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 ination in the vast majority of tumors it is probably only in the SNCL that such approaches can be seriously contemplated at present.

Our use of the highly sensitive PCR technique to identify breakpoint locations provides a new dimension on diagnostic techniques and the detection of minimal disease states. We have been able to detect the presence of a specific translocation in as few as 1 cell per million.

The demonstration that EBV has a direct role in the pathogenesis of the SNCL, and identification of the pertinent mechanisms would be of major importance to human viral oncology, and could also lead to new therapeutic approaches to EBV associated SNCL, which, once again, could provide a paradigm relevant to other tumors. Although discovered in 1964, the role of EBV in the pathogenesis of Burkitt's lymphoma has remained unclear ever since. Yet progress in understanding the biology and molecular biology of the virus, coupled to advances made in the molecular characterization of the SNCL, has provided an opportunity which did not previously exist, to elucidate the nature of the association of EBV with Burkitt's lymphoma.

Therapeutic Studies

The demonstration that short duration, intensive chemotherapy provides effective treatment for patients with malignant lymphomas would provide major benefits in terms of less toxic cost, less hospital inpatient time and less disturbance to the lifestyles of patient and family. More importantly, however, we hope to demonstrate that patients with a high tumor burden will have an improved outcome. Our dose intensity analysis strongly suggests that in the SNCL, the dose rate is crucially important to outcome, and that only two to three cycles of chemotherapy are necessary. The demonstration that GM-CSF not only ameliorates the toxic side effects of chemotherapeutic agents, but permits an increase in dose intensity could provide a double benefit if the increased dose intensity could be shown to translate into a survival advantage. This approach could well be applicable to other rapidly growing neoplasms including most of the tumors of childhood as well as a number of adult malignancies. This approach could also be transferrable to the less developed countries which account for three quarters of the worlds population and 85% of the worlds children less than 15 years of age. In such countries, where supportive care is frequently inadequate, such that a high percentage of patients would die when the intensity of therapy is increased, decreased toxicity alone would almost certainly result in a marked improvement in survival. Our collaborative studies with centers in developing countries will permit the exploration of this concept.

PROGRESS REPORT AND FUTURE DIRECTIONS

1. Molecular Analysis of Chromosomal Breakpoint Locations

Differences in the location of breakpoints on chromosomes 8 and 14 between endemic and sporadic tumors.

Non-random chromosomal translocations, the most important being an 8;14 translocation, provide a critical element in the pathogenesis of the small non-cleaved lymphomas. Our studies of the significance of the breakpoint locations on chromosomes 8 and 14 have provided important information. Burkitt's lymphoma has a much higher incidence in endemic regions (primarily equatorial Africa) than in other parts of the world (sporadic), but, as we have shown, there are a number of clinical and biological differences between these two subtypes of the disease. The endemic variety is nearly always associated with EBV, and has a high frequency of jaw tumors while the sporadic variety is much less often associated with EBV (15%-20%) and uncommonly presents or relapses in the jaw. We have now demonstrated that these two forms of the disease are associated with molecular genetic differences, and have located the breakpoints to small regions in and

around the c-*myc* allele involved in the translocation. These studies on over 60 tumors have been carried out primarily by Southern blot analysis, supplemented by S1 protection studies and Northern analysis in the case of cell lines. We have demonstrated that chromosomal breaks occur in the first intron of the c-*myc* gene in some 40% of sporadic tumors, but not at all, to date, in endemic tumors. The vast majority of sporadic tumors have breakpoints within or close to c-*myc*, whereas the majority of endemic tumors have breakpoints much further away from c-*myc* (referred to as "far 5' breakpoints subsequently).

We have also demonstrated that whereas in endemic tumors the majority of breakpoints fall outside the switch μ immunoglobulin region on chromosome 14, approximately one third of sporadic tumors have switch μ breakpoints. approximately twice as frequent. In addition, the majority of switch μ breakpoints are associated with breakpoints within or close to the c-*myc* gene, although the reverse does not apply - non-switch μ breakpoints are equally distributed between far 5' breakpoints and breakpoints in or close to c-*myc*. These findings have a number of important implications. Firstly, they complement our clinical observations, and together, they indicate that there are important biological and probably etiological differences between sporadic and endemic SNCL. Secondly, they strongly suggest that the mechanism of deregulation of c-*myc* differs in endemic and sporadic tumors. Breakpoints within or close to c-*myc* result in loss of the normal promoters, or of regulatory sequences which drive the promoters. Breakpoints outside the switch μ region lead to the recognized immunoglobulin enhancer being on the same chromosome as c-*myc*, while switch breakpoints may result in alternate promoters or regulatory elements acting on the c-*myc* gene.

Biological Correlates of Breakpoint Locations

We have progressed quite rapidly in our epidemiologic studies on breakpoint locations observed in SNCL from different geographical and ethnic groups. During the last year we have characterized a number of Burkitt lymphomas from South America. Based on the data obtained from these, it appears that there may be a clustering of breakpoints on chromosome 8 in a region of less than 300 bp upstream of exon I. Such breakpoint clusters have earlier been described for 18;14 translocations and for bcr abl translocations. Recently, Dr. Potter's group have also identified a cluster in the mouse analog of the SNCL - murine plasmacytoma. Identification of this cluster certainly provides important clues to (a) regulation of the "myc" loci and (b) chromatin topology of the myc gene.

Comparison of breakpoint locations from well over 50 SNCLs characterized in our laboratory, demonstrate three major classes of breakpoints.

A) Far 5' breakpoints. These occur quite frequently in endemic BL and leave the myc gene intact, insofar as being contiguous. Studies are underway to determine if there occur mutations further downstream of the breakpoints that would functionally result in a misregulated myc, not different from the biological consequences of breakpoint within the myc loci.

Alternatively, there is a possibility that breakpoints in the far 5' region may disrupt a region critically involved in the regulation of the myc gene. Such regions occurring far upstream of other genes have been described, e.g., locus activation region of globin genes located 50-65 K6 upstream of B-globin on chromosome 11. To characterize this, we would need to clone breakpoint regions from various endemic tumors.

An additional open question that needs to be answered pertains to the molecular basis of differing breakpoint locations in the endemic versus sporadic tumors. Two possible candidates that may influence differences in breakpoint patterns include EBV (since >90% of endemic Burkitt's are EBV positive) and ethnicity (since Burkitt's is endemic in Africa).

The influence of EBV on breakpoint location though probable, is less likely because no EBV positive sporadic tumors with far 5' breakpoints have been described. Since we have no access to Burkitt tumors from the Black population outside the endemic region, the question of ethnicity remains unanswered.

B) Immediate 5' breakpoints. These appear to be the more frequently observed breaks in tumors from South America although well over 40% of tumors from the U.S. also have similar breakpoints. The most interesting feature of this breakpoint region is its involvement in negative regulation of the myc gene. This would suggest that an 8;14 translocation resulting from such a breakpoint may be more frequent because some other breakpoints (within this region) may not result in deregulation of myc and thus in development of a detectable clone of 8;14 cells. In support of this, we have observed a clustering of breaks in this region. Alternatively, it is possible that the clustering is a result of chromatin accessibility of this region. In addition to the clustering, breakpoints, in this region, result in a shift of promoter usage.

C) Intron breakpoints. There appears to be an equal distribution of these and the immediate 5' breakpoints in tumors from the U.S. Clearly, this locus would remove all constraints on myc regulation that the 5' and the 1st exon characterize. In this sense, both the immediate 5'and the intron breaks achieve the same end result.

Clinical Correlates of Breakpoint Locations

Anatomical Location and Prognosis

We are planning a collaborative study with the POG group to examine the significance of different subtypes of SNCL determined by the breakpoint locations on chromosomes 8 and 14. We are interested in determining if different mechanisms of deregulation of c-*myc* lead to different clinical presentations, or different responses to chemotherapy. If such correlates can be made, this information could prove to be of value to the design of treatment protocols.

2. Use of PCR to Detect Translocations - a Diagnostic and Clinical Tool

We have been able to detect breakpoint locations and viral association with PCR by using repeat sequences within the switch u region as one of the amplicons (oligonucleotides) and repeat regions from the EBV genome. We have been able to distinguish different breakpoint regions within the c-*myc* gene by this method, and also, to detect one cell per million with such translocation. Recently we have been able to perform the same analysis on fixed tissue. This technique should therefore permit the analysis of archival tissue for molecular epidemiological studies help assess the roles of various viral associations with SNCL, provide a highly specific diagnosis (based on the presence of a chromosomal translocation) and can be explored for its utility in the clinic as a means of detecting minimal quantities of disease before (e.g in CSF) and after (residual disease) therapy.

3. Involvement of Other Genetic Lesions in SNCL

We have also been interested in two other loci that may be involved in the pathogenesis of Burkitt's. One of these was characterized by us as a relative predispositional allele. In our earlier studies, this allele of a pseudogene on chromosome 13 was found to be present at a higher frequency in SNCL DNA. We have now determined that higher frequency of this allele in SNCL DNA is principally due to an increase in the germline frequency and have characterized loss of heterozygosity of this allele in other hematological malignancies. Furthermore, we also show that the frequency of this allele is

higher in black individuals than in caucasians. Whether this reflects on the endemicity of Burkitt's in Africa or on the ethnic differences of breakpoint locations remains to be seen.

In a subset of Burkitt's breaks on chromosome 8, involving translocations with chromosome 2 and chromosome 22 (variant translocations), the myc gene is left intact. We are interested in determining how the breakpoint region of the variant loci disrupts myc regulation. At present we are using analogous regions from murine variant plasmacytomas to probe human cDNA libraries, to determine potential transcribing regions far 3' myc. (Sequence identity between this region in the mouse and genome has been described.)

4. Pathogenetic Role of EBV in Small Non-Cleaved Lymphomas

Direct Examination of Influence of EBV on c-myc Expression

In addition to making correlations between breakpoint location and EBV association, we are more directly studying the potential mechanisms whereby EBV could provide an essential pathogenetic element. One possible scenario is that latently expressed EBV genes influence c-*myc* expression directly, i.e via the regulatory elements of c-myc. This is a likely possibility, since several latently expressed EBV genes are transactivators - i.e influence the expression of other EBV genes. Indeed, the mechanism whereby EBV effects B cell transaction is likely to involve the transactivation of cellular genes - possibly even c-*myc*. We have hypothesized that structural changes in the c-*myc* gene may sometimes be sufficient in themselves to effect deregulation and neoplasia, but in other cases an effect of EBV on one or more of the c-*myc* regulatory elements may be essential. We plan to examine the possible effect of EBV on c-*myc* by several different experimental approaches.

As our primary approach, we have infected several EBV negative Burkitt cell lines with EBV (both the prototype B95.8 strain and P3HR1, which has a deletion in EBNA-2). We have also stably transfected EBNA-1 in several EBV negative Burkitt cell lines. We shall study the expression of the endogenous MYC gene in these cells, both by Northern blotting and by RNAse protection assay. We wish to see if the presence of EBV or EBNA-1 increases MYC expression or alters its regulation in conditions of stress, e.g., during conditions of serum deprivation. We also wish to see if exon 1 of MYC, which is still on chromosome 8, gets transcribed in the presence of EBV.

We have made numerous constructs of MYC regulatory elements upstream of luciferase, which is being used as a reporter gene. We have used MYC regulatory elements from both normal MYC gene and MYC genes obtained from Burkitt cell lines. In addition, we have cloned enhancer sequences from both heavy and light chain immunoglobulin genes, in appropriate orientation, in these MYC-luciferase constructs. We plan to study the expression of these MYC-immunoglobulin hybrid constructs in EBV negative and EBV converted cell lines. We plan to see what effect, if any, the presence of immunoglobulin enhancers has on MYC promoter activity.

We also plan to study directly the interaction of EBNA-1 with MYC regulatory sequences, by gel retardation and DNAse foot printing analysis.

Use of Antisense Oligomers Directed Against Specific EBV Genes

We have commenced experiments in which the effect of antisense molecules directed against specific lytic as well as latent EBV genes is being studied in Burkitt's lymphoma. At present, we have utilized antisense oligonucleotides directed against the restricted early antigen (EA-R) of EBV to successfully inhibit the lytic cycle of the virus. This technology will help to further characterize the molecular cascade of gene expression involved

in the EBV lytic cycle, and quite possibly be of therapeutic importance in combating EBV associated diseases. We are also interested in pursuing this antisense approach from the standpoint of inhibiting latent viral genes in Burkitt's lymphoma cell lines. If a latent EBV gene is essential to the malignant state, antisense treatment will result in failure of the cell to proliferate. In the case of EBNA-1, necessary for EBV plasmid maintenance, an alternative possibility is that ENV genomes may be lost from the cell line. Such findings, in addition to their theoretical importance could once and for all determine the role of EBV with Burkitt's lymphoma, and in turn have therapeutic implications for EBV associated tumors.

5. Development of Oligonucleotides Directed Specifically Against SNCL Cells

We have pursued 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 the means of specifically inhibiting the translocated c-myc gene in SNCL, while not affecting the c-myc gene of normal cells. We have chosen to use an antisense oligomer directed against 1st intron c-myc sequences, since these are not present in normal c-myc transcripts, but are present in SNCL which have breakpoints 3'of the normal promoters of c-myc, when transcription is initiated at the cryptic promoter site within the first intron. Using a 21 base oligomer derived from an intron region immediately adjacent to the second exon, we have been able to inhibit proliferation completely in cell lines (ST486 and JD38), in which intron sequences are present in mRNA, but not in lines (KK124 and MC116) lacking such intron sequences. Other oligomers, such as the equivalent sense sequence, have no effect on either cell line. We have also shown a reduction in c-myc protein in this cell line when treated with the anti-intron antisense oligomer.

Having shown that selective cell kill can be obtained in vitro, we are now in the process of testing the effectiveness of oligonucleotide therapy in animal models. In previous years we have developed a xenograft model of SNCL in nu/nu mice and information obtained from those studies has been very valuable in designing therapeutic screens for antisense oligonucleotides synthesized in our laboratory. Initial experiments were designed to standardize delivery of antisense oligomers subcutaneously using micro osmotic pumps to tumor bearing nude mice. No acute toxicity was observed in these studies. Interestingly, in a limited set of tumor bearing mice, some antisense oligomers showed inhibition of tumor growth. Although these results appear promising, we need to expand our studies to a larger number of mice and also incorporate in the study various SNCLs with breakpoints distributed in the 1st intron, before any definitive conclusions can be reached.

The ability to selectively turn off or down regulate transcripts from the tumor causing translocation also provides us for the first time with a model to study the molecular consequences of such an inhibition. Would such an inhibition result in activation of the normal myc allele (which is repressed in tumor cells)? Would such an inhibition reduce or revert tumorigenicity?

To approach these questions, we are in the process of designing SNCL cell lines with intron breaks that have been stably transfected with an expression system for expressing the antisense oligomer from a controlled promoter system. Additionally, we intend to design transgenic mice that carry and express deregulated myc loci with an intron breakpoint and cross them with transgenics that express the antisense oligomers in B cells, to determine the effect of tumor associated myc inhibition on tumor incidence.

In principle, the use of tumor cell specific antisense oligomers can be applied to other lymphomas which result from a specific chromosomal aberrations. We also intend to design oligomers for such neoplasia and screen them in vitro for tumor specificity.

6. Therapeutic Studies

Our clinical and therapeutic studies have provided a wealth of ination on childhood lymphomas. One of the original objectives of our work was to identify factors indicative of prognosis, and this information is now being used in defining patient eligibility for the latest protocol. Analysis of the results of patients treated according to protocol 77-C-145 with a cyclophosphamide/doxorubicin/ vincristine/prednisone combination followed by a prolonged (42h) infusion of methotrexate at the nadir of myelosuppression have shown that tumor burden (as measured by clinical stage, serum LDH or Il-2 receptor level) and dose intensity are the most important prognostic factors.

Recent Findings

Recently, we have completed analyses of the associated disease patterns and relevance to treatment of testicular and bony sites of tumor. In addition, we have demonstrated that obtaining multiple bone marrow samples is as important to the determination of bone marrow involvement in young patients with lymphoma as it is in adults - a finding which goes against accepted dogma. Having previously demonstrated that tumor burden appears to be the most important prognostic factor, we have shown that circulating interleukin-2 receptor levels provide an objective measure of tumor burden more specific than serum lactate dehydrogenase (LDH) and more accurate than stage. We believe that such objective markers of tumor burden provide a more valid means of assessing comparability of different published patient series, and have shown that the results of protocol 77-C-145 are similar to those of Total Therapy B when LDH is used to divide the patients into different subgroups. We plan to develop additional assays for the presence of elevated levels of serum protein molecules expressed by lymphoma cells (e.g common acute lymphocytic leukemia antigen and various B and T cell associated antigens) in order to determine whether such assays provide even more specific and precise measures of tumor burden.

Dose Intensity Analysis

We have largely completed an analysis of dose intensity in protocol 77-C-145 (largely a question of cycle duration in this case). We have shown that this kind of analysis provides ination which is highly pertinent to the design of treatment protocols. For example, the dose intensity of drugs in cycles 1 and 2 is highly significantly associated with treatment outcome, although such an association is not present when subsequent cycles are considered. Similar conclusions are arrived at when only the SNCL are examined, and since these tumors represent the majority of the patients, it is reasonable to conclude that additional therapy beyond three cycles does not contribute to outcome in the SNCL. Such a conclusion cannot at present be generalized to the lymphoblastic lymphomas, and indeed, may well not apply in this case.

In this analysis we have been unable to demonstrate a significant association of treatment outcome with adriamycin, vincristine and prednisone dose rate in the SNCL, indicating that cyclophosphamide and methotrexate are the most important drugs in this tumor. Preliminary analysis suggests that this is not so for the lymphoblastic lymphomas, where vincristine and adriamycin may be very important drugs.

Of additional interest was the finding that both partial responders and patients with bone marrow involvement had lower average dose intensities than other patients. There was significant overlap between these groups of patients. This raises the possibility that such patients would benefit from more rapid initiation of subsequent therapy cycles. Delays incurred in

their treatment resulted from delays in white blood cell count recovery. Armed with this ination, it may prove possible to develop new treatment strategies designed to overcome this problem.

Development of a Treatment Regimen for Patients with non-Lymphoblastic Lymphomas

We have developed a new protocol for the treatment of patients with non-lymphoblastic lymphomas. The high risk component of this protocol is based on two regimens piloted in clinical trials. The first of the pilot protocols, was a modified, slightly intensified version of 77-C-145. The second pilot protocol, instituted as a forerunner of the new protocol consisted of a the new drug combination consisting of Etoposide, Ifosfamide and high dose Cytarabine. This regimen was shown to be active, even in patients previously treated with the 77-C-145 regimen and probably, therefore, resistant to the combination to be used as the alternating arm of the new protocol. Low risk patients will be treated with a simpler regimen.

Low risk patients.

Patients with localized or completely resected abdominal disease, will be treated with only 3 cycles of therapy based on modified 77-C-145 - i.e cyclophosphamide/doxorubicin/vincristine alternating with an infusion of methotrexate over 24 h. The intent with low risk patients is to reduce treatment duration as much as possible (half the number of cycles given previously) while ensuring that more than 90% of patients continue to achieve long term survival. The new protocol will involve no radiation and should avoid the potential side effects which are sometimes encountered when high cumulative doses of some drugs (e.g doxorubicin) are received.

High risk patients

All patients not eligible for the low risk protocol will be treated with a regimen consisting of alternating cycles studied in the two pilot protocols. In view of empirical data emanating from Germany, where patients are treated effectively with only 12 weeks of therapy, and our own data suggesting that there is no advantage to prolonged durations of treatment, patients will receive only 4 cycles of therapy.

Evaluation of the effectiveness of GM-CSF in ameliorating toxicity and permitting increased dose intensity.

In view of the anticipated high degree of toxicity of this protocol, and the consequent likelihood that delays in therapy will be incurred with consequent reduction in dose intensity, we have designed a randomized study in one arm of which patients will receive GM-CSF. This should provide several opportunities. These include the possibility to determine: 1) whether GM-CSF will lessen the degree of myelosuppression and consequently the incidence of fever and infection in patients treated with the new protocol. 2) Whether lessened myelosuppression will translate into an increased dose intensity (i.e mgs of drug/M^2 administered per week). 3) Whether the increased dose intensity will translate into a survival advantage. In addition, patients in the control arm will also receive additional therapy.

The high grade B cell lymphomas represent a particularly appropriate model in which to attempt to shorten the interval between therapy cycles with GM-CSF because of the rapid regrowth of these tumors, which is one reason for

chemotherapy failure. The chosen protocol design enables us to make both a retrospective comparison of survival in equivalent patient groups treated according to protocol 77-C-145 and also a comparison of survival in patients treated with and without GM-CSF. Although rather more patients will be

required for the latter, the precise number will depend upon the degree of difference, if any, between the two arms.

7. 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. 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.

Recently we have shown that in Egypt, lymphoblastic lymphoma is very uncommon in children (less than 10% of all lymphomas). On the other hand, based on the phenotyping of 186 cases, 50% of the cases of acute lymphoblastic leukemia in this country are of T cell type, regardless of age. Common acute lymphoblastic leukemia is reciprocally reduced in frequency (39%), while null cases and B cases accounted for the remaining cases. We are pursuing this finding by carrying out further phenotypic analysis in Egypt and molecular analysis in this laboratory. We are interested in determining which T cell receptor genes are rearranged, and whether we can find evidence of breakpoints within the T cell receptor genes.

We are also in the process of expanding these studies to ALL samples collected from India. These are being genotyped in this laboratory. Analysis of available data from these samples has also revealed a high frequency of 6q- involvement. We intend to pursue the 6q- involvement at the molecular level and in collaboration with Dr. Ricardo Dalla-Favera try and define the biological basis of its association with ALL. To streamline our international collaborative efforts, we have also arranged a symposium with a view toward bringing together clinical and basic research experiences of our various collaborators and determine future goals.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06891-02 PB

PERIOD COVERED

October 1, 1989, to September 30, 1990

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

Solid Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc E. Horowitz	Senior Investigator	PB, NCI
Others:			
	Linda L. Weaver	Nurse Specialist (Res)	PB, NCI

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, NCI (E. Glatstein); Surgery Branch, NCI (S. Rosenberg); Lab Pathology, NCI (M. Tsokos); Cardiology, NHLBI (R. Bonow)

LAB/BRANCH

Pediatric Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Research into new therapeutic strategies for the treatment of pediatric solid tumors is focused on bone and soft-tissue sarcomas including Ewing's sarcoma, peripheral neuroepithelioma, rhabdomyosarcoma and osteosarcoma. These common pediatric tumors remain diagnostic and therapeutic challenges for which new approaches are needed. The sarcomas also 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.

PHS 6040 (Rev. 1/84)

GPO 914-918

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 54 protocol entries with the following diagnoses: Ewing's sarcoma (n=16), PN (n=14), primitive sarcoma of bone (n=6), rhabdomyosarcoma (n=7), other soft tissue sarcomas (n=6), and other (n=5). Thirty-six patients had central axis lesions and 27 metastatic disease at diagnosis. The numbers are too small and the duration of follow-up too short to judge the efficacy of this treatment. Response to the four pre-irradiation induction chemotherapy cycles (VAdrC-IE-VAdrC-IE) have, with the exception of two patients, been excellent (> 50% tumor reduction). There have been 21 protocol failures with progressive tumor in 18 and 3 deaths from toxicity (sepsis 1, cardiomyopathy 1, bleeding 1). The toxicity of this protocol has been significant. 96% of treatments have been associated with grade IV neutropenia (AGC nadir < 500) and in 59%, infection. Although the majority of infections have been fever, without a source, the incidence of sepsis is 7% with one toxic death from septic shock. The myelosuppression has resulted in delays in treatment. Instead of the scheduled

treatments every 21 days the average interval between treatments is 25 days. During or after radiation therapy the average interval between treatments is 28 days. Cardiac toxicity has also been significant. The patients are prospectively evaluated by radionuclide angiography (MUGA). There have been two episodes of clinically apparent cardiomyopathy; one resulting in death. The majority have a drop in MUGA scan ejection fraction to the lower levels of normal as they approach the cumulative 550 mg/m2 called for in the protocol. In some patients adriamycin was discontinued early because of the ejection fraction changes.

Although it is premature to judge the efficacy of this treatment as a general statement it is unlikely that significant gains will be realized by the introduction of new drugs if they result in a degree of toxicity that precludes their optimal utilization. We are therefore developing ways to decrease myelosuppression and cardiac toxicity in order to allow maximal benefit from VAdrC-IE by increasing dose intensity over time.

Protocol 88-C-165 - A Randomized Placebo-Controlled Trial of Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor in Pediatric Patients Following Intensive Combination Chemotherapy

This protocol was initiated as a randomized double blind study of rh-GM-CSF in patients on the sarcoma protocol to learn whether it will significantly reduce myelotoxicity and resultant delays in therapy. Patients received rh-GM-CSF at 10 uG/kg subcutaneously daily beginning 24 hours after completion of the chemotherapy regimen and continuing for 10 days. Seven patients have been entered in the study. The results were "unblinded" when it became clear that the effects of the agent precluded a true double blind comparison. From the 6 patients receiving the GM-CSF we have learned that it will not obviate neutropenia. In 20 cycles analyzed, the GM-CSF was discontinued after ten days with an absolute neutrophil count still below 500 in every case. From these initial patients the protocol has been amended in order that the study be randomized but not blinded. The GM-CSF dose has been 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. Ten patients have been entered on the amended study.

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. Fifteen 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.

Publications:

Bader JL, Horowitz ME, Dewan R, Watkins E, Triche T, Tsokos M, Kinsella T, Miser T, Steinberg S, Glatstein E. Intensive combined modality therapy of small round cell and undifferentiated sarcomas in children and young adults: Local control and patterns of failure. Radiother & Oncol 1989;16:189-201.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06892-01 PB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Rhabdomyosarcoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Helman	Senior Investigator	PB, NCI
Others:	G. Crouch	Special Volunteer	PB, NCI
	C. Minniti	Visiting Associate	PB, NCI

COOPERATING UNITS *(if any)*
National Cancer Institute, LMB, DCDB (I. Paston), St. Jude Children's Research Hospital (P. Houghton), Washington University School of Medicine (W. Daughaday)

LAB/BRANCH
Pediatric Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Summary of Work

We are studying the molecular mechanisms involved in the pathogenesis of rhabdomyosarcoma. This neoplasm probably arises due to a developmental disturbance during muscle formation. Since much has been learned recently about the molecular mechanisms underlying the commitment to muscle lineage and the mechanisms involved in normal muscle development, the study of rhabdomyosarcoma offers a unique opportunity to evaluate the relationship between differentiation arrest and the development of this pediatric embryonal tumor. For example, the activation of genes such as MyoD and myogenin have been shown to be required for the commitment of stem cells to myogenic differentiation. Additionally, several growth factors including TGF-beta and insulin-like growth factors have been implicated in the normal growth and maturation of muscle tissue. Our current focus has therefore been aimed at identifying the roles that such growth factors and regulatory genes may play in the development of the striated muscle tumor, rhabdomyosarcoma. In particular we have focused on the role of insulin-like growth factor II in the development of this tumor since previous work has shown that this growth factor is expressed at abnormally high levels in these tumors compared to normal muscle. We have also been evaluating various agents in an attempt to in vitro differentiate tumor cell lines. These studies are aimed at identifying particular lesions within the normal differentiation pathway that may occur in the development of rhabdomyosarcoma. We are also using cDNA cloning approaches to identify potential molecular mechanisms which may distinguish between the unregulated continual growth of the embryonal tumor, rhabdomyosarcoma, compared to the normal regulated growth of normal embryonal human muscle.

GPO 814-918

Accomplishments and Results:

1. The role of insulin-like growth factor II (IGF-II) in rhabdomyosarcoma: We have identified IGF-II as an autocrine growth factor in rhabdomyosarcomas. This growth factor is over-expressed in 12 of 12 tumors evaluated to date. In addition, several cell lines have been demonstrated to secrete authentic IGF-II into culture media, and these tumors have been demonstrated to contain typical type I IGF receptors which are known to mediate the mitogenic response of IGF-II. We subsequently were able to show that a monoclonal blocking antibody to the type-I receptor could substantially inhibit the in vitro growth of rhabdomyosarcoma cell lines both of the embryonal and the alveolar subtype. Interestingly, we were also able to demonstrate that the IGF-II secreted by the tumors was also capable of stimulating motility in these cell lines suggesting that the same growth factor may play a role not only in the unregulated growth of this tumor but also contribute to the high metastatic potential that these tumors have since motility is a major step in the metastatic pathway. Surprisingly, the blocking monoclonal antibody to the type-I receptor was not able to inhibit IGF-II induced motility in these tumors. Ongoing studies are aimed at determining the signaling pathway by which this ligand stimulates such motility.

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 IGF-II to bind to the type-I receptor, and we have also shown using a radioligand assay that suramin does not specifically interfere with the receptor. We have demonstrated that this compound causes growth inhibition in numerous rhabdomyosarcoma cell lines and that exogenously administered IGF-II to suramin treated cells can partially reverse this growth inhibition. These data suggest that suramin may be an important tool in further studying the IGF-II autocrine growth loop in rhabdomyosarcoma.

In collaboration with the Laboratory of Molecular Biology, DCBD, we have created an IGF-I-PE40 oncotoxin. Since IGF-II appears to be an autocrine growth factor that is mediated through the IGF-I receptor, and other embryonal tumors such as neuroblastoma and Wilm's Tumor have also been shown to have such an autocrine growth loop, we reasoned that specifically targeting pseudomonas exotoxin to this receptor may be of interest. We have demonstrated that this genetically engineered oncotoxin binds specifically to the type-I IGF receptor and is capable of killing tumor cells bearing such receptors on their cell surface, including three individual rhabdomyosarcoma cell lines. We are currently working to improve the binding of this IGF-I-PE40 oncotoxin molecule by structurally modifying the protein. In addition, we are attempting to fuse the PE40 toxin molecule to the moneclonal antibody that is capable of binding to the type-I receptor.

Because IGF-II is normally highly expressed in fetal muscle while expression is markedly diminished in normal adult muscle, it is currently unclear whether the high level expression found in rhabdomyosarcoma simply reflects the embryonal nature of this tumor or whether dysregulation of IGF-II expression may be a primary event in the pathogenesis of this tumor. Since there are known alterations on chromosome 11 band p15 in embryonal rhabdomyosarcoma and that is precisely the region where the IGF-II gene has been mapped, we are interested in evaluating the IGF-II gene at the structural level. We have recently cloned regulatory regions of the IGF-II gene from a rhabdomyosarcoma tumor and ongoing experiments are aimed at determining whether there are structural alterations in the cis-regulatory regions that may prohibit the down regulation of IGF-II expression in these tumors. Therefore, these cloned regulatory regions are being evaluated in reporter gene assays as well as sequenced in there entirety and compared to the sequence obtained from regulatory regions of this gene in normal tissue.

2. In Vitro Growth Differentiation of Rhabdomyosarcoma Cell Lines. We initiated studies on the effects of retinoic acid (RA) since this compound has been reported to be a limb morphogen in the developing chick limb-bud. In vitro treatment of rhabdomyosarcoma cell lines with all trans retinoic acid resulted in a greater than 70% inhibition of cell growth using nanomolar concentrations of RA. Interestingly, treatment of the same cell lines with 13-cis retinoic acid resulted in only a 30% decrease in cell growth. This growth inhibition was not accompanied by any morphological or biochemical evidence of differentiation. It therefore appears that these cells are sensitive to retinoic acid in a stereo-specific way and that growth inhibition is not accompanied by evidence of differentiation. Once again these results have been similar in both alveolar and embryonal rhabdomyosarcoma cell lines.

We have subsequently treated cells with low-dose ara-c, since this has been shown to in vitro differentiate hematopoietic cells. Treatment of rhabdomyosarcoma cell lines for four days with 0.5 uM ara-c resulted in 80-90% growth inhibition. Furthermore, after the cells are taken out of ara-c media and placed in normal growth media containing 10% serum, there is no evidence of recovery of cell growth after 14 days in growth media lacking ara-c. Plating efficiency studies have also shown that treatment for four days with low dose ara-c completely inhibits the ability of surviving cells to plate. Of further note, low dose ara-c treatment of the embryonal rhabdomyosarcoma cell line, RD, resulted in biochemical evidence of differentiation with increased expression of the muscle specific genes alpha actin, desmin, and MyoD. Based on these observations, a phase II study using low dose ara-c in relapsed rhabdomyosarcoma patients is being contemplated.

We have just completed the construction of cDNA libraries from normal human embryonal muscle as well as from a human embryonal rhabdomyosarcoma tumor. Screening studies are currently underway to identify two categories of expressed genes which may be of significance in the underlying pathogenesis of this tumor. One set of genes will be identified which are expressed in embryonal rhabdomyosarcoma but not in human fetal muscle suggesting that these genes should be turned off during normal muscle development. The second category of genes we will identify will be those that are expressed in normal fetal muscle but not expressed in the embryonal rhabdomyosarcoma suggesting this type of gene is normally up regulated during development and is inappropriately off in these embryonal tumors.

Publications:

Reeves S, Helman LJ, Allison A, and Israel MA. Molecular cloning and primary structure of human glial fibrillary acidic protein, Proc. Natl. Acad. Sci. USA 1989;86:5178-5182.

El-Badry OM, Romanus JA, Helman LJ, Cooper MJ, Rechler MM, and Israel MA. Autonomous growth of a human neuroblastoma cell line is mediated by insulin-like growth factor II, J Clin Invest 1989;84:829-839.

Helman LJ, Cohen PS, Averbuch SD, Keiser HD, and Israel MA. Neuropeptide Y expression may distinguish between malignant and benign pheochromocytoma, J Clin Oncol 1989;7:1720-1725.

Levine MA, Smallwood PM, Moen Jr. PT, Helman LJ, and Ahn TG. Molecular cloning of B3 a third form of the G-Protein B polypeptide, Proc Natl Acad Sci USA 1990;87:2329-2333.

Cooper MJ, Hutchins GM, Cohen PS, Helman LJ, Mennie RJ, Israel MA. Human neuroblastoma tumors correspond to the arrested differentiation of chromaffin adrenal medullary neuroblasts, Cell Growth and Diff 1990;1:149-159.

El-Badry OM, Minniti C, Kohn EL, Houghton PJ, Daughaday WH, and Helman LJ. Insulin-like growth factor II acts as an autocrine growth and motility factor in rhabdomyosarcoma tumors, Cell Growth & Diff 1990;1:325-331.

Helman LJ, Sack N, and Israel MA, Nucleotide sequence of pG2, and adrenal specific mRNA, Nuc Acid Res 1990;18:685.

Levine MA, Dempsey MA, Helman LJ, and Ahn TG. Expression of Chromogranin-A messenger ribonucleic acid in parathyroid tissue from patients with primary hyperparathyroidism, J Clin Endocrinol Metab 1990;70:1668-1673.

Helman LJ and Thiele CJ. The Biology of Solid Tumors. In: Pediatric Solid Tumors: Important Concepts in Biology and Therapy. Horowitz ME and Pizzo PA. eds. Ped Clinics of North America. Philadelphia: WB Saunders Co, in press.

Helman LJ and Horowitz ME. Rhabdomyosarcoma. In: Tonini GP, Sansone R, and Thiele CJ. eds. Molecular Genetics of Pediatric Solid Tumors. Basic Concepts and Recent Advances. London: Harwood Academic Publishers, in press.

Cohen PS, Cooper MJ, Helman LJ, Thiele CJ, Seeger RC, and Israel MA. Neuropeptide Y expression in neonatal neuroblastoma tumors may mimic its developmental regulation in the human adrenal medulla, Cancer Res, in press.

El-Badry OM, Helman LJ, Chatten J, Steinberg, SM, Evans A and Israel MA. Insulin-like Growth Factor II-Mediated Proliferation of Human Neuroblastoma, Submitted.

Prior TI, Helman LJ, FitzGerald DJ, and Pastan I. Cytotoxic Activity of a Recombinant Fusion Protein Between Insulin-like Growth Factor I and Pseudomonas Exotoxin, Cancer Res, Submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 00650-35 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Service Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Glatstein	Branch Chief	ROB, NCI
Others:	T. Goffman	Head, Clin. Ther. Sec.	ROB, NCI
	K. Straus	Senior Investigator	ROB, NCI
	A. Raubitschek	Senior Investigator	ROB, NCI
	B. Kelly	On-Site Coordinator	ROB, NCI
	J. Delp	Chief Technologist	ROB, NCI

COOPERATING UNITS *(if any)*
Cancer Nursing Service, CC

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	10	PROFESSIONAL:	4	OTHER:	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:

J. Smith	Clinical Nurse	CNS, CC
R. Smith	Cancer Nursing Specialist	CNS, CC
L. Dachowski	Clinical Nurse	CNS, CC
E. Fuetsch	Clinical Nurse	CNS, CC

Methods Employed

Formal and informal consultation with referring physicians and application of radiotherapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice, as well as modified programs when necessitated by concomitant adjuvant therapies.

Major Findings

Just under 700 patients were seen in formal consultation this year. In addition, between 400 and 500 telephone conversations provided ad hoc advice on treatment for a variety of problems and general information, including nursing management and follow-up for radiation therapy related problems. Approximately three visits per month from nursing staff to observe delivery of radiation therapy and simulation process. Approximately 450 patients will be treated this fiscal year with most of these being protocol patients in the Radiation Oncology Branch, or on collaborative studies.

Significance to Biomedical Research and the Program of the Institute

This project represents the ROB's direct contribution to clinical research and patient care. It also represents ROB's efforts to assist physicians and patients with problems which generally defy simple medical solutions.

Proposed Course

To continue.

Publications

1. Minna J, Pass H, Glatstein E, Ihde DC. Cancer of the lung. In: De Vita VT, Hellman S, Rosenberg SA, eds. Principles and practice of oncology. Volume 1. Philadelphia: JB Lippincott Company, 1989;591-705.

2. Chang AE, Rosenberg SA, Glatstein E, Antman KH. Sarcomas of soft tissues. In: De Vita VT, Hellman S, Rosenberg SA, eds. Principles and practice of oncology. Volume II. Philadelphia: JB Lippincott Company, 1989;1345-1398.

3. Kinsella TJ, Trivette G, Rowland J, Sorace R, Miller R, Fraass B, Steinberg SM, Glatstein E, Sherins RJ. Long-term follow-up of testicular function following radiation therapy or early stage Hodgkin's disease, J Clin Oncol 1989;7:718-724.

4. Mitchell JB, Russo A, Cook JA, Glatstein E. Chapter 12: tumor cell drug and radiation resistance: does an interrelationship exist? Ozols RF, ed. Drug resistance in cancer therapy. Boston: Kluwer Academic Publishers, 1989;189-203.

5. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, Rosenberg SA, Coltman CA, Tubiana M. Report of a committee to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting, J Clin Oncol 1989;7:1630-1636.

6. Raubitschek A, Glatstein E. The never-ending controversies in Hodgkin's disease. Int J Rad Oncol Biol Phys 1989;17:1115-1117.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06310-11 RO

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Surgery vs. Radiation Therapy in Treatment of Primary Breast Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. Straus Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*
Medicine Branch, NCI
Rehabilitation Medicine, CC
Cancer Nursing Service, CC
Biostatistics and Data Management Section, NCI

LAB/BRANCH: Radiation Oncology Branch

SECTION: Clinical Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 6	PROFESSIONAL: 3	OTHER: 3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The purpose of this study is to determine whether a breast-conserving treatment program of limited surgery and definitive radiation offers equivalent local control and survival to mastectomy in patients with early stage breast cancer. After work-up confirms localized disease, patients are randomly assigned to either primary surgery or primary irradiation. Patients treated with mastectomy are offered breast reconstruction. All patients undergo complete axillary node removal; those patients with pathologically positive lymph nodes, and those with negative lymph nodes who are estrogen receptor negative receive chemotherapy.

PHS 6040 (Rev. 1/84) GPO 914-918

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	Clinical 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 cosmetic and functional results of local treatment will be carefully evaluated. The psychological, sexual, and sociological impact of mastectomy vs. lumpectomy and radiation will be noted. Ability to combine aggressive chemotherapy with either local treatment in node positive patients and node negative, ER negative will also be assessed.

Methods Employed

Patients with stage T1-T2, N0-N1, M0 primary untreated breast cancer are candidates for the study. They will be randomized to receive either lumpectomy, axillary dissection, and radiation therapy or total mastectomy with axillary node dissection. Patients receiving mastectomy will be offered breast reconstruction. Patients with pathologically positive lymph nodes, and ER negative patients with negative lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 11 years. It is now open for follow-up only. Two hundred and fifty-six patients have been entered, of whom 128 have randomized to mastectomy, and 128 to radiation. Median follow-up is 78 months. No differences have been seen as yet between the surgery arm and radiation arm in terms of overall survival (85%/89% at 60 months 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.

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

Barth RJ, Danforth DN, Venzon DJ, Straus KL, d'Angelo T, Merino MJ, Gerber NL. Level of axillary involvement by lymph node metastasis in stage I, II, breast cancer lacks prognostic significance.In: Proceedings of the 36th Annual Meeting. Washington, D.C.: The Society of Surgical Oncology, 1990;261.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06320-11 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Response of Mammalian Cells to Chemotherapy Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Cook	Staff Fellow	ROB, NCI
	J. Gamson	Biologist	ROB, NCI
	S. Hahn	Clinical Associate	ROB, NCI
	D. Kaufman	Senior Investigator	ROB, NCI
	J. Liebmann	Clinical Associate	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Experimental Phototherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Several chemotherapy agents with proven utility such as anthracyclines, bleomycins, alkylators, neocarcinostatin, nobel metal derivatives, VP-16, and hypoxic radiosensitizers are being studied. The detoxification mechanisms, modification of cellular response by altered intercellular redox status, and oxygen metabolism in sensitive and resistant cells are of interest to the area of cancer treatment and directly related to our studies. Deleterious species produced by the antineoplastic drugs and cellular response to these species, as well as sulfhydryl containing compounds as they relate to metabolism, activation, and detoxification of antineoplastics are being explored. It has been demonstrated that depletion of glutathione levels either by directly conjugating or inhibition of de novo synthesis results in sensitization of cells by adriamycin, bleomycin, cisplatin, VP-16, alkylators, and hypoxic radiosensitizers. Alternatively, increasing glutathione levels by providing direct precursors results in protection of cells from the above reagents. Rescue of cells after treatment by supplying glutathione directly by modifying the molecule such that it becomes membrane permeable is being studied. We have senthesized a series of glutathione esters and have demonstrated in preliminary studies that these esters rapidly increase introcellular GSH levels. As a result of elevated GSH, cells are made markedly resistant to cisplatin and melphalan. Not only is introcellular GSH levels increased but also there is a marked elevation of introcellular cysteine. After we have selected the most efficient GSH ester we will proceed to determine if there is a differential increase in GSH levels in tumor as opposed to normal cell lines. Following these studies we hope to determine whether or not differential elevations in GSH and tumor versus normal tissues in animals is possible. Timing of delivery of the rescue agents, how the rescue agents interact with other biochemical pathways, cellular clearance and in vivo clearance are being investigated.

Project Description

Objective: The objective of this project is to determine the importance of biochemical modulation of selected cellular redox compounds upon chemotherapeutic drug cytotoxicity.

Methods Employed

In vitro cell culture and in vivo murine tumor models will be exposed to the various reagents mentioned above and assayed by conventional clonogenic assay, dye markers, tumor dose response, and survival advantage. In the in vivo studies, both thymic and athymic mouse are available to investigate murine and human tumor response. Standard biochemical enzyme assays, synthetic organic chemistry techniques, high performance liquid chromatography, and molecular biology techniques will and are being used.

Major Findings

Preliminary studies have indicated that exogenous glutathione applied to mice treated with cisplatin has been protective with respect to survival. Ongoing studies will seek to determine if such an effect exists in tumor-bearing animals. We have found that glutathione ester is extremely efficient in elevating intercellular GSH and cysteine levels. Treatment of cells with this ester results in a marked resistance to melphalan and cisplatin. We have also shown that glutathione depletion renders cells markedly sensitive to xanthine/xanthine oxidase. Surprisingly GSH elevation either by GSH esters or OTZ does not afford protection. The mechanism underlying these observations may shed light on how cells detoxify superoxide and hydrogen peroxide via glutathione and related enzymes.

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.

Publication

1. Alegria AE, Samuni A, Mitchell JB, Riesz P, and Russo A. Free radicals induced by adriamycin-sensitive and resistant cells: a spin-trapping study, Biochemistry 1989;28:8653-8658.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-11 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Radiosensitization and Chemosensitization of Aerated and Hypoxic Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	J. A. Cook	Staff Fellow	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

A major portion of this study has dealt with the importance of cellular redox systems such as glutathione (GSH) and related enzymes to the cell's defense against ionizing radiation and chemotherapy drugs. Recent studies have clearly shown that inherent GSH levels do not significantly contribute to the radiation response. Previous work from this laboratory has indicated that inherent GSH levels do have a marked impact on chemotherapy drugs and specific nitroimadazole radiation sensitizers. More recently we have shown that cell lines high intercellular GSH are not sensitized as much as cell lines with a low GSH levels to hypoxic preincubation with misonidazole followed by aerobic exposure to melphalan. These findings are of particular importance since misonidazole is being used clinically as a sensitizer for drugs such as melphalan.

Several laboratories have shown that the intercellular levels of GSH in human tumor lines are much higher than rodent cell lines. It has also been shown that high intercellular GSH levels afford resistance to a number of chemotherapy drugs. Our laboratory and others have questioned whether or not human tumor cells *in vivo* have high intercellular GSH levels. We have now evaluated some 40 biopsies from human lung cancer and have found that the GSH levels in the tumor are not markedly different from GSH levels taken from normal lung. An exception has been identified, namely, squamous lung cancer. Biopsies taken from these tumors indicated a subpopulation of cells with extremely high GSH levels. In order to make these measurements we have spent considerable time working out techniques. A combination of cell disaggregation and staining cells with a GSH specific stain, monochlorobimane, along with HPLC techniques have enabled us to identify subpopulations within tumor cell digest and establish their GSH levels. These approaches and techniques should prove useful in clinical trials where agents such as buthionine sulfoximine are being used to deplete tumor cell GSH. These techniques should enable accurate assessment of tumor cell populations from patients.

Project Description

Objective: The objective of the proposed 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 their implications for the clinic.

Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to radiation or selected chemotherapy drugs, either under aerated or hypoxic conditions. Cellular GSH will be measured by spectrophotometric methods and cellular levels altered by drugs that specifically modulate the GSH cycle. Particular attention will be placed toward optimizing flow cytometric assays for GSH determination of fresh human tumor biopsy material.

Major Findings

We have developed a sensitive technique to measure GSH levels in subpopulations taken from digests of human tumor biopsies. We have compared the GSH levels from tumor cell populations to those of normal lung cells. Our finding has been GSH levels of cells taken from tumor and normal lung are approximately the same with the exception of squamous carcinoma of the lung. These tumors appear to have much higher GSH levels than normal lung. The techniques we have developed will be useful in clinical trials where GSH levels from populations of cells taken from tumor might be correlated with the ultimate treatment outcome. The technique will also be useful in testing the extent of GSH depletion afforded by buthionine sulfoximine in clinical trials where this particular drug is being used inconjunction melphalan treatment.

Significance to Biomedical Research and the Program of the Institute

Agents such as buthionine sulfoximine which inhibits GSH senthesis are currently being evaluated in clinical trials. In order to assess the efficacy of such approaches accurate tumor cell measurements from patients is imperative. The techniques developed in our lab over the past two years are appropriate for these studies. These techniques will be made available to investigators conducting these trials and with on our own institute we will be exploring with or not GSH levels in tumors is a predictor of the overall treatment outcome.

Proposed Course

More studies will be conducted at the cellular level on a more efficient means of GSH modulation. A major continued effort will be directed toward the measurement of GSH (and related enzymes) in human tumor and normal tissue.

Publications

1. Carmichael J, DeGraff W, Gamson J, Gazdar AF, and Mitchell JB. Radiation sensitivity of human lung cancer cell lines, Eur J Cancer Clin Oncol 1989;25:527-534.

2. Mitchell JB, Russo A, Cook JA, and Glatstein E. Tumor cell drug and radiation resistance: does an interrelationship exist? In: Ozols RF, eds. Drug resistance. New York: Kluwer Academic Publishers, 1989;189-203.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-10 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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
	E. Lamoreaux	Computer Specialist	ROB, NCI
	N. Wersto	Radiation Physicist	ROB, NCI
	K. Yeakel-Orr	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, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

This section continues to provide expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to special clinical needs. Regular checking of dosimetric and technical set-up aspects of radiation treatment will continue.

1. The improvement of the quality assurance (QA) program for the three Varian accelerators (Clinacs 4, 18, and 20) and the Scanditronix Microtron M22 is an ongoing effort. A new quality assurance detector using five ionization chambers has been integrated into the QA program. This device consolidates output, energy and symmetry checks and will be useful for electrons as well as photons.

2. Adaptation of the radiation equipment and special supporting equipment for patient treatment and its implementation is a continuing effort, continually adjusted also to the needs of the ongoing and new clinical research programs.

PHS 6040 (Rev. 1/84)

GPO 914-918

3. The Microtron has been repaired and acceptance testing has been completed.

4. The computer programs for clinical radiation treatment planning are being rewritten in C-language for implementation on a Macintosh II system. This project is nearing completion for photon beam treatment planning. A powerful "toolbox" promises to be very helpful on a wide variety of applications, including monoclonal antibody dosimetry.

5. Supporting patient treatment and evaluation of clinical research.

Project Description

Personnel:

Objectives: To ensure highly flexible and quality physics support for radiotherapy.

Methods Employed

The locally developed highly efficient system for daily and periodic quality assurance is continually used for monitoring the performance of three linear accelerators, the Microtron, the simulator, and the CT scanner. Special mechanical supports and measuring devices are used to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning have been simplified and improved.

The Section continues to provide non-routine in vivo patient dosimetry by means of thermoluminescent dosimeters and diodes. Such ad hoc measurements are usually concerned with doses to sensitive organs, and are sometimes crucial to the continuation of a treatment technique.

Maior Findings

This is a continuing project, developing in part in line with developing or new clinical research. Beam monitoring locally developed and other quality assurance support jigs enable daily monitoring of output, beam flatness, symmetry, and alignment of light field and x-ray fields for all three linear accelerators. The method allows simple documentation of performance. Our system continues to impress visitors. The dosimetry of photon beam total-body irradiation, as well as that of total-skin electron beam irradiation for mycosis fungoides, has stabilized.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important aspect is the capability to image irregular fields shaped by individualized specially defined shielding blocks. This is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

The use of locally designed and developed equipment and methodology continues to be a major factor in quality control of equipment, methodology and treatment documentation. This is especially important in view of the generally highly complex clinical studies in this Branch. Reliability of treatment delivery is being improved by implementation of a computer controlled hand-held bar code reader system, developed in-house by Robert Miller.

Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies. The CT scanner is now the principal source of patient data for treatment planning.

Proposed Course

1. Continuation of adaptation of the computer programs to the new radiation machines, with emphasis on an updated system based on the Macintosh II.

2. Integration of alternative imaging systems such as MRI and PET into the updated treatment planning system.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06330-10 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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:	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

This is ongoing research and development. The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams to the most general characteristics is fundamental to radiotherapy. The radiation field model has been described before. It takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field." This concept is applied to the beam-modifying devices as well. One virtue of this approach is that it requires few experimental data and thus can be implemented very easily.

The implementation of our treatment planning programs on a Macintosh II system is continuing. The characteristics of this system require a drastically different approach as to the pure calculation part. The project is nearing completion for photon beams, but the "toolbox" will be directly applicable to other applications, including monoclonal antibodies dosimetry and imaging.

Project Description

Objectives: To extend and verify unified calculative models for the description of absorbed dose produced by beams of ionizing radiation, including photon beams as well as electron beams, as a basis for computer-assisted treatment planning, with special attention to high energy x-ray and electrons.

Methods Employed

Special attention will be paid to verification of the model for the revitalized Microtron, to the 6 MV and 21 MV x-ray beams as well as the electron beams. For this purpose, the newly updated Therados RFA-7 radiation field scanner is proving very useful.

Maior Findings

In x-ray dose field modeling, the description of electron transport correction has proved to be highly significant especially in high energy x-ray treatment with small fields in the thorax. Two publications are in preparation. The new electron beam model is both simpler to implement and has been shown to be more accurate than any other published model. All of these results are being incorporated in a new clinical treatment planning system built around a Macintosh II computer. The latter development has been held up by software problems.

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. Cooperation with a commercial organization providing hundreds of community hospitals has been approved and will shortly be initiated.

Proposed Course

1. This project is to be continued, with the emphasis of inhomogeneities in photon and ectron beams. In regard to electron beams, the influence of inhomogeneities needs further experimental work and algorithmic implementation.

2. Implementation on a Macintosh II portable system is to be continued. (see Z01 CM 06378-03 RO.)

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06351-08 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Response of Mammalian Cells to Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
	J.A. Cook	Staff Fellow	ROB, NCI
	T.E. Goffman	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

When certain halogenated pyrimidines such as bromodeoxyuridine (BrdUrd) and iododeoxyuridine (IdUrd) are incorporated into cellular DNA, cells become more sensitive to ionizing radiation and chemotherapy drugs. This observation has led to several clinical studies over the years and recently at the NCI to evaluate whether selective sensitization of tumors could be achieved by IdUrd infusion followed by radiation. An important question arises in these studies regarding the extent to which the drug is incorporated into cells. Work continues to develop techniques to quantify IdUrd incorporation into tumor and normal tissues. The IdUrd monoclonal antibody has proven useful in flow cytometry studies to accurately predict the labeling index (proportion of cells in S phase) and the laboratory is currently involved (with the clinical IdUrd study) in assessing this potentially important clinical parameter. We have obtained biopsied material from at least 10 patients who have received protracted IUdR intravenous infusion. Our findings to date have been that the replacement of thymidine with IUdR has been relatively low from most all patients ranging from 1-10%. However, it is to early to make a statement regarding the extent of replacement with the overall radiotherapy treatment outcome. We will continue to collect and evaluate IUdR replacement data from patients over the next year. Of significance was the observation that one patient who had received a five day infusion of IUdR with a replacement of 10% had a dramatic complete response of a large bulky head and neck lesion. Use of the IUdR antibody and image analysis has enabled us to assess each patient with respect to the hetergeneity of IUdR incorporation. We have automated this process such that it is feasible to determine a labelling index and a measure of heterogeneity from an entire tissue section. In addition to use of the IUdR antibody we are also using the Ki-67 antibody which should complement our studies by giving us an estimate of the growth fraction of the tumor.

Project Description

Objectives: To quantitate the amount of IdUrd in tumor vs. normal tissue by flow cytometry, HPLC, and image analysis. With these techniques we will be able to determine if there is a relationship between the tumor cell IUdR replacement and the overall radiotherapy treatment response. With these techniques, optimal timing schedules of incorporation for maximum differential radiosensitization will be determined.

Methods Employed

A monoclonal antibody for IdUrd and HPLC assays will be used to quantitate incorporation of IdUrd in tissues. Standard cell survival techniques have been used. Image analysis will be preformed using a fluorescent microscope linked to laser excitation and computer image analysis systems.

Major Findings

It is to early to make a definitive statement regarding the incorporation of IUdR into patient's tumor and the treatment outcome. However, we have collected data from 10 patients which indicate that the IUdR replacement is extremely variable from patient to patient ranging from 1-10% replacement. It is interesting to note that of those patients who receive high replacements approaching 10% have all had a good clinical response. More patients will have to acculmulated before definite statements can be made. By using the IUdR antibody and image analysis we have demonstrated marked heterogeneity with respect to presence of S-phase cells in tumor biopsies from patients. These studies have also been conducted with the Ki-67 antibody which will hopefully give us a measure of the tumor growth fraction.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding as to quantities and timing of IdUrd required to radiosensitize cells from tumor and normal tissue in a clinical setting. This parameter may be useful in selecting appropriate treatment approaches.

Proposed Course

Continue work on cellular quantitations of IdUrd. Evaluate cell survival of other mammalian cells to halogenated purines and pyrimidines and work out timing of incorporation for maximum differential sensitization in *in vivo* models. The influence of biological response modifiers on IdUrd incorporation will be studied.

Publications

1. Mitchell JB, Russo A, Cook JA, Straus KL, Glatstein E. Radiobiology and clinical application of halogenated pyrimidine radiosensitizers, Int J Radiat Biol 1989;56:827-836.

2. Cook JA, Mitchell JB. Viability measurements in mammalian cell systems, Analytical Biochemistry 1989;179:1-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06353-08 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charactars or less. Title must fit on one line between the borders.)*
Metal Chelate Conjugated Monoclonal Antibodies for Tumor Dx & Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	O. A. Gansow	Senior Investigtor	ROB, NCI
Others:	M. Brechbiel	Chemist	ROB, NCI
	T. McMurry	Senior Staff Fellow	ROB, NCI
	G. Pippin	Staff Fellow	ROB, NCI

COOPERATING UNITS *(if any)*
Laboratory of Cellular and Molecular Biology, NCI; Metabolism Branch, NCI; Johns Hopkins Medical School, Baltimore, MD (M. Strand); Argonne National Laboratory, Argonne, IL (R. W. Atcher)

LAB/BRANCH
Radiation Oncology Branch

SECTION
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in several animal model systems: one is a tumor virus induced leukemia of mice; another is human tumor xenographs in nude athymic mice.

The cytocidal agents being employed are various radionuclides. Their relative efficacy when conjugated to monoclonal antibodies is being assayed and compared to that of monoclonal antibodies alone or conjugated to toxins. The several radionuclides chosen for study span the range of nuclidic properties available, thus Copper-67 represents a weak, short range, low energy beta emitter, Yttrium-90 is a long range, high energy beta emitter, Bismuth-212 is a short-lived, alpha emitter and Lead-212 provides both short and long range beta emissions and the subsequent alpha emission of its Bismuth-212 daughter. The syntheses of the chelating agents required for linkage of these isotopes to antibody is now complete.

The Bi-DOTA complex has been shown to be stable in an animal model system.

The new bifunctional DOTA chelating agent has also been shown to be stable for Lead-203 in vivo. Tumor images have been obtained in the LS-174T tumor model system

These studies will provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytocidal agents to tumors, as well as metastases and as well will allow improved diagnostic imaging of malignancies.

Professional Personnel Engaged on the Project

D. Colcher	Senior Investigator	LCMB, NCI
T. Waldmann	Chief	MET, NCI
M. Strand		John Hopkins
R. W. Atcher		Argonne

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 testing bifunctional chelating agents for stability in vivo. Last year we could report that Y-90 was stably linked to antibody. This year we report studies with lead and bismuth alpha emitting radionuclides.

1. The antibody 103A was linked to the chelating agent DOTA and labeled with Bismuth-206. When testing in normal and leukemic mice, uptake in the kidney demonstrated that the bismuth-antibody conjugate was stable, excellent tumor targeting was observed.

2. The bifunctional chelating agent DOTA was linked to antibody B-72.3 and labeled with Lead-203. Tissue distribution studies demonstrated that the chelate was stable in vivo. Good tumor images were obtained of the LS-174T tumor in nude mice by gamma camera imaging.

3. New chelating agents, the hexa lactams were shown to be stable complexes for both lead and bismuth.

4. A careful study of linkage of isothiocyanate derivatives of chelates was published. This provided the required data for formulation of protocols for preparation of human doses of chelate linked antibodies.

5. Enhanced in vivo targeting of antimyosin antibodies to cardiac infarcts was observed when new and improved chelating agents were used.

6. The selective elimination of alloresponsive T-cells was demonstrated by linkage of an alpha emitter to antibody anti-Tac.

Proposed Course

Studies of the therapeutic efficacy of the several radionuclides now under investigation are in progress employing: 1) a model for leukemia in which normal mice have been infected with Rauscher leukemia virus; and 2) a human xenograph solid tumor model in mice. Based on these studies, we will be able to select the most appropriate radionuclide for radioimmunotherapy of the specified disease to be treated.

Radiobiology studies of relative in vitro therapeutic efficacy and dosimetry will be performed.

Since protocols for production of clinical doses of chelate linked Yttrium-90 labeled antibody are in place, treatment of lymphoma, leukemia and colon cancer with radiolabled antibody should be underway this year.

Publication

1. Gansow O, Brechbiel MW. US Patent 4,831,175: Backbone Polysubstituted Chelates for Forming a Metal Chelate-Protein Conjugate, May 16, 1989.

2. Gansow O, Brechbiel MW, Mirzadeh S, Colcher D, and Roselli M. Chelates and antibodies: current methods and new directions. In: Goldenberg DM, ed. Cancer imaging with radiolabeled antibodies. Boston: Kluwer Academic Publishers, 1990;153-171.

3. Khaw BA, Gansow O, Brechbiel MW, O'Donnell SM, and Nossiff N. Use of isothiocyanatobenzyl-DTPA derivatized monoclonal antimyosin fab for enhanced in vivo target localization, J Nuclear Medicine 1990;31:211-217.

4. Kozak RW, Fitzgerald DP, Atcher RW, Goldman CK, Nelson DL, Gansow OA, Pastan I, and Waldmann TA. Selective elimination in vitro of alloresponsive T cells to human transplantation antigens by toxin or radionuclide conjugated anti-IL-2 receptor (Tac) monoclonal antibody, The J of Immunology 1990;144:3417-3423.

5. Maagerstadt M, Gansow OA, Pannell LK, Vogtele F, and Kiggen W. Preparation, ^{252}-Cf-plasma-desorption mass spectrometry and radiometal exchange of the Pb^{2+} and Bi^{3+} complexes of a hexalactam macrocycle, Nucl Med Biol 1990;17:409-412.

6. Mirzadeh S, Brechbiel M, Atcher RW, and Gansow OA. Radiometal labeling of immunoproteins: covalent linkage of 2-(4-isothiocyanatobenzyl)diethylenetriaminepentaacetic acid ligands to immunoglobulin, Bioconjugate Chemistry 1990;1:59-65.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06356-07 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Treatment of Malignant Brain Tumors with Interstitial Radiotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	K. Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, NINDS

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section and Physics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.1	.15

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.)*

Median survival of high-grade gliomas remains less than a year, despite multi-modality treatment. Cure is considered anecdotal. These tumors usually have extended beyond the limits of a complete surgical resection, and the dose of conventional external beam radiotherapy has been limited by surrounding normal brain tolerance. We believe that we can achieve a higher radiation dose to the tumor by placing radioactive seeds of Iodine-125 directly into the tumor bed, with a sharp fall-off of radiation to the surrounding normal brain. Hopefully, this will achieve a much better therapeutic ratio, especially when delivered at low dose rates.

Project Description

Professional Personnel Engaged on Project:

E. Oldfield	Branch Chief	SN, NINDS

Objectives

To develop a technique of interstitial implantation of intracranial tumors; to determine the acute effects and complications of such treatment; to explore the efficacy of such therapy; and to develop patient selection guidelines for future applications.

Methods Employed

Patients with primary untreated high-grade gliomas of less than 5 cm diameter receive approximately 4000-4500 rads of external beam radiotherapy prior to interstitial implantation of radioactive Iodine-125 seeds. High-grade gliomas recurrent after prior external beam radiotherapy are treated with interstitial implantation of Iodine-125 only without additional external beam radiation. Using a Brown Robert Wells stereotactic frame and a customized template device, silastic catheters loaded with radioactive seeds of Iodine-125 are stereotactically positioned in the tumor. Catheters are then anchored to the dura, and the bone defect is closed. Catheters are left in place to deliver an appropriate radiation dose, and then are removed at the time of a second, minor surgical procedure, approximately 1 month later.

Major Findings

Twenty-two patients have been enrolled in the protocol: 10 with primary glioblastoma multiforme, 7 with recurrent glioblastoma multiforme, 3 with primary anaplastic astrocytoma, and 2 with recurrent anaplastic astrocytoma. Two patients remain alive, 8 and 14 months after the implant procedure. Twenty patients have died 2-41 months after implant procedure. Median survival of all patients is 12.8 months after implantation. Patients with previously untreated primary glioblastoma multiforme have a median survival of 16.3 months after diagnosis, which compares favorably to reported figures in the literature for other means of treatment of primary glioblastoma multiforme, where median survival is approximately 10 months. Median survival of patients with recurrent tumors is 9.3 months after implant, which also compares favorably with reported data in the literature.

The technique for the stereotactic placement of multiple catheters containing multiple radioactive sources has been developed. Only 3 of 22 patients have

required reoperation for symptomatic radiation necrosis/tumor, which compares favorably with a rate at 40% to 50% reported elsewhere in the literature. This technique can be used to implant a tumor in any cranial site, excluding the posterior fossa which is technically inaccessible. It can be adapted to a variety of isotopes and a variety of tumor configurations.

Significance to Medical Research and the Program of the Institute

This study helps to provide information on dose and dose rate effects on both tumor and normal brain after interstitial implantation of radioactive sources.

Proposed Course

It is proposed to study a total of 30 patients. We wish to explore the development of advanced computer algorithms for radiation treatment planning and dose display. Depending on the results of treatment of the first 30 patients, a decision will be made about whether to enter into additional studies incorporating interstitial implantation of brain tumors.

Publications

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06357-07 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Studies on Intraoperative Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Glatstein	Chief	ROB, NCI
Others:	W. Sindelar	Senior Investigator	SB, NCI
	H. Pass	Senior Investigator	SB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC

COOPERATING UNITS *(if any)*
Surgery Branch
Cancer Nursing Service, CC

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	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.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Personnel:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
R. Smith	Cancer Nursing Specialist	CNS, CC

Objectives: These are Phase I and II studies assessing the role of intraoperative radiation therapy as an adjunct to surgical resection in various primary tumor sites, including pancreas, stomach, and retroperitoneum, where local failure following surgery alone is extremely high. Additional pilot studies are ongoing to determine the role of intraoperative radiation therapy with tumors with high-risk of local recurrence.

Methods Employed

Patients are considered for entry on the randomized studies with combined surgical resection and intraoperative therapy that have specific malignant lesions with the abdomen and retroperitoneum, and lack evidence of metastatic spread. In general, the control arm of these studies receives resection with post-operative conventional fractionated radiotherapy, and the experimental arm receives in addition, intraoperative radiation therapy, as well as misonidazole, a known radiosensitizer of hypoxic cells, a single injection of 3.5 gm/m^2. Patients are followed closely to assess local toxicity, and patterns of recurrence.

Major Findings

With over 100 patients having been randomized to receive intraoperative radiation therapy at the NCI, there is no trend to suggest an improvement in local control, disease-free survival, or overall survival. Local control can be made to look quite good, if one talks only about the retroperitoneum. However, the marked predilection for carcinomatosis or sarcomatosis of the peritoneal surface itself, negates this potential gain. Until this problem can be overcome, intraoperative radiation therapy will not be useful on a large scale. Potentially, intraperitoneal chemotherapy, pre-operative radiation therapy, or intraoperative photodynamic therapy might be useful in overcoming this problem.

Significance to Biomedical Research and the Program of the Institute

Intraoperative radiation therapy studies are the first prospective randomized trials looking at this method of delivering radiation therapy.

Proposed Course

With the renovations of the electronics of the Microtron, we hope to continue these pilot trials. However, until we are able to deal realistically with the problem of peritoneal seeding, this modality will probably not prove to be useful. If we can overcome the problem of peritoneal seeding, this may represent a useful advance in a number of abdominal neoplasms. Photodynamic approaches to prevent peritoneal seeding are presently in Phase I studies.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06358-07 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Radiolysis, Photolysis and Sonolysis of Cells and their Constituents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Riesz	Research Chemist	ROB, NCI
Others:	M. K. Cherukuri	Visiting Associate	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Radiation Oncology Branch

SECTION: Experimental Phototherapy

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The photochemistry of the sensitizers N-formylkynurenine(NKF), Kynurenine(KU), 3-hydroxykynurenine(OHKU), riboflavin(RF) and flavin mononucleotide(FMN) which are endogenous to human eye lenses has been studied. These sensitizers have been found to act by both type I (electron transfer) and type II (singlet oxygen) photochemical reactions. The singlet oxygen quantum yields and the superoxide quantum yields have been estimated by spectrophotometric methods.and these quantum yields for the kynurenine derivatives were found to be significantly less compared to the flavin derivatives, which is in agreement with their respective triplet state quantum yields. Based on ESR and spin trapping experiments, it was found that the yield of the DMPO-superoxide adduct increased when the photochemical reaction was carried out in the presence of suitable electron donors for all the sensitizers studied. The relative yields of superoxide anion radical have been monitored by the cytochrome c reduction assay in the presence of EDTA and DETAPAC which were found to be suitable electron donors for NFK, RF and FMN. It was found that the superoxide anion radical yields increased 2-5 fold in the presence of millimolar concentrations of either EDTA or DETAPAC. The results indicate that the presence of electron donors could shift the mechanisms of photochemically induced cataract in lens from Type II to Type I. Our studies of the chemical effects of ultrasound in relation to hyperthermia combined with radiation therapy were continued. The 50 kHz sonolysis of argon-saturated water-acetone and water-acetonitrile mixtures was studied by EPR and spin trapping over a wide range of solvent composition. For both systems a single maximum was observed for the spin adduct yield of methyl radicals and of the radicals formed by H-abstraction from acetone and acetonitrile. Methyl radicals from acetone are formed by C-C bond scission in the collapsing argon bubbles. For acetonitrile C-H bond scission at high temperature is followed by H-addition to the triple bond and the decomposition of this intermediate radical to form methyl radicals.

Project Description

Objectives: The effects of ionizing and ultraviolet radiation and of ultrasound on biological macromolecules and their constituents are being investigated. Ionizing radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals, or by the "indirect effect" where radical species are hydrated electrons, hydrogen atoms, and hydroxyl radicals.

In the chain of events that lead to loss of biological activity, free radicals play an important role. Chemical compounds have been discovered which significantly modify radiation effects. These include: (a) electron affinity sensitizers which act on hypoxic tumor cells; (b) halogenated pyrimidines which are incorporated into DNA; and (c) cancer chemotherapy agents of the intercalating or alkylating type which sensitize tumor and normal cells. Studies of the mechanism of action of radiosensitizers and radioprotectors are necessary to design improved combinations of chemotherapy and radiation therapy.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity in macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

In the last few years, it has become apparent that superoxide anion radicals and hydroxyl radicals are found in many biological systems in the absence of either ionizing radiation or UV-photolysis. Recent reports have indicated that radicals are produced in the presence of certain anti-cancer drugs such as Bleomycin and Adriamycin. The significance of radical reactions is therefore not confined to radiation biology. It has also been shown that damage to tissues following ischemia appears to occur during reperfusion with oxygenated blood. This damage is generally considered to be due to the excessive production of superoxide radicals and hydrogen peroxide. In support of this hypothesis, it has been shown that in several model systems superoxide dismutase, catalase or allopurinol (a xanthine oxidase inhibitor) protect ischemic tissue from oxidative damage during reperfusion.

Methods Employed

Nucleic acids, proteins and their constituents were gamma-irradiated either in the solid state or in aqueous solutions in a 800-curie Colbalt gamma-source. Electron spin resonance studies were carried out with a Varian E-9 Spectrometer connected to an IBM-XT computer. For photolysis studies at specific wavelengths, a 1000-watt high

pressure Xenon arc source and monochromator were employed. For ultrasound exposures, aqueous solutions were insulated in a non-perturbing cylindrical cell with 1 mil mylar windows in an anachoic ultrasound exposure apparatus at 30 ± 0.5 degrees. Specimens were exposed to either continuous wave or tone bursts of 1 MHz ultrasound to simulate both therapeutic and diagnostic exposure conditions. In the spin trapping method, the short-lived free radicals react with a diamagnetic scavenger (the spin trap) to produce longer-lived radicals (the spin adduct) which can be conveniently investigated by e.s.r. In our studies, 2-Methyl-2-Nitrosopropane, 5,5-Dimethyl-1-Pyroline-N-Oxide, and 3,5-dibromo-2,6-dideuterio-4-nitrosobenzenesulfonate were employed as the spin traps.

Major Findings

I. Photochemistry of Endogenous Human Eye Lens Sensitizers (with C. Murali Krishna, J.S. Zigler, Laboratory of mechanisms of ocular diseases (LMOD, National Eye Institute) and D. Balasubramanian, (Centre for Cellular and Molecular Biology, Hyderabad, India)

The photochemistry of the sensitizers N-formylkynurenine(NFK), kynurenine(KU), 3-hydroxykynurenine(OHKU), riboflavin(RF) and flavin mononucleotide(FMN) which are endogenous to human eye lenses has been studied. These sensitizers have been found to act by both type I (electron transfer) and type II (singlet oxygen) photochemical reactions. The singlet oxygen quantum yields and the superoxide quantum yields have been estimated by spectrophotometric methods. The singlet oxygen and the superoxide anion radical quantum yields for the kynurenine derivatives were found to be significantly less compared to the flavin derivatives, which is in agreement with their respective triplet state quantum yields. Based on ESR and spin trapping experiments, it was found that the yield of the DMPO-superoxide adduct increased when the photochemical reaction was carried out in the presence of suitable electron donors for all the sensitizers studied. The relative yields of superoxide anion radical have been monitored by the cytochrome c reduction assay in the presence of EDTA and DETAPAC which were found to be suitable electron donors for MFK, RF and FMN. It was found that the superoxide anion radical yields increased 2-5 fold in the presence of millimolar concentrations of either EDTA or DETAPAC. For NADH, it was found that the SOD inhibitable superoxide anion radical yield from NFK and KUA increased 2- and 38-fold, respectively in the presence of 1 mM reduced nicotinamide adenine dinucleotide (NADH). The results indicate that the presence of electron donors could shift the mechanisms of photochemically induced cataract in lense from a Type II to a Type I mechanism depending on the relative concentrations of oxygen vs. endogenous electron donors. Further work on the crosslinking of lens crystallins by these sensitizers in the presence and absence of electron donors is being carried out.

II. Sonochemistry of Acetone and Acetonitrile in Aqueous Solutions (with Alasdair J. Carmichael, Radiation Biochemistry Department, Armed Forces Radiobiology Research Institute, Bethesda, MD)

Acoustic cavitation involes the formation, growth and implosive collapse of gas bubbles in liquids. The very high temperatures (5000K) and pressures (500

atmospheres) in local hot spots with lifetimes of a few microseconds lead to the thermal dissociation of water vapor into H atoms and OH radicals and to pyrolysis products from volatile organic solutes. The high temperature gradients in the interfacial regions of collapsing gas bubbles may also lead to pyrolysis products from solutes present at sufficiently high concentrations. The H atoms and OH radicals which escape into the bulk of the solution at ambient
temperature react with solutes to yield products identical to those observed in aqueous radiation chemistry. The 50 kHz sonolysis of argon saturated water-acetone and water-acetonitrile mixtures was studied by EPR and spin trapping with 3,5-dibromo-2,6-dideuterio-4-nitrosobenzenesulfonate over a wide range of solvent composition. For both systems a single maximum was observed for the spin adduct yield of methyl radicals and of the radicals formed by H-abstraction from acetone and acetonitrile. Methyl radicals from acetone are formed by C-C bond scission in the collapsing argon bubbles. For acetonitrile C-H bond scission at high temperature is followed by H-addition to the triplet bond and the decomposition of this intermediate radical to form methyl radicals. Since Anbar has shown (Science 161, 1343, 1961) that sonoluminescence and acoustic cavitation occur during the impact of liquid water on water with linear velocities similar to those of collapsing ocean waves, the sonochemistry of nitriles is of interest to chemical evolution studies.

III. An extensive invited review chapter was written for Advances in Sonochemistry, Vol. 2, T.J. Mason, Ed., on "Free radical generation by ultrasound in aqueous solutions of volatile and non-volatile solutes."

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 therapy and chemical and biological effects of ultrasound.

Publications

1. Kondo T, Riesz P. Sonochemistry of nitrone spin traps in aqueous solutions: evidence for pyrolysis radicals from spin traps, Free Radicals in Biol and Med 1989;7:259-268.

2. Murali Krishna C, Kondo T, Riesz P. Sonochemistry of alcohol-water mixtures: spin trapping evidence for thermal decomposition and isotope exchange reactions, J Phys Chem 1989;93:5166-5172.

3. Alegria AE, Lion Y, Kondo T, Riesz P. Sonolysis of surfactants in aqueous solutions: probing the interfacial region of cavitation bubbles by spin trapping, J Phys Chem 1989;93:4908-4913.

4. Kondo T, Riesz P. Hydrogen atom formation by ultrasound in D20 solutions of nitrone spin traps, Free Radical Res Comm 1989;7:11-18.

5. Alegria AE, Samuni A, Mitchell JB, Riesz P, Russo A. Free radicals induced by adriamycin-sensitive and resistant cells: a spin trapping study, Biochem J 1989;28:8653-8658.

6. Kondo T, Murali Krishna C and Riesz P. Pyrolysis radicals formed by ultrasound in aqueous solutions of nucleotides: a spin trapping study, Int J Radiat Biol 1990;57:23-33.

7. Riesz P, Kondo T, Krishna CM. Free radical formation by ultrasound in aqueous solutions: a spin trapping study, Free Rad Res Comm 1990 (in press).

8. 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, 1990 (in press).

9. Riesz P, Kondo T, Krishna CM. Sonochemistry of volatile and non-volatile solutes in aqueous solutions: EPR and spin trapping studies, Ultrasonics 1990 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06360-07 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Radionuclide Generators to Produce the Iridium-194 Beta Emitter

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:	T. J. McMurry	Senior Staff Fellow	ROB, NCI

COOPERATING UNITS *(if any)*

Oak Ridge National Laboratory (S. Mirzadeh)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	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.)*

The project investigates the design, manufacturing, testing, and use of a novel radionuclide generator for biomedical applications. The generator has a long shelf-half-life, many years, and produces a 20-hour radionuclide daughter which emits high-energy beta particles that have suitable characteristics for labelling proteins through bifunctional chelates.

In the 194-Os/194-Ir generator containing the parent nuclei, 194-Os has a half-life of 6.0 years which beta-decays, EmaxB=100 KeV, to the 19.15-hour 194-Ir daughter. The 194-Ir daughter decays with the emission of 2.2 MeV beta particles to the ground state of 194-Pt (86%) and to the 328.5 KeV first excited state with emission of 1.9 MeV beta particles (9.2%). There is a 328.5 KeV gamma-ray which follows the decay of 194-Ir with 13% absolute aboundance. The absence of high intensity gamma-rays in the decay of 194-Ir, with the exception to the 328.5 KeV, makes this beta emitter nuclei very attractive from the point of view of dosimetric considerations. On the other hand, the presence of 328.5 KeV gamma-rays makes 194-Ir a superior nuclei to 90-Y for tumor imaging.

This year, Dr. Mirzadeh moved from the NIH to Oak Ridge National Laboratory where he has now completed work on the Iridium-194 radionuclidic generator.

Substantial amounts of the isotope have been produced (2 millicuries). Work is now underway to form stable complexes of Ir-192 for eventual linkage to monoclonal antibodies.

Objectives

To develop a radionuclide generator system which will produce 194-Ir for attachment to proteins through bifunctional chelates and to devise methods for labeling proteins for use in immunotherapy.

Methods Employed

Osmium-194 was produced in a nuclear reactor by irradiating enriched Os-192. After irradiation, Os target was dissolved, purified and loaded into a suitable chromatographic column. The daughter 194-Ir was eluted with suitable solvents. The yield of the elution of the daughter, the breakthrough of the parent, radiation and chemical resistance of the column generator, radiochemical, and radionuclidic purity of the product were measured.

Next, 10 mg of enriched Os-192 (99.395%) was irradiated at BNL HFBR for a period of 21 days (a full reactor cycle), at position "Modified V16" with thermal neutron flux of 8.25×10^{14}/sec. cm^2. The preliminary results indicate that a 20-mCi generator can be produced by irradiating 100 mg of enriched Os-192 for a period of 3 months (3 reactor cycles). Presently, the decay of Os-194 is followed to obtain an accurate measure of its production cross-section. These measurements were used to prepare Os-194 for use in the Ir-194 generator.

Significance to Biomedical Research and the Program of the Institute

The development of the 194-Os-Ir generator for production of 20-hour 194-Ir would increase access of the biomedical community to a high-energy beta emitter as a radiotherapeutic agent. The radionuclide has the advantage of clearing the body rapidly.

Proposed Course

Since the radionuclidic generator for Ir-194 is now available, we will proceed to develop the chemistry required to link the radionuclide to immunoproteins.

1) Suitable chelating agents will be prepared and tested. Initially a series of bifunctional amines will be prepared and sent to Oak Ridge where collaborators will try to form the radioactive Iridium complexes and conduct in vitro tests of chelate stability.

2) For those chelates which are stable, bifunctional ligands will be prepared for use in linking iridium to proteins.

3) Proteins labeled with Ir-194 will be studied in vivo.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06361-06 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Phototherapy of Intracavitary Spaces

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	H. Pass	Senior Investigator	SOB, NCI
	P. Smith	Senior Investigator	BEIB
	W. Frauf	Senior Investigator	BEIB
	C. Black	Associate	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The use of hematoporphyrin derivative and other photosensitizing agents in combination with light activation is currently being investigated as an anti-tumor modality for the treatment of intraperitoneal and intrathoracic tumors. A major advantage of this modality is the apparent selective retention of the sensitizing dye within tumors. A murine ascites ovarian carcinoma and a human ovarian tumor that grows as an ascites tumor has been used to study the characteristics of drug distribution in the peritoneal cavity. Likewise, murine models have been 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 are being studied. We have shown that Phototherapy can be used to effectively treat a murine ascites tumor. We have also shown that in both the murine and the canine model, the peritoneal serosal surface is tolerant of at least 0.5 J/cm^2 and that this work can be extended to human subjects. In the murine system, we have shown that the thoracic cavity, like the peritoneal cavity, is exquisitely sensitive to treatment with red light (630 nm). The dose rate must be controlled to minimize heat build up (less than 150 mW fiber output from a forward projecting optical fiber is usually tolerated). We are exploring the use of photoimmunotherapy as an additional means of drug delivery. We are exploring the use of chemiluminescence as a means of light delivery to the cavitary spaces.

GPO 914-918

Project Description

Objective: 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. To determine the best means of delivering light and sensitizer and to establish means to better quantitate light delivered to the tumor and normal tissue (dosimetry), to originate means of improving or circumventing phototoxicity to normal tissue, and to provide means to remove viruses outside of cells or cells containing viruses either inside the cell or incorporated into the human DNA in banked blood intended for human use are secondary objectives of the study.

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 affixed to either Porphyrin C or hexaethylmethylporphyine-3-propionic acid 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 or ovarian tumors that have been developed for growth in an athymic murine model system. General searches for sensitizers that absorb light at longer wavelengths (>600 nm) are being sought that also have the characteristics of being lipid membrane permeable and favorably partition to nucleic acid oligomers. Such sensitizers are investigated for viricidal effect.

Major Findings

Preliminary work in a cell culture system that has been pretreated with HPD shows that chemiluminescence agents provide enough light to be effectively used as a light source. We have evaluated a number of chemiluminescent agents and are still in the process of identifying the most efficient agent. The agents in and of themselves do induce cytotoxicity and of course this is a concern. Plastic models of a canine thoracic cavity suggest that intralipid (fat emulsion) can be used for real-time simultaneous equal light distribution to the pleural surface when three or more fiber sources are concurrently used. Studies using these models have led to determination of toxcitiy in animals of treating the plural surface. The animal studies have been completed and the Radiation Oncology Branch in cooperation with the Surgery Branch have begun pilot 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.

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. Russo A, Mitchell JB. Future directions of photodynamic therapy. In: Phototherapy of human tumors, (in press).

2. Mitchell JB, Cook JA, Russo A. Biological basis for phototherapy. In: Phototherapy of human tumors, (in press).

3. Manyak MJ, Nelson LM, Solomon D, Russo A, Thomas GF and Stillman RJ. Photodynamic therapy of rabbit endometrial transplants: a model for treatment of endometriosis, Fertil Steril 1989;52:140-145.

4. Bernstein EF, Thomas GF, Smith PD, Mitchell JB, Glatstein E, Kantor GR, Spielvogel RL and Russo A. Response of black and white Guinea pig skin to photodynamic treatment using 514 nm light and photofrin II, J Dermatol, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06378-05 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

QA of Treatment Delivery by Means of Overlayed Digitized Simulator & Port Films

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	B. Chin Arora	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	.25	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The quality assurance of the consistency of radiation treatment delivery with the prescription is a continual concern, locally as well as nationally. The ROB already employs graticules projecting onto all simulator films and all corresponding portfilms. A project has been started to overlay differently processed digitized films to increase the quality of information, as well as to decrease the volume of documentation to be retained.

The system should be of great interest to inter-institutional studies as well. The project has been on hold until recently because of delays in acquisition of essential hardware due to lack of funds. It is nearing completion now.

GPO 914-918

Project Description

Objectives: 1) To improve the quality of documentation on the proper implementation of beam treatment set-ups.

2) To intergrate this information with the MacII based treatment planning system.

3) To condense the amount of documentation to be kept, and to increase its objectivity and exchangeability.

Methods Employed

1. Take x-ray films at the simulator, in the planned beam positions, including graticules projected onto the films.

2. Follow similar procedure at the treatment machine, producing port films with graticules.

3. Digitize both categories of films taking care to use the same orientation, cetering and magnification, with help of the graticules projected onto all films, and enter the data into a MacII computer system.

4. Apply appropriate computer enhancement of both simulator films and the corresponding port films.

5. Use overlay techniques to bring out salient anatomical features, graticules, block delineation, etc.

6. Using the computer, do measure significant deviations.

7. Store the results, properly labeled.

8. Evaluate the quality of treatment delivery.

Maior Findings

Great progress has been made over the last decades in diagnostic imaging and computerized treatment planning. The overall quality assurance, control and documentation of actual treatment delivery is not at the same level. This project promises major improvement.

Significance to Biomedical Research and the Program of the Institute

1. Quality assurance and verification will become much more efficient, self-contained and attractive to use.

2. Documentation will be much more compact and easier to use.

3. Quality assurance of joint studies will be much easier and more objective.

Proposed Course

To implement the system in the Macintosh II environment and start a pilot project.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06379-04 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Phase I Study of Photodynamic Therapy for Surface Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR; Laboratory of Pathology, NCI; Diagnostic Radiology Department, CC

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	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.)*

Photodynamic therapy involves the use of a light activated compound which localizes in tumor, followed by the activation of this compound by light for cytotoxic effects for the treatment of cancer. The current protocol uses the intravenous administration of the Photofrin II preparation of the hematoporphyrin derivative, the only currently approved photosensitizer for use in humans. This is followed by the delivery of light to the affected area using optical fibers coupled to an argon/pumped dye laser. Hematoporphyrin derivative selectively localizes in tumor compared to certain normal tissues. Selective retention of the photosensitizer in combination with focal light delivery to the involved area permits selective destruction of tumor with minimal effect on uninvolved normal tissue. Hematoporphyrin derivative photodynamic therapy may be clinically useful in a number of anatomic sites involved by tumor.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on Project:

H. Pass	Senior Investigator	SB, NCI
W. Sindelar	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR
W. Travis	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	DR, CC

Objectives

This is a Phase I study designed to assess the toxicity and effectiveness of photodynamic therapy with Photofrin II and laser light in treatment of surface malignancies. Physical parameters of light distribution in tissue are being measured, as well as photosensitizer pharmacology.

Methods Employed

Patients with surface malignancies, cutaneous or mucosal, that are not curable by conventional therapy are eligible for this protocol. Patients receive the Photofrin II photosensitizer by intravenous administration 1.5 - 2.5 mg/kg. Laser light is then delivered in single or multiple fractions to the involved tumor area, using optical fibers for surface illumination, endoscopic treatment, or intraoperative treatment, depending on the patient's clinical problem.

Major Findings

Patients with recurrent tumors involving skin and patients with tumors obstructing the bronchus comprise the majority of patients treated (A small number of patients with intraperitoneal tumors and pleural tumors have been treated in a pilot fashion to permit the design of formal, phase I photodynamic therapy in each of these anatomic areas).

Twenty patients with recurrent breast cancer on the chest wall have been treated on this protocol. Four patients (20%) experienced a complete regression of tumor while 9 patients (45%) experienced a partial response, defined as reduction of tumor greater than 50%. Unfortunately the duration of the complete responses was generally less than 6 months, while the duration of the partial response was only 4 months. A major problem in the use of this modality for the treatment of these breast cancers is the limited light penetration of the light wavelength currently employed. Other patients 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.

In patients who have been treated on this protocol for tumors obstructing the bronchus, twelve patients had metastatic tumors and 6 patients had tumors of the bronchus or the trachea. Treatment was considered successful in 13 of 18 patients (72%) with collapsed lobes re-expanding or re-opening distal airways. Two patients (11%) had a transient re-expansion of previously obstructed areas for less than 2 months. One patient had a mixed response with 1 of 2 obstructed bronchi re-expanding and 2 patients (11%) had no response to treatment.

Nine patients with disseminated intraperitoneal tumors received the hematoporphyrin derivative prior to laparotomy. This was a pilot group of patients prior to the initiation of a formal Phase I study of Surgery and Photodynamic with Laser Light and Photofrin II for Intraperitoneal Malignancies. Of these first 9 patients, 6 were able to get tumor debulking and intraperitoneal photodynamic therapy at progressively increasing light doses from 0.2 - 0.6 J/cm^2 without toxicity. On the basis of the findings in these patients, a formal study of photodynamic therapy for intraperitoneal malignancies was initiated.

Seven patients with tumors involving the pleural space received the photosensitizer prior to thoracotomy/median sternotomy, at which time tumor was debulked to< 5mm and at increasing light doses from 5.0 to 15.0 J/cm^2 without significant toxicity. On the basis of the findings in these patients, a formal study of Surgery and Intraperitoneal Photodynamic Therapy for Pleural Malignancies has been initiated.

Treatment-related morbidity includes sunburn in five patients, full thickness skin necrosis in 2 patients requiring surgical repair or burn treatment, and moderate discomfort in the treatment field requiring medication. In the patients with bronchial lesions, 1 patient died of massive hemoptysis from recurrent tumor in 3 months after treatment. The contribution of photodynamic therapy to this complication is uncertain. One patient developed a radiographic infiltrate after treatment which resolved on antibiotics and 1 patient developed a pneumothorax which was successfully treated.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially curative therapy for selective groups of patients with malignant disease. In particular, patients with a tumor that is accessible to light either by superficial, endoscopic or interstitial illumination may benefit from treatment. Intraoperative treatment is both practical and potentially efficacious. Photodynamic therapy is being explored for use in multiple anatomic sites including the superficial tumors in the urinary bladder, tumors involving peritoneal and pleural surfaces, gynecologic malignancies, brain tumors, and selected skin cancers.

Proposed Course

Phase I study of photodynamic therapy are in progress in the peritoneal and pleural cavity, as well as in the urinary bladder. On completion of these trials we would hope to move on to Phase II studies in the following sites, peritoneal cavity, pleural cavity, and bladder. Long range plans also include examination of other photosensitizers which may be activated by light with deeper tissue penetration in which may have less cutaneous photosensitivity.

Publications

1. Pass HI, DeLaney TF, Smith PD, Bonner R, Russo A. Bronchoscopic phototherapy at comparable dose rates: early results, Ann Thorac Surg 1989;47:693-9.

2. Pass HI, Mitchell J, Russo A, DeLaney TF, Glatstein E. Photodynamic therapy. In: Wiernik PH, ed. Mediguide to oncology. New York: L Dellacorte, 1989;1-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06381-04 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modeling of Time-Dose Response of Human Tumors and Normal Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	T. Goffman	Radiotherapist	ROB, NCI
	J. Mitchell	Radiobiologist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	E. Glatstein	Radiotherapist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The purpose of radiation therapy is tumor control. In view of clonogen proliferation it makes sense to deliver the necessary dose in as short a time as possible. The limiting factor in tumor treatment is normal tissue reaction: normal tissue reactions must not exceed "tolerance" level. The development and application of high technology particularly in computers and computer-based and assisted imaging has stimulated great progress in tumor localization and treatment planning, and even in the technology of delivery and its quality assurance, i.e., the spatial aspects of the issue. Decision-making as to the amount of dose and its distribution over time (by fractionation or protraction) is still essentially empirical, however. The present project continues the development and exploration of a theoretical description of time-dose response of tumors as well as normal tissues. Its basic concepts have been published in 1988[1]. Since the, further developments have concentrated on extension of the Linear-Qudratic model. This has resulted in some further publications. The extension concerns a unified description of the influence of incomplete repair and comprises a description of the influence of tumor proliferation and stem cell/transition cell repopulation. Currently a study is being completed regarding the implications of high dose arate vs. low dose rate brachytherapy. This work includes the development of an interactive computer program to search for high dose rates compatable with constant tumor control as well as no more severe toxicity.

GPO 914-918

Project Description

Objectives: To develop a mathematical formalism describing:

1. The attrition of functioning normal tissue cells.
2. The survival rate, per single dose, of viable stem cells.
3. The inter-fraction and post-treatment course repopulation including an account of the sublethal damage repair of viable stem cells.
4. The survival rate of clonogenic tumor cells per single-dose.
5. The effective dose for early as well as late reacting normal tissues.
6. The inter-fraction and post-treatment growth pattern of the clonogenic cells as well as the gross tumor.
7. Extension of the model to cover both high-dose rate fractionated (beam) therapy and protracted therapy including brachytherapy.

Methods Employed

1. The alpha/beta (2-parameter) model is applied for the single-dose response of stem cells and clonogenic tumor cells.

2. Radiation damage is assumed to consist of lethal and sublethal damage.

3. Linear attrition over time is assumed for functioning normal tissue cells as well as non-clonogenic tumor cells.

4. Normal tissue cell loss and replacement is under homeostatic control.

5. Clonogenic tumor cells are assumed proliferate exponentially over time.

6. Stem-cell proliferation is triggered only after some distress signal related to functionality cell levels drop below a certain threshold.

7. Normal tissue tolerance in interpreted as the lower limit of normal tissue functionality: the normal tissue functioning cells dropping below some fraction of their normal count.

Major Findings

1. Initially, a "two component" mathematical model was developed and was shown to be promising. A major paper was published in 1988.

2. Since then, further development has concentrated on extension of the "Linear Quadratic" (LQ) model of single dose response; in particular, we have developed a generalized description of the relative importance of "extra lethal damage" resulting from incompletely repaired sublethal damage still existing at the time of delivery of new dose. The mathematical description covers both high dose rate fractionated (external beam) therapy and high dose rate as well as low dose rate brachytherapy. A paper on the mathematical aspects is now under review for publication in Medical Physics.

3. Interactive computer programs have been developed which enable automatic search for acceptable parameters, based on reasonable estimated ranges of certain key parameters, α and β, cell doubling times, etc.

4. It is possible to simulate time-dose response patterns for conventional and unconventional fractionation schemes, which are reasonably consistent with published findings in some clinical trials.

5. Recent work has concentrated on high dose rate (HDR) vs. low dose rate (LDR) brachtherapy, which is of particular interest in view of technological developments in small "hot" sources for afterloading equipment. It now seems possible to determine "acceptable" ranges of dose rates and corresponding total doses in regard to early and late reactions of normal tissues, depending on the relative dose levels at these tissues.

Significance to Biomedical Research and the Program of the Institute

1. The present model shows promise as a tool toward understanding of time-dose response to conventional or "standard" treatment schedules, as well as some hyper-fractionation schemes and other non-standard schemes.

2. The model promises to become useful to explore, by simulation, other unconventional schemes, and provide reasoned guidance to at least avoid work results especially as regards to late reactions and tumor.

3. The developments as to high dose rate vs. low dose rate treatment are of great practical significance, as they may help change the logistic, economic and possibly the clinical r results of brachytherapy.

Proposed Course

1. Continuation of theoretical studies

2. Study of clinical data.

3. Organisation of an NCI sponsored workshop.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 06382-04 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Therapy with Radiolabelled Antibodies: Technical & Dosimetric Aspects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* (Name, title, laboratory, and institute affiliation)

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	N. Wersto	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	J. Carrasquillo	Nuclear Medicine Physician	NM, CC

COOPERATING UNITS *(if any)*
Nuclear Medicine Department, CC; Diagnostic Radiology Department, CC.

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.0	8.5	1.5

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

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

Administration of radiolabelled antibodies is a relatively new treatment modality for certain forms of cancer. Much of this field is developmental in nature. In particular, the dosimetry of tumor masses, especially at the microscopic level, is at yet unknown. The Radiation Physics and Computer Automation Section is actively assisting in the implementation of clinical protocols. Current research is in two major areas.

Imaging of the organ-specific distribution patterns on a temporal basis is fundamental to the understanding of antibody kinetics and for large volume radiation dosimetry (at the total organ level). The ability to accurately localize biodistribution patterns using nuclear medicine imaging techniques and to accurately register these images with respect to other imaging modalities (CT or MRI) is essential for obtaining quantitative results.

Dosimetry of microscopic tumor masses is approached through the use of computer modeling. The results, where possible, will be validated using quantitative autoradiography.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objectives: To localize the sites of retention of radiolabelled antibodies and to determine the deposition-retention kinetics as well as the clearance pathways. To determine normal organ radiation doses and tumor dose, if possible on a microscopic level for alpha, beta and gamma emitting radionuclides. To determine the optimum combination of imaging modalities for localization and to determine the lower limits of detection of tumor masses with external imaging devices.

Methods Employed

This project will use small animal models to determine the metabolic pathways of various antibodies and their deposition-retention-excretion kinetics. Phantom studies will be conducted to determine the optimum imaging modalities and their lower limits of detection. These will be confirmed using large animal models. Computer models for determining dose distributions on a microscopic level and for alpha emitting radionuclides will be developed and tested with animal models. Patients under treatment will be imaged, as appropriate, and will be bioassayed using external counting techniques. Biopsies will be taken and used to validate metabolic and dosimetric models for each radiolabelled antibody.

Major Findings

Studies at other institutions indicate that therapy with radiolabelled antibodies offers little advantage over conventional forms of radiation therapy in the treatment of large tumor masses, due to the inhomogeneous distribution pattern of organ uptake. This results in large dose gradients within the treated site. Antibody therapy shows real promise, however, in the treatment of small tumor masses, especially microscopic disease. The problem with this approach is that the size of these masses makes them difficult to localize using traditional nuclear medicine imaging techniques. It may be possible to image these masses by employing other imaging modalities, either singly or in combination. Also, the dose calculational formalism for distributed radionuclide sources (MIRD), may no longer be valid under these conditions, since the range of the particulate radiations may be greater than the dimensions of the tumor mass and the distribution of radioactivity may be inhomogeneous. A new formalism will need to be developed for alpha emitting radionuclides, as their energy deposition pattern differs significantly from beta-gamma emitters.

Significance to Biomedical Research and the Program of the Institute

Radiolabelled antibodies are a new, exciting potential treatment modality. They offer the promise of selectively irradiating tumor masses, while delivering minimal radiation doses to normal tissues. This represents the ideal form of radiation therapy. It is possible that, for some forms of cancer, radiolabelled antibody therapy will supplant chemotherapy as the treatment of choice for microscopic disease.

Proposed Course

To be continued. The SPECT camera dedicated to this project is to be replaced by a dual head, opposed crystal camera. This will permit us to simultaneously acquire AP and PA whole-body scans and will simplify correction for self-attenuation. Depending on the model purchased, this unit may be SPECT capible. A laser system to facilitate patient alignment has been installed in the room where the current camera will be located. Phantom studies will shortly commence, first employing simple geometries.Existing humanoid phantoms which will permit imaging via multiple modalities are inadequate. Effort will be directed towards development of an organ phantom which accurately retains its internal anatomy and which can be adequately scanned by CT and MRI as well as gamma cameras, SPECT and PET. Image processing techniques will be developed to correlate images from different scanning modalities to aid in diagnosis and treatment planning. Two alternative methods for quantitating whole-body clearance of gamma-emitting radioisotopes will be instituted and compared. The first uses a dedicated microcomputer with both multichannel analysis and multichannel scaling capabilities, while the latter is a much simpler, less expensive system utilizing a portable, data-logging radiation detector.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06383-04 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Development of an Improved Treatment Chair for Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Ovadia	Visiting Scientist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH Radiation Oncology Branch

SECTION Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

This project is intended to design a treatment chair which overcomes the inherent design limitations of commercially available chairs. This chair will function independently of the treatment couch so as to permit opposed field treatment in any orientation in an extended isocentric fashion (the center of rotation will be at a distance greater than the standard isocenter of the accelerator). It will be capable of accurate, reproducible rotation and translation in the lateral, longitudinal and vertical planes. If possible, the chair will function with a standard radiotherapy simulator to permit proper localization, immobilization and treatment planning.

The chair is being designed on the "tool platform" principle. That is, the chair will function as a platform, allowing the attachment of various additional devices which can be placed in such a manner that they permit proper immobilization of the patient without unduly restricting treatment delivery .

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objectives: To develop an independent treatment chair to permit multiple-field radiation therapy at either standard or extended SSD.

Methods Employed

The Radiation Therapy Machine Shop fabricates any chair components and accessories that are needed. Selected patients are placed in the chair for simulation and for their course of therapy. Any problems associated with immobilization and repositioning are analyzed on a daily basis and the necessary modifications are made.

Major Findings

The initial version of the treatment chair permitted opposed-field treatments and could be used with the simulator as well as with any treatment unit. Treatment of some forms of cancer with the patient seated is advantageous. The original design of the chair was excessively limited by the stipulation that it operate with the simulator. The hydraulic vertical motion was imprecise and the positioning of the patient by hand was operationally difficult. The center of rotation of the chair was also at an undesirable location, which precluded its use in an isocentric manner. A new elevating mechanism has been designed and implemented. This consists of an electrically-driven, precision scissors which provide a great degree of stability and a more precise control of vertical position. Patient positioning has been improved by using a back rest and a silastic seat cushion. A new back rest "Tennis Racket" is being built to allow for the marking of posterior set-up and alignment points on the patient. An "extended isocentric" mounting is being explored for the chair. This will provide for complete clearance of all obstacles, which in the past have limited the rotational freedom of the chair. A distance of up to 120 cm. can be accommodated with the current simulator.

Significance to Biomedical Research and the Program of the Institute

Treatment of the mediastinum with the patient seated can minimize the amount of lung in the irradiated field, minimizing complications. A combined Waldeyer's/mantle field treatment is possible in this position. Also, low dose rate mantle fields can be used by placing the chair at an extended SSD.

Proposed Course

Currently, the chair has undergone a major modification which incorporates a base-mounted turntable to provide isocentric positioning of the patient. This greatly simplifies the initial set-up and treatment. The vertical stability has also been greatly improved. These modifications still permit the chair to be used with our current simulator. A new elevating mechanism will be

developed to give precise control over patient positioning in the vertical direction. Also, the possibility of providing motor driven controls will be explored and additional attachments for positioning and immobilizing the patient will be developed.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06386-03 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Radioimmunotherapy of Peritoneal Cancer with I-131 Labeled B72.3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Raubitschek	Senior Investigator	ROB, NCI
Others:	J. Carrasquillo	Head, Antibodies Project	NM, CC
	R. Neumann	Chief	NM, CC
	J. Reynolds	Senior Investigator	NM, CC
	J. Schlom	Chief	LTIB, NCI
	D. Colcher	Senior Investigator	LTIB, NCI

COOPERATING UNITS *(if any)*

Nuclear Medicine Department, CC; Laboratory of Tumor Immunology and Biology, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.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.)*

In cooperation with the Department of Nuclear Medicine, and the Laboratory of Tumor Immunology and Biology, we have initiated clinical trials for the treatment of peritoneal carcinomatosis. A classical phase one study has begun using escalating doses of I-131 labeled antibody administered intraperitoneally.

Since last year's report we have only treated an additional four patients as well as continue to follow the patients treated initially. We have now reached a dose of 150 mCi of Iodine-131 labeled b72.3. With the increased doses we have noticed the appearance of two new symptom complexes. One we relate to a mild peritonitis occurring approximately one week after treatment and manifest by mild abdominal tenderness and the accumulation of ascites. These symptoms resolve spontaneously over the following few weeks requiring no medical intervention. We have also noticed the onset of diarrhea several days after the administration of the isotope. This also resolves without treatment over a few weeks. This is probably a direct effect of the radiation on the mucosal surface of the gastrointestinal track.

Dose limiting toxicity appears still to be bone marrow, with patients with extensive previous chemotherapy being limited to 125-150 mCi. Patients without extensive pretreatment may be able to receive as high as 175 mCi, although this dose level has not been reached yet.

The clinical protocol has been recently revised by Dr. Carrasquillo to more accurately define the maximum tolerated doses. A follow-up protocol using a higher affinity antibody CC49, with concomitant administration of intraperitoneal gamma interferon is being written.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06387-03 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Development of Superoxide Dismutase Mimics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	A. Samuni	Visiting Scientist	ROB, NCI
	C. Black	Associate	ROB, NCI
	C. Krishna	Associate	ROB, NCI
	Stephen Hahn	Associate	ROB, NCI
	J. B. Mitchell	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

There has been recent interest in the administration of superoxide dismutase (SOD) for ablation of oxygen mediated tissue damage. It is known that oxygen radicals such as superoxide and hydroxyl radical are important radiolysis products in an oxygenated aqueous environment. To date, there is no means of easily studying the effects of augmenting SOD cells. We have found that certain nitroxides, particularly oxazolidine containing nitroxides, may act as low molecular weight, cell permeable, superoxide dismutase mimics. We have demonstrated pH dependence of the oxazolidine nitroxide (OXANO) SOD mimic in both a Xanthine oxidase and cesium source superoxide generating systems. Similarly, the rates of SOD like activity of the oxan system has been determined. The scope of the chemistry is being investigated by synthesizing analogues. Likewise, the biochemistry of the reaction is under investigation. We have shown that these compounds protect mammalian cells *in vitro* against hydrogen peroxide and hypoxanthine/xanthine oxidase cytotoxicity. The compounds have been demonstrated to be non-toxic and capable of penetrating intracellular sites. We have recently demonstrated that nitroxides are also radiation protectors for mammalian cells and preliminary work indicates that they protect against whole body radiation in mice. Preliminary studies also indicate that nitroxides can have a marked influence on the cytotoxicity mediated by a number of chemotherapy drugs.

GPO 914-918

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 studied. We hope to demonstrate that nitroxides will be useful agents against toxicity mediated by chemotherapy drugs and ionizing radiation. A major objective of the next year will be 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 mechnism of action and the use of analogues in *in vivo* systems.

Methods Employed

Electron spin resonance spectroscopy allows the study of free radical chemistry and biology. The study of short lived oxy-radicals (spin trapping) or the rate of interaction of superoxide with oxazolidine nitroxides (stable spin labels) is well suited to the use of electron spin resonance. 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. Immediate use of polymorphonuclear white blood cells to investigate the oxygen burst phenomenon and the interpretation of DMPO reactions, as well the use of superoxide dismutase mimics in changing the effects of the oxygen burst. Murine systems will be used to investigate the pharmacology, biodistribution, and metalism of the different nitroxides.

Major Findings

We have shown that nitroxides protect against oxidative stress. Mammalian cells were protected against hydrogen peroxide and superoxide generated by hypoxanthine/xanthine oxidase. Further we have shown that cells can be protected against ionizing radiation with use of nitroxides. Our preliminary data also indicates that nitroxides afford protection against ionizing radiation to animals. These findings are important in that they afford the opportunity to learn more of the mechanisms of action of agents that impose oxidative stress and may have practical use in clinical situtations where protection against oxidative stress is desirable. This would apply for radiation and perhaps for selected chemotherapy drugs as well.

Significance to Biomedical Research and the Program of the Institute

The study of nitroxide and how they protect against agents which impose oxidative stress will further our understanding and hopefully offer clinical avenues to explore the protection of normal tissues against radiation or chemotherapy. The superoxide dismutase mimics may have applications in the area of coronary reperfusion, arthritis treatment, inflammation resolution, and decreasing harmful effects of the anthracyclines and bleomycin antineoplastic agents (respective cardiac and lung toxicities), as well as changing the dose response to radiation-induced damage.

Proposed Course

To explore the breath of the chemical and biochemical reactions of a series of nitroxide analogues. To synthesize additional analogues which have different characteristics to explore the cellular effects of having superoxide dismutase activity in different sites (membrane, cytoplasm, nucleus, mitochondria) and to investigate the dose modifying effects of such superoxide dismutase mimics on ionizing radiation and chemotherapy drugs.

Publications

1. Samuni A, Krishna CM, Riesz P, Finkelstein E, Russo A. Superoxide reaction with nitroxide spin-adducts, Free Radic Biol Med 1989;6(2):141-8.

2. Mitchell JB, Samuni A, Krishna CM, DeGraff WG, Ahn MS and Russo A. Biologically active metal-independent superoxide dismutase mimics. Biochemistry 1990;29:2802-2807.

3. Samuni A, Mitchell JB, Samuni U, DeGraff W, Krishna CM and Russo A. Nitroxide SOD-mimics: modes of action, Free Radical Research Communication, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06388-03 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Treatment of Superficial Carcinoma of the Bladder with Photoradiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Bladder cancer is subdivided into four groups: 1) Superficial high-grade disease (TIS); 2) superficial disease (T1); 3) superficially invasive into the muscle (T2); and 4) tumor extending into deep muscle and/or perivesical fat (T3A/T3B). Standard therapy for superficial disease confined to the mucosa or submucosa consists of transurethral resection and intravesical chemotherapy (thiotepa, mitomycin C, BCG). Recurrence rates may range from 30%-85% depending upon the grade of tumor and multiplicity of lesions. The concept of a full field defect in patients with carcinoma in-situ in association with a solitary papillary tumor is supported by the high incidence of invasive disease developing within two years following resection alone. Five-year survival rates for patients developing invasive disease (T2/T3A) range from 31%-52%. Early control of superficial disease offers a potential advantage towards reduction of the overall death rate in bladder malignancy. Carcinoma in-situ refractory to intravesical chemotherapy is a particularly troublesome clinical entity, as patients are at high risk for the development of invasive disease and may require removal of the urinary bladder (cystectomy). Recent work with hematoporphyrin derivative ($^{H}p^{D}$) sensitized photodynamic therapy of the bladder mucosa suggests high cytotoxic effect, but low systemic toxicity. This modality may permit treatment of superficial carcinoma of the bladder as well as carcinoma in-situ which may permit bladder preservation with cure of tumor.

GPO 914-918

Project Description

Professional Personnel Engaged on Project:

W. M. Linehan	Senior Investigator	SB, NCI
M. Walther	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR

Objectives

This is a Phase I trial designed to determine the feasibility of treating patients with superficial bladder carcinoma with a combination of hematoporphyrin derivative ($^{H}p^{D}$) and laser light, and to judge tumor response.

Methods Employed

Eligible patients will receive hematoporphyrin derivative by intravenous injection. They will subsequently undergo a cystoscopy at which time light will be delivered to the bladder. Following treatment, both cystoscopy and urine cytology will be done regularly to assess response to treatment. If partial responses are observed without serious side effects, repeat treatment will be performed. Patients who develop recurrence or invasive bladder cancer will be taken off protocol and referred for appropriate treatment. In the past year we have began to accrue patients for this protocol. Four patients have been treated to date. Two patients remain free of disease 3 months after treatment, 1 patient recurred focally in the bladder 6 months after treatment and was able to have this focal area of recurrence resected with a cystoscope and is currently free of disease, and 1 patient still has less than 3 months of follow-up. All patients have had transient significant bladder irritation secondary to treatment which has been managed symptomatically. One patient continues to have a irritated bladder 3 months after treatment. Additional patients will need to be studied with refinement and treatment and dosimetry technique.

Major Findings

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially useful mode of curative therapy for selected patients with superficial carcinoma of the bladder. If this is achievable without requiring that the patients have their urinary bladder removed, this will represent a major advance in treatment of superficial carcinoma of the bladder.

Proposed Course

We propose to study 8 additional patients on this protocol. We are developing a system for monitoring the dose of light during the treatment, which we think will improve treatment. The patients will need to be followed for at least a year before we can have any assessment of efficacy.

Publications

None to date.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06390-02 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Bifunctional Chelates for Gallium (III)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. J. McMurry Senior Staff Fellow ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

We have initiated a project with the intent of developing bifunctional chelates to specifically sequester trivalent radionuclides such as Gallium (III). Bifunctional chelates provide a means for conjugating radionuclides to monoclonal antibody, resulting in a potentially site-specific radiopharmaceutical. To this end, we have chosen to synthesize new bifunctional chelates which incorporate functionalized catechol (1, 2-dihydroxbenzene) binding subunits. Chelates of this type form highly stable 3:1 complexes with trivalent metals at physiological pH, yet typically have little affinity for divalent metals, thus making the tris (catecholates) inherently more selective complexing agents than the more familiar polyaminocarboxylates (e.g., EDTA).

We have recently completed the synthesis of a new macrocyclic tris (catecholate) chelate which incorporates a side arm for attachment to monoclonal antibody. Preliminary thin layer chromatography evidence indicates the chelate efficiently extracts Ga-67 from Ga-67 (citrate) at pH 6.6.

We have successfully linked the new bifunctional catecholate to monoclonal antibodies and have labeled the ligand with gallium and indium. Evaluations of immunoreactivities and initiation of animal tissue distributions have begun.

Professional Personnel Engaged on the Project:

G. Pippin	Staff Fellow	ROB, NCI
O. Gansow	Senior Investigator	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. These 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

We have synthesized the first macrocyclic tris (catecholate) chelate with appropriate functionality necessary to attach the chelate to monoclonal antibody. The efficacy of this ligand is demonstrated by its ability to remove Ga-67 from the citrate ligand at pH 6.6.

We have demonstrated that this ligand may be linked to proteins and radiolabeled with radionuclides of gallium and indium.

Significance to Biomedical Research and the Program of the Institute

Several Gallium isotopes have desirable properties for applications in nuclear medicine. In particular, Ga-67 78.3 hr, (EC 100%, 93(38%), 185(24%) KeV) and Ga-68 (68 min., B^+, 90% (1.89 MeV, 100%) are suitable for gamma imaging and PET scanning, respectively, while the energetic positron emission of Ga-66 (9.45 hr, B^+,56%(4.2 MeV,51.2%), EC 44%) combined with its half-life of 9.5 hours make it an attractive candidate for radioimmunotherapy. While simple inorganic complexes (e.g., Ga-67 (citrate)) of Ga-67 and Ga-68 are used clinically, it is anticipated that conjugation with monoclonal antibody will greatly enhance the utility of Gallium isotopes. By developing a selective and stable bifunctional chelate for attachment of Gallium to monoclonal antibody, we hope to contribute to the development of site-specific radiopharmaceuticals, in particular, for the treatment of AIDS-related lymphoma.

Publication

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06391-01 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

IIUdR as a Radiosensitizer in Unresectable Sarcomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Goffman	Head, Clinical Therapy Section	ROB, NCI
Others:	J. Mitchell	Deputy Chief	ROB, NCI
	A. Russo	Head, Exp. Phototherapy Sec.	ROB, NCI
	J. Cook	Senior Staff Fellow	ROB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC
	S. Rosenberg	Chief	SB, NCI
	S. Steinberg	Head	BDMS, NCI

COOPERATING UNITS *(if any)*

Cancer Nursing Service, CC
Surgery Branch
Biostatistics and Data Management Section

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5-6	4	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

This protocol has accrued 7 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, although the tenacity of private practice oncology keeps many patients from ever being seen here. There have been no significant toxicities to date. No publication planned at this time with such small numbers.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Professional Personnel Engaged on the Project:

J. Mitchell, Ph.D.	Deputy Chief	ROB, NCI
A. Russo, M.D., Ph.D.	Head, Exp. Phototherapy Sec.	ROB, NCI
J. Cook, Ph.D.	Senior Staff Fellow	ROB, NCI
R. Smith, R.N.	Cancer Nursing Specialist	CNS, CC
S. Rosenberg, M.D.	Chief	SB, NCI
S. Steinberg, Ph.D.	Head	BDMS, NCI

Objectives: a. The main objective is to assess the results of patients treated with IUdR as a radiosensitizer in the management of patients with unresectable sarcomas and compare the result to similarly irradiated patients without the radiosensitizer; local control and survival will be the important endpoints.

b. In selected patients, to obtain a biopsy after infusion to allow for cell kinetic quantification using flow cytometric techniques and thymidine replacement estimates.

Methods Employed

Patients with non-metastatic unresectable sarcomas of various types will be randomized to be treated with radiation therapy plus Iododeoxyuridine (IUdR), a radiosensitizer.

Major Findings

Too soon to show.

Proposed Course

Continuation of current studies.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06392-01 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

IUdR as a Radiosensitizer in Unfavorable Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Goffman	Head, Clinical Therapy Section	ROB, NCI
Others:	J. Mitchell	Deputy Chief	ROB, NCI
	A. Russo	Head, Exp. Phototherapy Sec.	ROB, NCI
	J. Cook	Senior Staff Fellow	ROB, NCI
	R. Smith	Cancer Nursing Specialist	CNS, CC
	H. Pass	Senior Investigator	SB, NCI
	S. Steinberg	Head	BDMS, NCI

COOPERATING UNITS *(if any)*

Surgery Branch
Biostatistics and Data Management Section
Cancer Nursing Service, CC

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3-6	4	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Overall, we have continued to accrue considerable numbers of patients on this protocol: the focus this year has been on obtaining biopsies as delineated in the original protocol for thymidine replacement. The results have been variable, but no toxicity was encountered in biopsy of these patients. A publication of these biopsies with discussion of thymidine quantitative replacement (as opposed to just labeling which we also have obtained) is planned this year. We continue to control a majority of unresectable sarcomas, many of whom now have received prior chemotherapy, for whom there is no practical option. Two publications relating to the sarcomas have been submitted within the last two months, but will not be counted here as we have not heard whether or not they have been accepted for publication.

Project Description

Professional Personnel Engaged on the Project:

J. Mitchell, Ph.D.	Deputy Chief	ROB, NCI
A. Russo, M.D., Ph.D.	Head, Exp. Phototherapy Sec.	ROB, NCI
J. Cook, Ph.D.	Senior Staff Fellow	ROB, NCI
R. Smith, R.N.	Cancer Nursing Specialist	CNS, CC
H. Pass, M.D.	Senior Investigator	SB, NCI
S. Steinberg, Ph.D.	Head	BDMS, NCI

Objectives: a. The objective is to assess the results of patients treated with IUdR as a radiosensitizer in the management of patients who have gross residual cancers, but are not being studied as disease-oriented protocols by the rest of the Cancer Institute. Comparisons will be made with historical controls by emphasizing local control and survival.

b. In selected patients whose tumor is accessible to biopsy, to obtain a biopsy after infusion to allow for cell kinetic quantification using flow cytometric techniques and thymidine replacement estimates.

Methods Employed

Patients with unresectable cancer of relatively low expected responsiveness to radiation therapy will be treated with the radiosensitizer, Iododeoxyuridine, (IUdR) plus irradiation and compared to historical controls.

Major Findings

Too soon to show.

Proposed Course

Continuation of current studies.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06393-01 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Study of Surgery and Photodynamic Therapy for Intraperitoneal Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. F. DeLaney Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, NCI; Biomedical Engineering Instrumentation Program, NCRR; Laboratory of Pathology, NCI; Diagnostic Radiology Department, CC; Radiation Oncology Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The patients with tumors diffusely seeding the peritoneal cavity have a poor prognosis with conventional treatment. Ovarian cancer is one tumor which presents with advanced disease diffusely involving the peritoneal surface in approximately 70% of the 23,000 women who have ovarian cancer annually in the United States. The disease free survival in these patients at 5 years is less than 10% with conventional management, which involves additional debulking surgery followed by multi-agent chemotherapy. We have been able to sterilize an ovarian tumor similar to this in a mouse model using intraperitoneal photodynamic therapy, i.e. a photosensitizer which localizes in tumor and can be activated by light to destroy cancer cells. We are interested in incorporating this strategy into the management of patients with ovarian cancer and have thus initiated this Phase I trial.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Professional Personnel Engaged on Projects:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIP, NCRR
R. Bonner	Biophysicist	BEIP, NCRR
P. Smith	Laser Physicist	BEIP, NCRR
L. Elwood	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	DR, CC
E. Glatstein	Branch Chief	ROB, NCI

Objectives

This is a Phase I study designed to assessed the toxicity and effectiveness of surgical debulking and hematoporphyrin derivative photodynamic therapy at the time of laparotomy in patients with primary or metastatic malignant tumors involving the peritoneal cavity.

Methods Employed

Patients with tumors seeding the surface of the peritoneal cavity who have no known curative options for their particular disease or stage receive Photofrin II photosensitizer by intravenous administration 1.5-2.5 mg/kg. 48-72 hours later the patients undergo laparotomy with surgical debulking of tumor. If tumor can be resected to less than 5 mm thickness, light is delivered to the entire peritoneal surface using appropriate optical fibers connected to lasers. Light dose and drug dose will be subsequently escalated.

Major Findings

Thirty-seven patients have received the photosensitizer and gone to laparotomy. Nineteen patients had ovarian cancer, 9 patients had sarcomas, 5 had carcinomas of gastro-intestinal organs, 3 had low grade borderline tumors, and 1 patient had an adrenal tumor. Resection/light delivery was successful in 26 of 37 patients: 16/19 with ovarian cancer, 8/9 with sarcomas, 0/5 with gastric-intestinal, 2/3 patients with borderline tumor, and in successful with the patient with the adrenal cancer. Light dose was subsequently escalated from 0.2 to 3.0 J/cm^2 to the entire peritoneal surface. Boost treatment has been given to the entire pelvis and omental remnant up to 10 J/cm^2. Photosensitizer dose has been increased from 1.5 mg per kg given 72 hours prior to surgery up to 2.5 mg per kg given 48 hours before operation. Five patients with ovarian cancer, 2 patients with sarcoma and 1 patient with borderline tumor remain free of disease between 2 and 12 months after treatment. The other patients have

all recurred. Malignant peritoneal washings have been sterilized and 4 of 7 evaluable patients. Six of 26 patients (23%) have had major postoperative complications. Three appear related to surgery (1 ureteral injury, 1 pancreatitis, 1 postoperative pelvic bleeding) and 3 complications may be related to the combination of surgery and phototherapy. These later 3 were all small bowel perforations. Two of these 3 patients had undergone partial small bowel resection and re-anastomosis. Five additional patients have received light doses similar or higher than the patients who have experienced the small bowel complications, so that the relative contribution of surgery and phototherapy to these complications is uncertain at this time.

Significance to Biomedical Research and the Program of the Institute

Because photodynamic therapy has shown curative potential in an animal model, we are quite interested in bringing this modality in to the clinic for the treatment of patients with ovarian cancer. We would like to incorporate this treatment strategy in to the second look laparotomy which is often done in these patients, currently without known therapeutic benefit. Over the course of this study we have developed an on-line light dosimetry system which we feel we'll have applicability to treatment with light activated compounds at various other sites, including the pleural cavity and bladder.

Proposed Course

We propose to finish this Phase I study of photodynamic therapy in the peritoneal cavity. Because of the large surface of the peritoneal cavity, this may require the use of alternative light wavelengths, which are produced at higher power by the current generation of laser systems.

Publications

1. DeLaney TF, Sindelar W, Smith P, Friauf W, Pass H, Russo A, Thomas G, Dachowski L, Cole J, Glatstein E. Initial experience with photodynamic therapy for intraperitoneal carcinomatosis. In: Sharp F, Mason WP, Leake RE, eds. Ovarian cancer: biological and therapeutic challenges. London: Chapman and Hall Medical, 1990;371-380.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06394-01 RO

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Developing Insights into Radiolabelled Antibody Dosimetry by Computer Simulation & Experimental Procedures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	A Raubitschek	Radiotherapist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.0	8.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Administration of radiolabelled antibodies is a relatively new treatment modality for certain forms of cancer. Much of this field is developmental in nature. In particular, the dosimetry of tumor masses and adjacent body structures and tissues is still a complex set of problems. The Radiation Physics and Computer Automation Section is attempting a novel approach employing computer aided convolution of CT density distributions into scout film images, and "loaded" with radioactivity, into simulated gamma camera images. By means of iterative procedures, starting from images of known activity distributions, it hoped to gain insight in the quantitative distribution of radioactivity on the basis of intrinsically low resolution gamma camera images of real patient MAb distributions.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objectives: To localize the sites, and to determine the amount of radiolabelled antibodies in patients. To determine normal organ radiation doses and tumor dose. To determine the optimum combination of imaging modalities for localization and to determine the lower limits of detection of tumor masses with external imaging devices.

Methods Employed

The MacTPS treatment planning system currently under development has led to the development of a versatile "toolbox" (a set of function modules which can be used much more widely than for "conventional treatment planning alone.)

This toolbox will be used to create special derivations of multi-slice CT scans, for instance simulated scout films and x-ray films. It is possible to simulate gamma camera, and also MRI images, of various "resolution" and "contrast". Attempts will be made to simulate "loading" voxels with radioactivity in a programmed fashion, to produce simulated images corrected for transmission and inverse square law attenuation. This phase is of iterative nature and methodology needs to be developed at this stage, to apply this method to patient information.

Once reasonable quantification of the localization of radioactivity is possible, the dosimetry aspect will be implemented by treating the source distribution as consisting of voxel sized mini-sources, the voxel size adjusted to the available image resolution.

Major Findings

This study was started in April 1990, so findings are limited as yet. Initial explorations have indicated that the underlying idea is feasible, and likely to be productive. In fact a search of the literature shows very little methodology or data that is clinically significant as an improvement over the very global MIRD system that is currently the principal tool in clinical Ab therapy.

Significance to Biomedical Research and the Program of the Institute

Radiolabelled antibodies are a new, exciting potential treatment modality. They offer the promise of selectively irradiating tumor masses, while delivering minimal radiation doses to normal tissues. Currently, there are severe limitations as regards knowledge of the time course of the distribution, and the distribution itself of radioactive labelled Abs. The principal limiting factor is limited resolution of current imaging techniques. The present method may help overcome some of the problems by combining high resolution information with knowledge of anatomy and physiologic processes, and controlled physical experiments with computer simulation techniques to arrive at possible and probable distributions of activity, and thus to useful dose calculations.

Proposed Course

To be continued. An acquired "body-organ phantom" has to be modified to enable reproducible measurements. For clinical purposes we need to develop "minidosimetry," rather than adopt either some existing microdosimetry method, or the too crude MIRD system. This minidosimetry may well turn out to be closely similar to some of the existing brachytherapy methods.

Publications

None so far.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06395-01 RO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Solution Chemistry of Metal-Ions Used in Radioimmunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. G. Pippin	Staff Fellow	ROB, NCI
Others:	T. J. McMurry	Senior Staff Fellow	ROB, NCI
	O. A. Gansow	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.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 fundamental solution chemistry of the copper(II) complexes of 1, 4, 7 - triazacyclononane-N,N',N" triacetate (NOTA) was studied in aqueous media. The prime goal was to determine the speciation of Cu(II) in NOTA solutions and to measure the chemical reactivities of the Cu(II)NOTA complexes. This information may be used to describe the chemical factors which influence the stability of ^{67}CuNOTA antibody conjugates in vivo.

1. Speciation of Cu(II)NOTA. Using the method of potentiometric titration, we determined the protonation constants (log K) of H_3NOTA to be 11.33, 5.73, and 3.28 in 0.1 M $NaClO_4$. In addition, the conditional protonation constant for the $CuNOTA^-$ complex was measured by conventional spectrophotometry, log K= 2.50. The later result indicates that the Cu(II) complex of NOTA undergoes a single-step protonation at pH ca. 2.5; the anion $Cu(NOTA)^-$ is the predominate Cu(II) species in solution above PH 4.0.

2. Kinetics of Cu(II)NOTA Isotopic Exchange. The rate parameters for the isotopic exchange of Cu(II)NOTA and ^{67}Cu(II) were measured until isotopic equilibrium was attained. The empirical form of the rate law showed both acid dependent and acid-independent pathways for exchange, the net rate being dependent on the concentration of CuNOTA. These results suggest

dissociation of the Cu(II) ion from the Cu(II)NOTA complex as the rate-limiting-step. Additional kinetics experiments which examine the dissociation of Cu(II) from Cu(II)NOTA are in progress.

The significance of the project is the ability to describe the chemical factors which may affect the retention of radioactive metal-ions bound to antibody conjugates in vivo.

Publication

1. Pippin CG, Mirzadeh S, Kumar K, Gansow OA. Kinetics of the isotopic exchange between copper(II)1,4,7-triazacyclonane-N,N',N" triacetate. Journal of Labeled Compounds and Radiopharmaceuticals: (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03800-20 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

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), P Pizzo (NCI), J Gardner (NIAMDD)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical 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.

Secondly, the Surgery Branch collaborates in the procurement of tissues 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 tissues.

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. Haas GP, Pittaluga S, Gomella L, Travis WD, Sherins RJ, Doppman JL, Linehan WM, Robertson C. Clinically occult Leydig cell tumor presenting with Gynecomastia, J Urol 1989;142:1325-7.

2. Fraker DL, Norton JA. The role of surgery in management of islet cell tumors, Gastrointestinal Endocrinology 1989;18:805-30.

3. Darling G, Goldstein DS, Stull R, Gorschboth CM, Norton JA. Tumor necrosis factor: Immune endocrine interaction, Surgery 1989;106:#6:1155-60.

4. Sheppard BC, Norton JA, Doppman JL, Maton PN, Gardner JD, Jensen RT. Management of islet cell tumors in patients with multiple endocrine neoplasia: A prospective study, Surgery 1989;106:1108-17.

5. Friedman B, Darling G, Norton J, Hamby L, Metcalfe D. Splenectomy in the management of systemic mast cell disease, Surgery 1990;107:94-100.

6. Fraker DL, Doppman JL, Shawker TH, Marx SJ, Spiegel AM, Norton JA. Undescended parathyroid adenoma: An important etiology for failed operations for primary hyperparathyroidism, World J Surg 1990;14:342-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03801-20 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

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)*

PI: S.A. Rosenberg	Chief of Surgery, NCI	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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 cancer and melanoma. The major emphasis in Surgery Branch cancer therapy is in adjuvant therapy with emphasis on the use of combined treatment modalities in addition to surgery.

SURGICAL SERVICES DEPARTMENT

ANNUAL STATISTICS

APRIL 1989 - MARCH 1990

TOTAL PROCEDURES	HOURS	INSTITUTES/OTHERS	TOTAL PROCEDURES	
407	1245.50	Ward (NCI)	112	Emergencies
808	1719.25	Consult (NCI)	130	Add-ons
52	104.25	Med. Br. (NCI)	413	Cancellations
1267	3069.00	TOTAL (NCI)	336	OPD's
			28	2WCSR
1267	3069.00	NCI		ICU-2J
235 1/2	1014.25	NHLBI	2	MICU-10D
146 1/2	804.75	NINCDS	49	Radiation
41	44.75	Med. Neuro		
66	138.75	NEI		
103	172.50	ENT		
32	99.50	NIDR	1967	Total Cases
12	41.25	Orthopedics	5499	Total Hours
53	85.50	NICHD		
11	28.75	Other (Dic, Trout, Kosloff, Vincent, Cattau, Redner, Brewer)		

MONTHLY SUMMARY

January	184	Total Procedures	July	163	Total Procedures
	472.75	Total Hours		504.25	Total Hours
February	165	Total Procedures	August	217	Total Procedures
	393.25	Total Hours		621.25	Total Hours
March	170	Total Procedures	September	160	Total Procedures
	420.75	Total Hours		467.50	Total Hours
April	162	Total Procedures	October	132	Total Procedures
	495.50	Total Hours		346.50	Total Hours
May	180	Total Procedures	November	127	Total Procedures
	537.75	Total Hours		398.75	Total Hours
June	163	Total Procedures	December	144	Total Procedures
	477.00	Total Hours		363.75	Total Hours

ss-169

1. Sargent ER, Gomella LG, Belldegrun A, Linehan WM, Kasid A. Epidermal growth factor receptor gene expression in normal human kidney and renal cell carcinoma, Journal of Urology 1989;142:1364-8.

2. Danforth DN Jr, Lippman ME, McDonald H, Bader J, Egan E, Lampert M, Steinberg SM, Swain SM. Effect of preoperative chemotherapy on mastectomy for locally advanced breast cancer, The American Surg 1990;56:6-11.

3. Rosenberg SA. Adoptive immunotherapy for cancer, Scientific American 1990; 262:62-9.

4. Ward B, McGarvey C, Lotze MT. Excellent shoulder function is attainable after partial or total scapulectomy, Archives of Surgery 1990;125:537-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03811-16 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

The Immunotherapy of Animal and Human Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. A. Rosenberg Chief of Surgery, NCI SURG NCI

Others: P. Brett (Medical Staff Fellow), A. Asher (Staff Fellow), B. Fox (Senior Staff Fellow), R. Zakut (Expert), P. Aebersold (Expert), J. Weber (Senior Investigator), J. Yannelli (Expert), N. Restifo (NCI Biotechnology Fellow) SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
14	8	6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Attempts are being made to develop new immunotherapeutic techniques for the treatment of advanced cancer. A variety of animal models are being used to test the effects of lymphokine activated killer cells, tumor infiltrating lymphocytes 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.

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, the combination of alpha-interferon and interleukin-2, the combination of interleukin-2 and tumor necrosis factor, and the value of colony stimulating factors in cancer treatment.

Newer efforts are directed at transducing new genes into tumor infiltrating lymphocytes that can increase their therapeutic effectiveness and clinical trials using these cells have been initiated.

1. Cameron RB, McIntosh JK, Rosenberg SA. Synergistic antitumor effects of combination immunotherapy with recombinant interleukin-2 and a recombinant hybrid interferon-alpha in the treatment of established murine hepatic metastases, Cancer Res 1988;48:5810-7.

2. Rosenberg SA, Schwarz S, Spiess P. Combination immunotherapy of cancer: Synergistic anti-tumor interactions of interleukin-2, alpha-interferon and tumor infiltrating lymphocytes, JNCI 1988;80:1393-7.

3. Weber JS, Rosenberg SA. Modulation of Murine Tumor Major Histocompatibility Antigens By Cytokines in vivo and in vitro, Cancer Res 1988;48: 5818-24.

4. Carter CS, Leitman SF, Cullis H, Muul LM, Nason-Burchenal K, Rosenberg SA, Klein HG. Technical aspects of lymphokine activated killer cell production, J Clin Apheresis 1988;4:113-7.

5. Kawakami Y, Rosenberg SA, Lotze MT. Interleukin-4 promotes the growth of tumor infiltrating lymphocytes cytotoxic for human autologous melanoma, J Exp Med 1988;168:2183-91.

6. Eisenthal A, Cameron RC, Rosenberg SA. The effect of combined therapy with lymphokine activated killer (LAK) cells, interleukin-2 and specific monoclonal antibody on established B16 melanoma lung metastases, Cancer Res 1988;48:7140-5.

7. Belldegrun A, Kasid A, Uppenkamp M, Rosenberg SA. Cellular and molecular characterization of a helper/inducer T cell clone with lytic specificity isolated from renal cell carcinoma ascitic fluid, Surg Forum 1988;39:698-700.

8. Ognibene FP, Rosenberg SA, Lotze M, Skibber J, Parker MM, Shelhamer JH, Parrillo JE. Interleukin-2 administration causes reversible hemodynamic changes and left ventricular dysfunction which are similar to those seen in septic shock, Chest 1988;94:750-4.

9. Rosenberg SA, Packard BS, Aebersold PM, Dolomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP, White DE. Immunotherapy of patients with metastatic melanoma using tumor infiltrating lymphocytes and interleukin-2: Preliminary report, N Engl J Med 1988;319:1676-80.

10. Aebersold P, Carter CS, Hyatt C, Johnson S, Ottaway K, Leitmen SF, Rosenberg SA. A simplified automated procedure for generation of human lymphokine-activated killer cells for use in clinical trials, J Immunological Methods 1988;112:1-7.

11. Topalian SL, Rosenberg SA. Tumor specific lysis by tumor infiltrating lymphocytes derived from melanomas, Surgical Forum 1988;39:413-4.

12. McIntosh JK, Mule JJ, Krosnick J, Rosenberg SA. Combination lymphokine therapy: synergistic antitumor effects of tumor necrosis factor, interleukin-2, and interferon-alpha against established murine subdermal and hepatic tumors, Surgical Forum 1988;39:452-4.

13. Fox BA, Rosenberg SA. Heterogeneous lymphokine activated killer cell precursor populations: Development of a monoclonal antibody which separates two population of precursors with distinct culture requirements and separate target recognition repertoires, Cancer Immunol Immunother 1989;29:155-66.

14. Topalian SL, Solomon D, Rosenberg SA. Tumor-specific cytolysis by lymphocytes infiltrating human melanomas, J Immunol 1989;142:3714-25.

15. Belldegrun A, Webb DE, Austin HA, Steinberg SM, Linehan WM, Rosenberg SA. Renal toxicity of interleukin-2 administration in patients with metastatic renal cell cancer: Effect of pretherapy nephrectomy, J Urol 1989;141: 499-503.

16. Mule JJ, Krosnick JA, Rosenberg SA. Interleukin-4 regulation of murine lymphokine-activated killer (LAK) activity in vitro: Effects on the interleukin-2 induced expansion, cytotoxicity and phenotype of LAK effectors, J Immunol 1989;142:726-33.

17. Puri RK, Travis WD, Rosenberg SA: Decreae in Interleukin-2 induced vascular leakage in the lungs of mice by administration of recombinant interleukin-1 in vivo, Cancer Res 1989;49:969-76.

18. Kawakami Y, Custer MC, Rosenberg SA, Lotze MT. Interleukin-4 regulates interleukin-2 induction of lymphokine activated killer activity from human lymphocytes, J Immunol 1989;142:3452-61.

19. Mule JJ, Rosenberg SA. Immunotherapy with cytokine combinations. In: DeVita VT, Hellman S, Rosenberg SA, eds. Important Advances in Oncology, Philadelphia: JB Lippincott, 1989;99-126.

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22. Barba D, Saris SC, Holder C, Rosenberg SA, Oldfield EH. Intratumoral LAK cell and interleukin-2 therapy of human gliomas, J Neurosurgery 1989;70: 175-82.

23. McIntosh JK, Mule JJ, Jablons DM, Nordan RP, Rudicoff S, Lotze MT, Rosenberg SA. The kinetics of interleukin-6 induction by the systemic administration of rhTNF - in mice, Ann NY Acad Sci 1989;557:572-5.

24. Fisher B, Packard BS, Read EJ, Carrasquillo JA, Carter CS, Topalian S, Yang JC, Yolles P, Larson SM, Rosenberg SA. Tumor localization of adoptively transferred Indium-111 labeled tumor infiltrating lymphocytes in patients with metastatic melanoma, J Clin Oncol 1989;7:250-61.

25. Belldegrun A, Kasid A, Uppenkamp M, Topalian S, Rosenberg SA: Human tumor infiltrating lymphocytes: Analysis of lymphokine mRNA expression and relevance to cancer immunotherapy, J Immunol 1989;142:4520-6.

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27. Eisenthal A, Rosenberg SA. The effect of various cytokines on the in vitro induction of ADCC in murine cells: Enhancement of the IL-2 induced ADCC activity by IL-1, J Immunol 1989;142:2307-13.

28. Jablons D, Steinberg SM, Roth J, Pittaluga S, Rosenberg SA, Pass HI. Metastasectomy for soft tissue sarcoma: Further evidence for efficacy and prognostic indicators, J Thoracic and Cardiovas Surg 1989;97:695-705.

29. Yang JC, Rosenberg SA. Surgery for adult patients with soft tissue sarcomas, Seminars in Oncol 1989;16:289-96.

30. McIntosh JK, Mule JJ, Krosnick JA, Rosenberg SA. Combination cytokine immunotherapy with tumor necrosis factor- , Interleukin-2 and interferon leads to synergistic antitumor effects in mice, Cancer Res 1989;49:1408-14.

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35. Rosenberg SA, Lotze MT, Yang JC, Linehan WM, Seipp CA, Calabro S, Karp SE, Sherry RM, Steinberg SM, White DE. Combination therapy with interleukin-2 and alpha-interferon for the treatment of patients with advanced cancer, J Clin Oncol 1989;7:1863-74.

36. Chang AE, Steinberg SM, Culnane M, Lampert MH, Reggia AJ, Simpson CG, Hicks JE, White DE, Yang JJ, Glatstein E, Rosenberg SA. Functional and psychosocial effects of multimodality limb-sparring therapy in patients with soft tissue sarcomas, J Clin Oncol 1989;7:1217-28.

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42. Belldegrun A, Rosenberg SA. Adoptive immunotherapy of urologic tumors. In: Lepor H, Ratliff TL, eds. Urologic Oncol, Boston: Kluwer Academic Publishers, 1989;213-33.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06654-13 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

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)
Others: Radiation Oncology Branch NCI

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

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

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

Patients with gastrointestinal carcinomas have been studied for evidence of 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 pancreatic cancers. Tolerance of various normal and surgically-manipulated tissues to intraoperative radiotherapy has been investigated in dogs to determine both acute and long-term toxicity from radiation effects. Clinical trials of intraoperative radiotherapy have been performed including feasibility and developmental studies, randomized trials in resectable and unresectable pancreatic carcinoma, randomized trials in gastric carcinoma, and randomized trials in retroperitoneal sarcomas. Tolerance of normal and surgically-manipulated tissues to photodynamic therapy using hematoporphyrin derivatives and laser light has been investigated in dogs to determine toxicity and to establish dose levels applicable for clinical practice. Clinical trials of intraperitoneal photodynamic therapy have been initiated for the treatment of peritoneal carcinomatosis and peritoneal surface malignancies, including ovarian carcinoma, metastatic gastrointestinal carcinoma, and sarcomatosis.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06657-08 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Metabolic Studies with Cytokines and Clinical Studies with Endocrine Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	J.A. Norton	Senior Investigator	SURG, NCI
Others:	H.R. Alexander	Expert	SURG, NCI
	C. Buresh	Biologist	SURG, NCI
	G. Doherty	Clinical Associate	SURG, NCI
	S. Carty	Clinical Associate	SURG, NCI
	C. Jensen	Clinical Associate (MSF)	SURG, NCI
	H. Langstein	Research Associate (MSF)	SURG, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Surgery Branch

SECTION
Surgical Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

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

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

The iv administration of low doses of recombinant human Tumor Necrosis Factor (rhTNF) to awake unrestrained rats increases plasma levels of glucagon, corticosterone, ACTH, norepinephrine and dihydroxyphenylglycol. Incubation of human adrenocortical cells with TNF causes cortisol secretion similar to incubation with ACTH. Local application of cachectin/TNF is detrimental to wound healing. Insulin therapy reverses the toxic effects of TNF including improvement of food intake, body weight and the cellular changes associated with TNF treatment. Tolerance to the toxic effects of rhTNF can be induced in animals by repetitive exposure to doses of rhTNF. In animals undergoing TNF anti-tumor therapy tolerance to the anti-tumor effects of rhTNF also develops limiting the treatment efficacy of TNF. Similarly, TB rats made tolerance to rhTNF by repetitive twice daily ip doses for 7-14 days appear to tolerate the cachectic effects of tumor and eat more, maintain body weight and survive longer than control TB animals. Tolerance is only induced by intermittent bolus doses of rhTNF. Continuous iv doses results in maintenance of severe anorexia, body weight loss and death, while bolus iv applications of the same dose does not. Tumors exist in an acidic environment with high concentrations of lactic acid as a result of anaerobic glycolysis. Macrophages exposed to a similar environment induce the gene for TNF and secrete excessive amounts of TNF as do macrophages exposed to photodynamic therapy. An antibody to interferon-gamma reverses the clinical parameters of cachexia in TB rats and allows cachectic TB rats to live longer than control TB rats.

Adrenal tumors are rare causes of Cushing's syndrome. Benign tumors are curable with resection but recovery of the HPA axis and cessation of replacement doses of hydrocortisone requires nearly 2 years. Malignant adrenal tumors generally recur locally or systemically. There is no curative therapy for recurrent adrenal cancer, but surgical resection of recurrent disease is associated with longer survival from the time of recurrence.

GPO 814-018

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06658-08 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Effect of Cytokines on Breast Cancer Cell Growth and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: D. N. Danforth, Senior Investigator, SURG, NCI
Others: M. Sgagias, IRTA Fellow, SURG, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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 effect of interleukin-1 (IL-1) on MCF-7 human breast cancer cell growth and metabolism. We found that IL-1 inhibits in a dose-dependent manner the growth of these cells. IL-1 acts to block cell growth in the G_0G_1 phase of the cell cycle. We also found the IL-1 blocks estradiol stimulated growth of these cells. This is dose-dependent for IL-1 and occurs for all concentrations of estradiol from 10-8 to 10-11M. IL-1 acts synergistically with the estrogen antagonist hydroxytamoxifen to further inhibit cell growth; this synergism with hydroxytamoxifen is also dose-dependent for IL01. IL-1 has been found to down-regulate the estrogen receptor (ER) in these cells by 38.0-44.0%. Down-regulation is demonstrated by both Scatchard analysis and enzyme immunoassay. Down-regulation occurs by 12 hours and persists through 48 hours of exposure, is dose-dependent, and occurs without a change in the receptor affinity constant (Kd). Down-regulation is blocked by cycloheximide, and thus requires continuous protein synthesis. IL-1 does not, however, alter expression of ER mRNA and therefore IL-1 is acting at the post-transcriptional level to down-regulate the ER. IL-1 did not block estradiol stimulation of progesterone (PgR) synthesis as determined by Scatchard analysis or EIA, and did not alter resting PgR levels. IL-1 also did not block estradiol down-regulation of the ER or ER mRNA. The effect of IL-1 on secretion of insulin-like growth factor was examined. IL-1 decreased resting levels of IGF, however, did not block estradiol stimulation of IGF secretion, further indicating that IL-1 selectively alters the estrogen responsiveness of these cells. We are currently studying the effect of IL-1 on growth and metabolism of MCF-7 cells in vivo in nude mice, as well as the effect of IL-1 on TGF-beta growth factor secretion, protooncogene erbB expression and EGF receptor expression, and modualtion of estradiol regulation of cell cycle phase distribution.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06659-08 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of Urologic Malignancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	W. M. Linehan	Head, Urologic Oncology Section	SURG, NCI
Others:	M. M. Walther	Senior Investigator	SURG, NCI
	G. H. Weiss	Senior Investigator	SURG, NCI
	P. Anglard	Exchange Scientist	SURG, NCI
	M. W. Ewing	NCI Biotechnology Fellow	SURG, NCI
	S. C. Liu	Chemist	SURG, NCI
	E. E. Trahan	Medical Technician	SURG, NCI

COOPERATING UNITS (if any)

Others:	Dr. B. Zbar	NCI
	Dr. C. E. Myers	NCI

LAB/BRANCH
Surgery Branch

SECTION
Urologic Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
6.5	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.)

We are studying the molecular genetics of human renal cell carcinoma, evaluating the effect of anti-growth factor agents on human genitourinary tumors and participating in studies of adoptive immunotherapy in patients with advanced malignancies. We have identified DNA sequence deletions in the short arm of chromosome 3 in both sporadic as well as a familial form of human renal cell carcinoma which indicate the presence of a renal carcinoma recessive oncogene at this location. We have also identified DNA sequence deletions at chromosomes 11, 17 and 13 at the retinoblastoma locus as well as at the NM23 site and have demonstrated that chromosome 3p retained in the renal tumors were inherited from the affected parent, consistent with our data that the RCC gene is located on chromosome 3p and that 3p loss represents the second step of a two-mutation process. The DNA sequence deletions observed at other chromosomal loci (11, 13 and/or NM23) may be important in progression or metastasis. We have evaluated patients at risk for familial renal cell carcinoma and have demonstrated that the VHL disease gene is located on the short arm of chromosome 3. We have developed, also in collaboration with Dr. B. Zbar, a procedure utilizing a molecular evaluation of lymphocytes which is over 95% accurate in detecting among at risk individuals who is affected with the familial form of renal cell carcinoma. In evaluating the effect of suramin on human genitourinary tumors we have found that this agent inhibits in vitro proliferation of human prostate carcinoma and that in an suramin plus TNF is more effective than either agent alone. We are currently evaluating the effect of suramin on growth-factor induced mitogenesis in these cells and on the molecular events associated with suramin-inhibition of prostate carcinoma growth. In collaboration with Dr. C.E. Myers of the Medicine Branch we have found that this agent has antitumor activity in patients with advanced hormone refractory prostate carcinoma. We evaluate and participate in treatment of patients with advanced renal cell carcinoma with IL-2 based immunotherapy and have characterized the use of cytoreductive surgery in patients with metastatic renal cell carcinoma treated with adoptive immunotherapy with interleukin-2 based therapy.

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21. Lerman MI, Latif F, Glenn GM, Daniel LN, Brauch H, Hosoe S, Hampsch K, Delisio J, Orcutt ML, McBride OW, Grzeschik KH, Takahashi T, Minna J, Anglard P, Linehan WM, Zbar B. Isolation and regional localization of a large collection (2,000) of single copy DNA fragments on human chromosome 3 for mapping and cloning tumor suppressor genes, Human Genetics, in press.

22. Perry RR, Keiser HJ, Norton JA, Wall RT, Robertson CN, Travis W, Pass HI, Walther MM, Linehan WM. Perioperative management of pheochromocytomas using metyrosine, Annals of Surgery, in press.

23. LaRocca RV, Stein CA, Danesi R, Jamis-Dow CA, Weiss GH, Myers CA. Suramin in adrenal cancer: modulation of steroid hormone production, cytotoxicity in vitro and clinical antitumor effect. J Clin Endocrinol Metab, in press.

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26. Linehan WM, Walther MT, Sargent ER, Gomella LG, Robertson CN, Wade TP, Anglard P, Weiss GH, Ewing MW, Liu S, LaRocca RV, Myers CE. Studies of the endocrine and paracrine effect of tumor produced factors in human genitourinary cancers. In: Karr J, Coffey D, Smith R, eds. Molecular and Cellular Biology of Prostate Cancer, New York: Plenum Press, in press.

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29. Linehan WM. Thoracoabdominal Radical Nephrectomy. In: Glenn JF, ed. Urologic Surgery, Philadelphia: JB Lippincott, in press.

30. Kragel PJ, Pestaner J, Travis WD, Linehan WM, Filling-Katz M. Papillary cystadenoma of the epididymis: A report of three cases with lectin histochemistry, Archives of Pathology & Laboratory Medicine, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06660-07 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
The Study of Interleukin-2 Based Immunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. C. Yang	Senior Investigator	SURG, NCI
Others:	D. Perry-Lalley	Microbiologist	SURG, NCI
	S. Marcus	Clinical Associate (MSF)	SURG, NCI
	J. Wei	Staff Fellow	SURG, NCI
	R. Sherry	Staff Fellow	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	3.0

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

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

Our laboratory is trying to improve the therapeutic efficacy of tumor infiltrating lymphocytes (TIL) by generating populations of cells with improved specificity as well as modifying their in vivo localization to tumor. Current efforts on improving cell populations involve the use of immunization regimens with a variety of cytokine adjuvants and extracting and growing cells from immunization sites and draining lymph nodes. We are also studying the mediators of TIL motility in vitro using a Millipore migration system. Whole animal in vivo trafficking of tumor-specific TIL is being performed using a fluorescent cell surface dye with analysis of TIL content of organs by fluorescent cytometry. These studies are being correlated with ongoing clinical trials trafficking indium-labeled TIL. Furthermore, we are studying the adoptive transfer of genetically-modified T-cells in an attempt to establish the IL-2 and antigen requirements to promote proliferation of these populations and modulate their expression of gene products. This is being performed using a mouse T-cell line modified with the human TNF gene.

Another project involves pre-clinical studies using a polyethylene glycol-modified form of IL-2, which has a prolonged circulating half-life. Murine models are used to examine the effect of PEG-IL-2 on adoptively-transferred cells. A Phase I trial using PEG-IL-2 has been conducted and a Phase III trial with IL-2 and PEG-IL-2 maintenance is underway.

1. Griffith KD, Read EJ, Carrasquillo JA, Carter CS, Yang JC, Fisher B, Aebersold P, Packard BS, Yu My, Rosenberg SA. In vivo distribution of adoptively transferred indium-111-labeled tumor infiltrating lymphocytes and peripheral blood lymphocytes in patients with metastatic melanoma, JNCI 1989;81:1709-17.

2. Yang JC, Perry-Lalley D, Rosenberg SA. An improved method for growing murine tumor-infiltrating lymphocytes with in vivo antitumor activity, J Biol Resp Mod 1990;9:149-59.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06661-07 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Immunologic Studies in Patients with Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.T. Lotze	Senior Investigator	SURG, NCI
Others:	M.C. Custer	Microbiologist	SURG, NCI
	M.G. Sanda	Clinical Associate (MSF)	SURG, NCI
	J.T. Rubin	Staff Fellow	SURG, NCI
	Y. Kawakami	Visiting Fellow	SURG, NCI
	H. Stotter	Visiting Associate	SURG, NCI
	M. Tran	Stay-In-School Student	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
5.0	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.)*

The major goal of our laboratory continues to be the development and evaluation of immunologic reagents in therapy of patients with cancer. A major aspect of our current work involves the evaluation of the two novel T cell growth factors, IL-4 and IL-7. IL-4 has been administered to over 73 patients in 84 treatment courses. Administration of IL-4 is associated with dose-related toxicity and evidence of a vascular leak syndrome. Both partial and complete responses have been noted in patients with melanoma and renal cell cancer have been noted in patients receiving combinations of IL-2 and IL-4.. IL-4 has profound effects on human monocytes including decreased expression of several phenotypic markers including the phosphoinositol-glycan linked CD14 while leaving other monocyte antigens such as CD13 unaltered. Functionally it decreases ADCC mediated by such cells both in vitro and in vivo induces the expression of CD23, the low affinity FC receptor for IGE. IL-7 has been evaluated for its role in T cell expansion and induction of the human lymphokine activated killer cell. It appears that IL-7 does indeed serve as a major T cell growth factor causing the proliferation of both activated T cells as well as large granular lymphocytes. The latter cells once stimulated with IL-7 produce IL-4 bioactivity as well as causes them to be stimulated to produce LAK activity. Ongoing studies in the administration of murine monoclonal antibodies to over 40 patients including L6, 17-1A and B72.3 are in progress. These studies have demonstrated that high doses of antibody can be given safely in association with IL-2 and that increased ADCC is mediated by cells obtained from patients receiving IL-2 in the context of our monoclonal antibody protocols. TGF beta has been evaluated in its ability to augment or alter the growth of human tumor infiltrating lymphocytes and a biologic assay has been modified to allow its ready assessment.

1. Rubin JT, Elwood L, Rosenberg SA, Lotze MT. Immunohistochemical correlates of response to recombinant Interleukin-2 based immunotherapy, Cancer Research, 1989;49:7086-92.

2. Rosenberg SA, Lotze MT, Yang JC, Aebersold PM, Linehan WM, Seipp CA, White DE. Experience with the use of high dose Interleukin-2 in the treatment of 652 patients with cancer, Ann Surg 1989;210:474-85.

3. McIntosh JE, Jablons DM, Mule JJ, Nordan RP, Rudikoff S, Lotze MT, Rosenberg SA. In vivo induction of IL-6 by administration of exogenous cytokines and detection of De Novo serum levels of IL-6 in tumor-bearing mice, J Immunology 1989;143:162-7.

4. Lotze MT. Disturbing homeostasis: Recent results of ongoing immunotherapy trials at the NCI, Biotechnology Therapeutics 1989;1:125-63.

5. Wiebke EA, Rosenberg SA, Lotze MT. Cytokines alter target cell susceptibility to lysis: I. Evaluation of non-MHC restricted effectors reveals differential effects on natural and lymphokine-activated killing, J Biol Resp Mod 1990;9:113-26.

6. Bock SN, Lee RE, Fisher B, Rubin JT, Schwartzentruber D, Wei JP, Callender D, Yang JC, Lotze MT, Pizzo PA, Rosenberg SA. A prospective randomized trial evaluating prophylactic antibiotics to prevent catheter-related sepsis in patients treated with immunotherapy, J Clin Oncoloy 1990; 8:161-9.

7. Jablons D, Bolton E, Mertins S, Rubin M, Pizzo P, Rosenberg SA, Lotze MT. Interleukin-2 based immunotherapy alters circulating neutrophil Fc receptor expression and chemostasis, J Immunology 1990;144:3630-6.

8. Huang CM, Ruddle M, Sliva C, Elin RJ, Lotze MT, Rosenberg SA. Changes in laboratory results with interleukin-2 therapy administered to cancer patients, Clin Chemistry 1990;36:431-4.

9. Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson F, Rosenberg, SA. Human gene transfer: Characterization of human tumor infiltrating lymphocytes as vehicles for retroviral mediated gene transfer in man, Proc Nat Acad Sci 1990;87:473-7.

10. Stotter H, Lotze MT. Cytolytic effector cells against human tumors: Distinguishing phenotype and function, Cancer Cells 1990;2:44-56.

11. Lotze MT, Custer MC, Bolton ES, Wiebke EA, Kawakami Y, Rosenberg SA. Mechanisms of immunologic antitumor therapy: Lessons from the laboratory and clinical applications, Human Immunology 1990;28:198-207.

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13. Lotze MT. Interleukin-2-basic principles. In: DeVita V, Hellman S, Rosenberg SA, eds. Principles and Practices of Biology Therapy, Philadelphia: JB Lippincott, 1990.

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15. Lotze MT. Preface - Current paradigms in cellular immunology: Implications for immunity to cancer. In: Lotze MT, Finn OJ, eds. New York: Wiley-Liss, 1990.

16. Lotze MT, Custer MC, Kawakami Y, Stotter H, Rubin JT, Bolton ES, Guedez L, Sanda MG. T cell growth factors and the expansion of lymphoid cells with antitumor activity in vitro and in vivo. In: Lotze MT, Finn OJ, eds. Cellular Immunity and the Immunotherapy of Cancer, New York: Wiley-Liss, 1990.

17. Lotze MT, Finn OJ. Current paradigms in cellular immunity: Implications for immunity to cancer, Immunology Today 1990;11:190-3.

18. Bolton E, Custer M, Lotze MT. Interleukin-4 alters monocyte phenotype in vitro and in vivo, Proc Amer Assoc Cancer Research, in press.

19. Kasid AT, Director E, Stovroff MC, Lotze MT, Rosenberg SA. Regulation of tumor necrosis factor- and lymphotoxin- mRNA expression in human peripheral blood lymphocytes, Cancer Res, in press.

20. Custer MC, Lotze MT. A biologic assay specific for Interleukin-4: Rapid fluorescence assay for IL-4 detection in supernatants and serum, J Imm Meth, in press.

21. Kragel AH, Travis WD, Feinberg L, Pittalugia S, Striker LM, Roberts WC, Lotze MT, Yang JJ, Rosenberg SA. Pathologic findings associated with Interleukin-2-based immunotherapy for cancer: A postmortem study of 19 patients, Human Pathology, in press.

22. Lotze MT. Interleukin-2 based immunotherapy of malignant melanoma in Therapy of Advanced Melanoma. In: Rumke P, Karger S, Basel AG, eds., in press.

23. Rubin JT, Rosenberg SA, Lotze MT. The efficacy of high dose Interleukin-2 based immunotherapy in man, in Interleukin-2. In: Guyre, ed. Plenum Press, in press.

24. Jablons DM, Donohue R, Kawakami Y, Young J, Lotze MT. Interleukin-3 induces proliferation but not lymphokine activated killer activity from human and murine mononuclear cells, Eur Cytokine Network, in press.

25. Kumar V, Kawakami Y, Stotter H, Lotze MT, Rosenberg SA, Hood L. T-cell receptor repertoire of tumor-infiltrating lymphocytes specific for human melanoma, UCLA Symposia Book, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06662-04 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Studies of Phototherapy for Thoracic Malignancies

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

COOPERATING UNITS (if any)

Others:	J. Mitchell	Deputy Branch Chief	ROB, NCI
	A. Russo	Head, Experimental Phototherapy Section	ROB, NCI

LAB/BRANCH

Surgery Branch

SECTION

Thoracic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-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.)

Our laboratory has continued investigation of photodynamic therapy for the treatment of thoracic malignancies by sensitization of malignant cells with dihematoporphyrin ether followed by illumination by 630 nM light. Since October 1989 we have established the sensitivity of different lung cancer lines in vitro. We have found minor variations in vitro as far as phototherapy sensitivity. We have characterized these changes with regard to dihematoporphyrin ether content, cell size, cell volume, cell protein as well as plating efficiency. We have published investigations concerning the treatment of murine macrophages with tumor necrosis factor and have revealed that photodynamic therapy stimulates these macrophages to increase tumor necrosis factor in the macrophage supernatants. This can be detected either in the presence of absence of endotoxin. We have also established the kinetics of such TNF production over a 24 hour period. These data represent the first demonstration of cytokine release due to photodynamic therapy and may explain indirect cytotoxicity of PDT as well as vascular effects. We are continuing investigations of the treatment of patients with endobronchial malignancies with PDT and we have now treated 18 patients with endobronchial disease with PDT. There is a 70% response rate in these patients and the patients are well palliated and photodynamic therapy of the bronchus has now become our preferred method of endobronchial therapy. We are also now performing photodynamic therapy for treatment of pleural malignancies. We have presently treated 7 patients with debulking of pleural malignancies and intraoperative photodynamic therapy. This is now being studied as an approved Phase I trial at the Clinical Center. Before performing these human studies we performed canine experiments in which we established the dose tolerance of normal thoracic tissue (esophagus, lung, heart, chest wall and diaphragm) to PDT.

PHS 6040 (Rev. 1/84) GPO 914-918

1. Evans S, Matthews W, Perry R, Fraker D, Norton J, Pass H. Photodynamic therapy stimulates murine peritoneal macrophages to produce tumor necrosis factor, Surg Forum XL 1989;421-3.

2. Evans S, Matthews W, Perry R, Fraker D, Norton J, Pass H. Effective photodynamic therapy on tumor necrosis factor production by murine macrophages, JNCI 1990;82:34-9.

3. Pass HI, Evans S, Matthews W. Kinetics of tumor necrosis factor production by phototherapy stimulated macrophages. Proc. Soc. Optical Engineering, in press.

4. Perry RR, Matthews W, Mitchell JB, Russo A, Evans S, Pass HI. Sensitivity of different human lung cancer histologies to photodynamic therapy, Cancer Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06663-01 SURG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effect of Tumor Necrosis Factor on Breast Cancer 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	IRTA Fellow	SURG, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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 effect of tumor necrosis factor (TNF) on MCF-7 human breast cancer cell growth and metabolism. We found that TNF inhibits growth of these cells in a dose-dependent manner. TNF acts by arresting growth in the G_0G^1 phase of the cell cycle. We also found that TNF completely blocks estradiol stimulation of growth. This is dose-dependent for TNF, and growth is blocked for all concentrations of estradiol. TNF down regulates the estrogen receptor in dose-dependent manner as demonstrated by both Scatchard analysis and EIA; this occurs without a change in the receptor affinity constant (KD). Down-regulation is blocked by cycloheximide and thus TNF requires continuous protein synthesis for this effect. TNF significantly increases resting levels of the progesterone receptor (PgR) in a dose-dependent manner. This effect is also blocked by cycloheximide. The increase in PgR levels in these cells is not accompanied by altered growth response to exogenous progestins. TNF, however, does not block estradiol stimulation of progesterone receptor synthesis, and does not block estradiol down-regulation of the ER. We also found that, in contrast to its effects on cells in the immune system, TNF does not act synergistically with IL-1 to inhibit growth of these human breast cancer cells. This is true for varying doses of either IL-1 and TNF. IL-1 transiently enhances TNF mRNA expression at 3 hours; this is not associated with increased secretion of TNF, and therefore TNF does not appear to mediate the inhibitory of IL-1. In contrast, TNF persistently enchanced TNF mRNA in these cells, beginning at 3 hours and lasting unchanged through at least 72 hours of exposure. This is associated with increased secretion of TNF into the media; TNF may therefore act in an autocrine manner on these cells to inhibit cell growth. IL-1 and TNF do not act synergistically to alter TNF mRNA expression. We are currently studying the effect of TNF on growth and metabolism of MCF-7 cells in vivo, the effect of TNF on gene expression of ER and PgR mRNA, the effect of TNF on expression and secretion of the growth factors TGF-beta and IGF and on EGF receptor expression, and modulation of these proteins by estradiol.

1. Sgagias MK, Kasid A, Danforth DN Jr. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibit growth and induce TNF mRNA in MCF-7 Human breast cells. Proceedings Seventy-first Annual Meeting, The Endocrine Society, Atlanta, Georgia, June 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06664-01 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Immune Recognition of Autologous Tumor by Human Tumor Infiltrating Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	S.L. Topalian	Senior Investigator	SURG NCI
Others:	D.J. Schwartzentruber	Senior Investigator	SURG NCI
	S.S. Hom	Clinical Associate (MSF)	SURG NCI
	M. Mancini	Biologist	SURG NCI
	A. Kasid	Visiting Scientist	SURG NCI
	S. Morecki	Visiting Fellow	SURG NCI

COOPERATING UNITS *(if any)*

HLA Laboratory, Department of Transfusion Medicine, Clinical Center, NIH (Toni Simonis)

LAB/BRANCH

Surgery Branch

SECTION

Human Tumor Immunology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-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.)*

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 metastatic melanoma. Elucidating the mechanisms by which TIL recognize and destroy tumor cells is essential to optimizing clinical protocols. The following are areas under study:

1. Mechanisms of tumor resistance to TIL killing. A melanoma tumor line resistant to lysis by autologous TIL was immunoselected from a TIL-sensitive melanoma. The variant appeared to have lost a cell surface determinant critical to TIL recognition. Experiments to define this determinant are in progress.

2. Nature of the melanoma tumor antigen recognized by TIL. TIL lysis of panels of HLA-matched melanomas revealed that multiple shared and possibly unique tumor antigens exist which can be recognized by TIL.

3. Nature of TIL response to tumor. In addition to direct lysis of tumor targets, some TIL are capable of producing a variety of cytokines upon specific stimulation with autologous tumor, but not with allogeneic tumors. This response has been documented in TIL derived from 3 of 4 melanomas, and 1/7 breast carcinomas investigated. Studies of colon carcinoma are in progress. Cell separation studies show that $CD8^+$ TIL are responsible for cytokine production as well as lysis.

PHS 6040 (Rev. 1/84) GPO 814-918

1. Topalian SL, Kasid A, Rosenberg SA. Immunoselection of a human melanoma resistant to specific lysis by autologous tumor infiltrating lymphocytes: Possible mechanisms for immunotherapeutic failures, J Immunol 1990;144: 4487-95.

2. Topalian SL, Rosenberg SA. Tumor infiltrating lymphocytes (TIL): Evidence for specific immune reactions against growing cancers in mouse and man. In: DeVita V, Hellman S, Rosenberg SA, eds. Important advances in oncology, Philadelphia: JB Lippincott Co., 1990;19-41.

3. Hom SS, Topalian SL, Rosenberg SA. MHC class I antigen restriction of tumor recognition by lymphocytes infiltrating human melanomas, Surg Forum, in press.

4. Skornick Y, Topalian S, Rosenberg SA. Comparative studies of the long-term growth of lymphocytes from tumor infiltrates, tumor-draining lymph nodes, and peripheral blood by repeated in vitro stimulation with autologous tumor, J Biol Response Mod, in press.

5. Morecki S, Topalian SL, Myers WW, Okrongly D, Okarma TB, Rosenberg SA. Separation and growth of human $CD4^+$ and $CD8^+$ tumor infiltrating lymphocytes and peripheral blood mononuclear cells by direct positive panning on covalently attached monoclonal antibody coated flasks, J Biol Response Mod, in press.

6. Topalian SL, Rosenberg SA. Adoptive cellular therapy: Basic principles. In: Devita V, Hellman S, Rosenberg SA, eds. Biologic therapy of cancer: Principles and practice, Philadelphia: JB Lippincott Co., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06665-01

PERIOD COVERED

October 1, 1989 to September 30, 1990

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

Studies of Antitumor Efficacy and Toxicity of Adoptive Immunotherapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. J. Mule	Microbiologist	SURG NCI
Others:	J.K. McIntosh	Medical Staff Fellow	SURG NCI
	D.M. Jablons	Medical Staff Fellow	SURG NCI
	R.J. Barth, Jr.	NCI Biotechnology Fellow	SURG NCI
	S.N. Bock	Staff Fellow	SURG NCI
	J.A. Krosnick	HHMI Research Scholar	SURG NCI

COOPERATING UNITS (if any)

Laboratory of Pathology, NCI (W.D. Travis)

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
6.0	6.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Preclinical studies in animals were undertaken to investigate the antitumor effects and toxicities of recombinant cytokines and effector cells in adoptive immunotherapy models of established cancer.

A. Tumor Necrosis Factor (TNF). We showed that the systemic administration of TNF alone at high dose or at lower doses with other cytokines (IL-2, IFN-alpha), or with chemotherapeutic agents can effectively reduce or eradicate serially passaged weakly-immunogenic sarcomas as well as spontaneous autochthonous tumors in mice. TNF does not affect transplants of normal skin or fetal cardiac transplants. Tumor-bearing (TB) mice were shown to have greater sensitivity to acute toxicity of high-dose TNF compared to non-TB mice. The lethal effects of TNF can be substantially decreased by treatment with fluid resuscitation and with direct therapy that is antagonistic to oxygen radical formation (i.e. bismuth subnitrate).

B. Interleukin-6 (IL-6). We showed that purified IL-6 when administered alone at high dose or at lower doses in combination with TNF mediated the regression of established weakly-immunogenic sarcomas in mice; toxicities were not observed at the doses of IL-6 used. The antitumor effect of IL-6 was mediated through a radiosensitive host component.

C. Tumor Infiltrating Lymphocytes (TIL). We have defined tissue culture conditions for the optimum growth of murine TIL with in vitro cytolytic specificity and increased therapeutic potency that incorporates purification of T cells from tumors, repetitive restimulation with irradiated autologous tumor cells in the presence of low concentrations of IL-2.

GPO 914-918

1. Krosnick JA, McIntosh JK, Mulé JJ, Rosenberg SA. Studies of the mechanisms of toxicity of the administration of recombinant tumor necrosis factor-alpha in normal and tumor-bearing mice, Cancer Immunol Immunother 1989; 30:133.

2. McIntosh JK, Mulé JJ, Travis WD, Rosenberg SA. Studies of effects of recombinant human tumor necrosis factor on autochthonous tumor and transplanted normal tissue in mice, Cancer Res 1990;171:629.

3. Mulé JJ, McIntosh JK, Jablons DM, Rosenberg SA. Antitumor activity of recombinant interleukin-6 in mice, J Exp Med 1990;171:629.

4. Barth RJ Jr, Bock SN, Mulé JJ, Rosenberg SA. Unique murine tumor-associated antigens identified by tumor infiltrating lymphocytes, J Immunol 1990;144: 1531.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 06666-01 SURG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Transfer using Cytokine (TNF and IL-2) into Tumors and Tumor I Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Attan Kasid, Ph.D.	Visiting Scientist	SURG, NCI
Others:	Jonathan Salo, M.D.	Clinical Associate	SURG, NCI
	Masahiro Ogasawara,	Visiting Fellow	SURG, NCI
	Osama El-Badry	NCI Biotechnology Fellow	SURG, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.0	2.0	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.)

Generation of recombinant retroviruses encoding human cytokine genes. A series of retroviruses that express the cytokine genes and a selectable marker gene, were constructed and tested for the efficiency of transduction as well as stable expression of the cytokine genes.

Insertion of cytokine genes into tumor infiltrating lymphocytes. The most efficient retroviral vector containing the human tumor necrosis gene was used to insert the TNF gene into several human TIL. The transduced TIL were extensively characterized for trhe stable integration and high phenotype, cytotoxicity, expression of other cytokines, and clonality using T-cell receptor gene rearrangements. A clinical protocol based on thes use of TNF-gene modified TIL in cancer patients is currently under review.

Insertion of cytokine genes into human and mouse tumors. Using retroviruses containing human TNF and IL-2 genes, we introduced the cytokine genes into mouse and human tumors and characterized the gene-modified tumors for the integration, expression, growth and other properties in vitro as well as the biological effects in vivo both in athymic and syngeneic animals. The gene transferred tumor cells were unable to form progressively growing tumors. The cytokine-gene transferred cells contained the intact proviral genome and expressed the cytokines in vivo.

Significance. The significance of the project lies in therapeutic TIL based on cytokine gene transfer for the treatment of human cancers. One insertion of genes into tumors was to evaluate the effects of such gene insertions into animal models.

GPO 814-818

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SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
OCTOBER 1, 1989 - SEPTEMBER 30, 1990

I. INTRODUCTION

In 1982 the Radiation Research Program (RRP) was established in the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH). The RRP is an extramural program having two branches: the Diagnostic Imaging Research Branch (DIRB) and the Radiotherapy Development Branch (RDB). The scientific mission of the Radiation Research Program is to develop research program for the extramural community in which radiation and related forms of energy are used in the diagnosis, staging, treatment and post-treatment evaluation of the patient with cancer. Included in the mission of the Radiation Research Program is the planning, development, administration, and evaluation of an extramural radiation research program. This is accomplished by establishing program priorities, allocating resources, maintaining project integration, evaluating program effectiveness, and representing the program in the administrative and scientific decision-making processes of the National Cancer Institute.

For scientific and administrative direction, the RRP relies heavily on the advice of the DCT Board of Scientific Counselors. The Program coordinates research activities with related programs at NCI and NIH, other Federal agencies, and national and international research organizations. The RRP provides a radiation research focal point for national and international extramural investigators.

II. PERSONNEL

A. Staffing

1. Office of the Associate Director

   John E. Antoine, M.D., Associate Director
   Ruthie S. Herrington, Secretary to the Associate Director
   Wendy R. Fredericks, Biologist
   Richard V. Stepney, Computer Specialist
   Wendy Fairall, Clerk-Typist

2. Administrative Office

   James Stoneman, Administrative Officer
   Cynthia Glagola, Administrative Technician

3. Diagnostic Imaging Research Branch

   Faina Shtern, M.D., Chief
   Matti Al-Aish, Ph.D., Program Director
   Roger Powell, Program Director
   Patricia Angelis, Branch Secretary

4. Radiotherapy Development Branch

Francis Mahoney, Ph.D., Acting Chief
Thomas Strike, Ph.D., Program Director
Sandra Zink, Ph.D., Cancer Expert
Catherine Bailey, Branch Secretary

B. Recruitments

Chief, Radiotherapy Development Branch
Radiation Oncologist, Radiotherapy Development Branch
Program Director, Radiotherapy Development Branch
Program Director, Diagnostic Imaging Research Branch

III. MAJOR ACTIVITIES

The Radiation Research Program continues to stimulate, develop, administer and evaluate basic science and clinical research areas in radiation therapy, nuclear medicine, diagnostic imaging, and their related subspecialty areas.

A high priority research area of the RRP is and has been the fast neutron clinical trials project. After many years of planning and development, Phase III trials have been designed and are successfully being carried out in Fast Neutron Clinical Trials. The institutions participating in these trials continue to be the University of Washington, Seattle, Washington; UCLA, Los Angeles, California; and the University of Texas Cancer Center, M. D. Anderson Hospital, Houston, Texas. The efficacy of neutron beam therapy for the treatment of malignant salivary gland tumors has been demonstrated. Data continue to be obtained from clinical trials in the treatment of localized prostate cancer, head and neck tumors, and radio resistant neoplasms. The RRP believes the trials can be concluded by 1992-93.

At the October 12-13, 1989, Division of Cancer Treatment Board of Scientific Counselors meeting, a Radiation Research Program review was carried out. Dr. Antoine provided a brief program overview including the research activities of the Radiotherapy Development Branch and the Diagnostic Imaging Research Branch. He stated that the mission of the Radiation Research Program is to develop research in the diagnosis, staging, treatment and post-treatment evaluation of cancer patients in whom radiation and related forms of energy are used.

The Radiotherapy Development Branch develops research in radiation oncology, including the scientific areas of conventional photon radiation, fast neutron radiation therapy, proton beam radiation therapy, hyperthermia, radiation sensitizers, radiation protectors, systemic radiation therapy (SRT), photodynamic therapy (PDT), boron neutron capture therapy (BNCT), radiobiology, radiation physics, and the use of expert systems in the radiologic sciences.

In the Diagnostic Imaging Research Branch, 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 radioimmuno diagnosis (RID). The major DIRB goal is the development of non-invasive, tissue specific diagnostic procedures and techniques.

Multi-institutional clinical trials in diagnostic radiology conducted by the Radiologic Diagnostic Oncology Group (RDOG). The objectives of the trials are to use single or multiple new imaging technologies to diagnose, stage, and monitor cancers and to develop an algorithm for the appropriate sequential, cost effective selection of diagnostic procedures. Institutions participate in the group through regular working group meetings and by accruing patients to the imaging protocols. Data from the first trial, RDOG 1, on lung and prostate cancer are now being analyzed. A manuscript has been submitted for publication. RDOG 2, now in progress, is examining the diagnosis, staging, and monitoring of colorectal and pancreatic tumors. The third trial will study musculo-skeletal and head and neck tumors. Dr. Antoine reiterated that the data from all of the three trials would be brought to the Board for review as they become available.

Workshops are used to develop proposals, and two recent DIRB-sponsored workshops have been held. One was on diagnostic imaging with results to be published in Investigative Radiology. The second workshop on the use of MRI and MRS in the non-invasive approach to the diagnosis and staging of cancer was helpful in the development of research directions for the DIRB.

The Diagnostic Radiology Coordinating Committee (DRCC) is centered in the Office of the Director of the NIH as mandated by Congress. The committee, chaired by Dr. Antoine, is charged with developing a five-year research plan for diagnostic radiology/imaging at the NIH. Additional functions of the DRCC include improving the NIH database and information dissemination. Extramural scientific advisors will be involved in the development of the research plan.

Scientific concepts were presented for discussion and review. They are:

- Imaging of the bone marrow tumors proposed request for grant applications (RFA) of three years. Proposed first year award: $500,000.

  In presenting the first concept Dr. Antoine noted evidence that MRI may be useful for early detection of bone marrow abnormalities in staging neoplastic disease. The objective of the RFA is to study the anatomy and physiology of normal and diseased bone marrow using the most advanced imaging technology such as MRI and CT. They will be evaluated in conjunction with the radionuclide bone scan which is still the standard technique for studying skeletal metastatic tumors and/or bone marrow involvement. Drs. Hendee and Hryniuk of the Board of Scientific Counselors urged that MRS be included under the RFA, and Dr. Antoine agreed to incorporate MRS into the tumor biology questions being asked.

- Digitization of chest radiography for lung cancer: proposed request for grant applications (RFA) of three years; proposed first year award of $600,000. Work is progressing in the use of digital techniques to increase the ability to discern and identify neoplasms. He noted that the Division of Cancer Prevention and Control (DCPC) is planning a screening study for lung cancer that would use conventional chest X-rays along with sputum cytology. Digital processing may improve the precision of chest X-rays and thereby improve the early detection of lung cancer. The RFA would support up to three grants for research on the application of digital processing of chest X-rays.

- Diagnostic imaging studies of tumor perfusion: Proposed request for grant applications (RFA); period of three years; proposed first year award of $600,000. Delivery of therapeutic agents and the perfusion of tumors using

MRI is being investigated. He identified the need to determine what imaging modalities would be best used to detect perfusion and the diffusion of neoplasms and thus increase the specificity of both diagnosis and therapy. Dr. Niederhuber, Chairman of the DCT BSC urged that the proposed research include biological as well as anatomic questions. A workshop on the scientific area of tumor perfusion did, in fact, address biological as well as imaging research questions.

- Planning and development for proton therapy research and treatment facilities: Proposed Request for Application (RFA); period of one year; proposed first year award of $1,500,000. Because of the excellent results reported in the treatment of clival chordomas, base of skull chondrosarcomas and uveal melanomas reported by the proton beam research team at the Harvard Cyclotron Laboratory, Cambridge, Massachusetts, further research in the use of proton beams for the treatment of neoplasms is scientifically justifiable. Congress has made available $1.5 million for proton beam research planning.

After discussion, all four of these scientific concepts were passed by the Board of Scientific Counselors.

- On November 14, 1989, the Radiation Research Program in conjunction with the Division of Cancer Biology and Diagnosis, sponsored a meeting on the early diagnosis of cancer. The meeting was suggested by the Electronics Industry Foundation (EIF) whose members are dedicated to the rapid technology transfer of exciting new diagnostic techniques for the diagnosis of cancer. State of the art mammography and innovative laboratory studies were discussed. Dr. Antoine served as the Chairman of that meeting and, hopefully, some joint industry/NCI projects will come from ongoing liaison and dialogue.

- A committee with a similar type mission is called the Diagnostic Decision Implementation Committee. Dr. Antoine is the RRP's representative on this Committee, which is charged with the identification of new diagnostic techniques and approaches which could benefit those having cancer. This committee meets on a regular basis to identify, discuss, and implement the use of new diagnostic techniques.

- At the February 12-13, 1990, meeting of the DCT BSC, Dr. Antoine introduced the concept of being able to do in vivo acoustic microscopy which would result in the capability of doing the biological and diagnostic assessment of tumors involving the human being. In vivo tissue imaging using acoustic microscopy has the potential for tissue-specific diagnosis comparable to conventional histology as seen with light microscopy. Dr. Antoine explained how this instrumentation operates and noted that it is currently in use at the University of California, Irvine, for making for dermatologic histologic diagnosis. Although the current electronics and computer technology are adequate to allow development of in vivo acoustic microscopy, instrumentation problems, including miniaturization of the transducer and obtaining improved resolution, need to be resolved. The integrated probe, signal transfer and image processing needed for tissue-specific diagnosis are not currently under development. Dr. Antoine added that there is also a need for better understanding of high frequency ultrasound wave tissue interaction, understanding of physical elastic properties of biologic tissue and the relationships to pathology, and definition of acoustic microscopy's diagnostic ability in viable tissue. Thus, there is sufficient understanding to allow programmatic description of the research and development needed for the

delivery of a prototype instrument. Many of the Board of Scientific Counselors' members stated the research should include biological research projects. This advice was taken by the Program with the understanding that additional information would be presented by the RRP at the next DCT BSC.

- Dr. Antoine then presented the scientific concept: Screening of Compounds as Radiosensitizers using a Colorimetric Assay: proposed new procurement RFP; period of 5 years; estimated annual award amount of $750,000. Dr. Antoine informed the Board that the present radiosensitizer screening contract is due for recompetition later in 1990. In order to increase the volume of the radiation sensitizer screening program, it was the recommendation of the RRP that a colorimetric assay be used to facilitate the throughput of compounds. This is in keeping with the colorimetric assay system developed by DTP at their Frederick, Maryland, facility.

  Radiation sensitizers continue to be investigated for use with radiation therapy. SR-2508 is currently in Phase III clinical trials in Europe and the United States with the investigation in the U.S.A. being carried out by the Radiation Therapy Oncology Group (RTOG). Other notable compounds that were identified by the radiation sensitizer contracts of RRP included BSO and Nicotinamide. SR-4233 appears to be not only a radiation sensitizer but a selective hypoxic cell cytotoxic agent. Phase I and II clinical trials are planned for the near future. Dr. Norman Coleman, Chairman of the DCT Advisory Committee on Radiation Modifiers pointed out that when one considers the improvement in local control of some cancers with radiation treatment, radiation plus a modifier has the potential for greater therapeutic advantage for a greater number of cancer patients. He also pointed out the potential benefit of this approach when used in conjunction with chemotherapy or in other multi-modal systemic therapies.

  Dr. James Mitchell of the Radiation Oncology Branch stated that an in vitro assay such as the MTT colorimetric assay used in the DCT drug screen might be feasible but would require development taking into account important factors such as an appropriate cell lines, cell inoculum size, exposure time to radiation, and drug concentration. Dr. Coleman agreed with Dr. Mitchell that there would need to be some initial developmental work and that a screen could be set up and implemented with very productive results. Expressing support for such a screen, Dr. Chabner suggested that the areas of greatest potential progress in cancer treatment in the next five years will involve combinations of radiotherapy and chemotherapy, and that screening is most likely the best way to identify new drugs that interact with radiation.

  Dr. Thomas Strike stated that the current screening contract is at Stanford University with Dr. Martin Brown being the Principal Investigator. While there have been no problems with the work quality, the number of compounds that can be screened is limited by the clonogenic assay being used. At the conclusion of the discussion the concept was approved.

  Dr. Antoine, at the request of DCT, again renewed the Fast Neutron Beam Clinical Trials project and informed the Board of Scientific Counselors the clinical trials will be completed by the year 1992-93. The institutions involved in the studies continue to be the University of California at Los Angeles (UCLA), M. D. Anderson Cancer Center at Houston, Texas, and the University of Washington, Seattle, Washington. Following a brief discussion of the project, Dr. Antoine introduced

Dr. Robert Parker from the University of California at Los Angeles who reviewed the research going on at his institution in the use of fast neutron beams. In presenting the ten-year update, Dr. Parker noted that overall fast neutron therapy is clearly successful in the treatment of malignant salivary gland tumors. There appears to be a significant advantage (20 percent) of fast neutrons over photons in stage C and D1 prostate cancer. Dr. Parker noted there appears to be some promise in the treatment of advanced rectal cancers by use of the fast neutron beam. He summarized the number and types of patients receiving neutron therapy at UCLA (144 on protocols, 225 available for long-term study), noting that because of problems with construction of the cyclotron, accrual to the Phase III clinical trials began only three years ago. He presented data to show that the UCLA contribution was about equal to those of the University of Washington and M. D. Anderson. Because of significant differences in facility location (on V.A. grounds), third party reimbursement, and costs of the Los Angeles area, Dr. Parker stated that it was more expensive to treat a patient at UCLA than at the other facilities.

In summary, Dr. Antoine recalled that an ad hoc subcommittee of the Board of Scientific Counselors, chaired by Dr. William Hendee, reviewed the Fast Neutron Program and recommended that the Phase III trials be completed as quickly as possible. He said UCLA's contribution for FY '90 is needed to complete the trials as scheduled and produce the data necessary for scientific credibility. He presented data to demonstrate that the patient accrual at UCLA had improved consistently. Dr. Antoine then asked the Board to consider a request that UCLA be permitted to exceed the previously negotiated limit of $6,500 per patient for reimbursement and be allowed to use both FY '90 dollars and the unexpended balance from FY '89. A motion was made and seconded to approve this request. The motion was approved.

- At the June 4-5, 1990, (DCT, BSC) Board meeting Dr. Antoine discussed four separate topics:

  (1) The use of the RO3 Grant Mechanism for innovative research in radiation oncology.

  (2) Improvements in the radiation sensitizer screening contract.

  (3) Ultrasound microscopy.

  (4) Radiation resistance.

At a previous DCT BSC an RFA for the study of drug resistance was introduced. It was drawn to the attention of the DCT BSC membership that radiation resistance was also a problem in tumor control and new molecular biology techniques available were now ready for use in the study of radiation resistance. Dr. Antoine informed the DCT BSC that a workshop will be held in the near future to assess the status of radiation resistance and what research needs to be done. The Board agreed this was an excellent topic for a workshop and urged the program to bring the findings and recommendations of that workshop to the DCT BSC when available.

Dr. Antoine then discussed the intended use of a colorimetric assay for the detection of radiation sensitizers answering specific BSC questions on its development. The program will rely heavily on advice from the DCT Advisory Committee on Radiation Modifiers presently chaired by Dr. Norman Coleman of the Joint Center in Boston, Massachusetts,

a joint working committee of RRP and DTP for the identification and development of new compounds, and the extramural radiation sensitizer research community.

At the direction of the DCT BSC, the Radiation Research Program developed an RFA for the use of the RO3 grant mechanism to facilitate innovative radiation research laboratory findings to the clinic. After discussion by the DCT BSC the Board gave its approval to this scientific concept.

The scientific concept for the use of acoustic microscopy in the study of tumor biology and diagnosis was reintroduced to the Board. By developing this instrument the possibility of in vivo assessment of individual tumor cells and subcellular components becomes possible. Major research goals include the minification of a transducer to be used on the end of a needle or catheter and sophisticated signal processing required for gigahertz ultrasonographic tissue analysis. Following an animated discussion the Board of Scientific Counselors approved this scientific concept.

As previously described, Dr. Antoine chairs the Diagnostic Radiology Coordinating Committee which is a trans-NIH coordinating committee having three functions, namely: develop a five year research plan, disseminate information, and improve the databases of NIH as they relate to diagnostic imaging/radiology research. This committee meets on a regular basis and, May 30-31, 1990, a meeting of extramural scientists to assist in the development of this plan was held. With the input of the extramural scientists the goal is to prepare the first draft of the five-year radiology/imaging research plan as soon as possible.

Exciting areas of research supported by the RRP 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 unit is under development 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.

  Data from the Heavy Ion Project at the Lawrence Berkeley Laboratory in Berkeley, California, 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.

- Intraoperative radiation therapy continues to be clinically investigated and appears to be effective in the treatment of advanced local gynecological and rectal tumors, retroperitoneal sarcomas and gastric cancers. Phase II and III Clinical Trials are being performed in the United States and internationally.

- Radiation modifiers: The radiation sensitizer contracts of RRP continue to identify and develop substances with radiosensitizing properties. Encouraging results with SR-2508 have been followed by Phase II and Phase III Clinical Trials presently being carried out by the Radiation Therapy Oncology Group

(RTOG), and other cooperative groups. The sensitizer program is being reevaluated and there is hope that a large scale automated radiation sensitizer screening program can be developed. The expertise, experience and advice of the Developmental Therapeutics Program (DTP) is being utilized in the development of a new screening program.

The radioprotector, WR-2721, continues to show a normal tissue protective effect, not only when used with radiation, but also with chemotherapy. Encouraging clinical results are being reported in the use of WR-2721 with chemotherapeutic agents (e.g., melanoma). Larger doses of chemotherapy can be given with the normal tissues protected. Improved therapeutic ratios are being reported but this scientific observation requires substantiation by further clinical investigation.

- Hyperthermia continues to show promise in the management of malignant disease. In addition to being used with radiation for the improved control of local tumors it is being investigated as an adjunct to chemotherapy in the treatment of systemic disease. In the treatment of localized neoplasms thermometry remains an invasive procedure requiring multiple probes be inserted into the patient's tumor. Research on the development of non-invasive thermometry is needed. Magnetic Resonance Imaging techniques may make non-invasive thermometry a reality. Persistent difficulties with adequate local deep heating may be overcome by the use of ultrasound techniques. A RRP workshop addressing these problems was held May 12 - 13, 1988. A Recommendation that NCI hyperthermia efforts be coordinated largely through RTOG were made to RRP by the participants of this workshop and this advice has been followed by the Program during 1988, 1989, and 1990.

- The exciting field of photodynamic therapy (PPT) is a research field in which systemically administered tumor seeking light sensitive compounds are used in conjunction with activating light, usually generated by a laser. Improvements in the light sensitizing compounds are being made and several new compounds are now entering the Decision Network of DCT, NCI. The potential for the treatment of closed space neoplasms such as carcinoma of the ovary, mesothelioma and bladder cancer is being explored. The effectiveness of this therapy in the reestablishment of airway in totally occluded bronchi caused by lung cancer has been demonstrated. Hopefully, this research area will mature into a treatment modality giving improved results in the treatment of tumors which commonly recur following conventional therapy , e.g., ovarian cancer. Industry-supported Phase III clinical trials are now being performed in lung, esophogeal, 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.

- A "Patterns of Care" study to evaluate the radiation therapy in the United States is being funded. This retrospective analysis will evaluate data from patients treated for breast, cervix, prostate, and recto-sigmoid cancer and Hodgkin's Disease. Previous patterns of care studies have proven helpful in identifying methods for the improvement of patient treatment such as in the treatment of prostate cancer, cervix cancer, and Hodgkin's Disease.

- The rapidly emerging research area of medical informatics, also known as "expert systems", is being exploited in the optimization of radiation treatment planning and delivery. Three contracts have been funded for the development of a system to rapidly and automatically extract anatomic features from diagnostic images, define and delineate tumors from normal tissues, define treatment volumes from tumor contours, optimize treatment plans, display three-dimensional images rapidly and interactively, and improve simulation verification. The impact of medical informatics on the field of radiation oncology and the radiologic sciences will be great, and an even greater impact on the field of medicine is anticipated.

- 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.
  Dr. Mishima presented his clinical data to a select group of NCI scientists and extramural investigators in November of 1989. These encouraging results will result in further research in the use of this innovative therapeutic approach.

- The exciting area of Systemic Radiation Therapy (SRT), such as radioimmuno therapy continues to be of high priority to the RRP. An RFA passed by the Board of Scientific Counselors for the establishment of a research group of investigators to develop the optimal dosimetry of this rapidly emerging therapeutic technique was released in October 1988 and several grant applications were funded in FY 89.

In diagnostic imaging:

- Diagnostic ultrasound has the potential for tissue characterization which has not been totally exploited. Program emphasis will be placed on research making tissue specific diagnosis using non-invasive techniques possible. Hopefully, ultrasound will be helpful in accomplishing this goal. This potential exists in the evaluation of breast disease where ultrasonographic techniques are readily used.

- Of the rapidly evolving imaging modalities, none is more dynamic than that of magnetic resonance imaging (MRI) and spectroscopy (MRS). A workshop on the use of MRI/MRS for the evaluation of tumors before, during and following therapy is planned.

- Another goal of the Diagnostic Imaging Research Branch of the Radiation Research Program is to develop the anatomic and functional diagnosis of neoplasms employing single and multiple modality imaging related technology. RRP supports grants dealing with the imaging modalities of CT, MRI, MRS, and others. In radionuclide technology exciting research areas include Single Photon Emission

Computed Tomography (SPECT), Positron Emission Tomography (PET), and Radionuclide Conjugate Diagnosis.

- PET scans are extremely helpful in obtaining functional and anatomic information. Biologically active substances such as Carbon 11, Nitrogen 13, Oxygen 15, and Fluorine 18, have been used to obtain information differentiating tumor tissue from normal tissue. This technology may make possible the differentiation of normal brain tissue from brain tumors and the determination of tumor viability following therapy such as radiation therapy. These findings require further investigation and verification. An RFA on the use of PET in the evaluation of brain tumors was released in 1989.

- Three dimensional anatomic diagnosis is made possible by the use of Single Photon Emission Computed Tomography (SPECT) units. It is anticipated that this will be an invaluable tool, not only for tumor identification and localization, but also may be useful in treatment planning.

- Perhaps the most exciting of the rapidly evolving radionuclide related research areas is that of radiolabeled ligands for use in tumor identification and treatment. The possibility of tumor specific radiodiagnosis is made possible by this technique. Radioimmuno diagnosis (RID) is a classic example of this approach.

The Radiation Research Program continues to carry out its mission in the development of innovative research for the radiologic sciences. It does this in support of the greater mission of the National Cancer Institute in the prevention, detection, and treatment of oncologic disease.

IV. SCIENTIFIC OVERVIEW

A. DIAGNOSTIC IMAGING RESEARCH BRANCH

The Diagnostic Imaging Research Branch (DIRB) of RRP, DCT, NCI continues to develop and administer basic and clinical diagnostic imaging research. Areas of research supported by the DIRB include nuclear magnetic resonance imaging (MRI) and spectroscopy (MRS), as well as MR microscopy, X-ray computerized tomography (CT), ultrasound, instrumentation development and image perception. Other research areas include digital radiography, 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 $36.3 million in 1990.

Magnetic resonance imaging/spectroscopy and nuclear medicine research continue to be two major areas of funding at DIRB. Areas of increasing interest and significance are the use of monoclonal antibodies in imaging and the collaborative clinical diagnostic imaging research. The following is a summary of the DIRB actual budget FY89 and estimated budget FY90.

FY89 AND 90 BUDGETS

			$ (in thousands)	
GRANTS	FY89	FY90	FY89	FY90
Coop. Agree.(RDOG I) (U01, RFA 86-CA-10)	6	6	1,281	1,243
Coop. Agree. (RDOG II) (U01, RFA 88-CA-02)	5	5	401	403
Traditional (R01)	104	93	21,084	21,608
Program Projects (P01)	10	9	10,097	8,062
Conf. & New Investigator (R13 & R23)	2	1	93	3
SBIR*	20	23	3,258	2,591
First Awards	7	7	642	573
RFA, 87-CA-33	2	2	233	215
RFA, 87- CA-36	3	3	529	476
RFA, 87-CA-20 (OSP)	3	3	384	356
RFA, 88-CA-10	3	3	400	417
TOTAL GRANTS	165	155	38,402	35,947
CONTRACTS				
SPECT Contract	1	1	177	179
SBIR	1	1	277	223
TOTAL CONTRACTS	2	2	454	402
TOTAL DIRB BUDGET	167	157	38,856	36,349

MAGNETIC RESONANCE IMAGING (MRI) AND SPECTROSCOPY (MRS)

Instrumentation and Technique Development:

The frontiers of research in this area have been extended both by continued progress in existing projects and by the appearance of new developments. Work on the SBIR-supported program at Advanced NMR Systems, Inc. in Woburn, Massachusetts was completed to become one of the fastest available MRI systems, which can depict any portion of the body in any plane in real time. Individual images can be made in as little as 20 milliseconds and "assembled" sequentially to create a moving picture of the beating human heart, lung and diaphragmatic motion, or the action of the temporomandibular joint.

Another project on fast MRI at the Mayo Foundation provides real- time imaging and reconstruction, which permit real-time observation of the MRI in a manner analogous to x-ray fluoroscopy. Another novel fast scanning sequence has been developed at Stanford University to minimize motion artifacts when using MRI imaging of the chest and abdomen for tumor diagnosis and staging.

Advances at the Massachusetts General Hospital in proton NMR chemical shift imaging have now been used to study the fat and water fractions and their relaxation times in bone marrow. Several leukemic patients have been under study to determine if these MRI parameters can be used to monitor therapeutic response. The same group of investigators have pioneered proton lactic acid imaging of normal and diseased tissue. MR imaging of lactate may enhance this modality as an in vivo tool for tissue characterization (e.g. differentiation of treatment-induced necrosis from viable tumor).

Outstanding physics and engineering contributions to the clinical applications of MRI have been made at the Medical College of Wisconsin at Milwaukee in the design, construction, clinical evaluation, and use of specially designed MR body and surface coils to fit around or next to different body parts. These coils are now routinely used to increase image quality in diagnostic images of the head, neck, spine, and abdominal organs as well as the shoulder, wrist, knee, and ankle joints. MR arthrography of the joints performed in combination with surface coils is a valuable new development in diagnostic medicine and in sports medicine. Special coils have also been developed for use in research and clinical studies in MR spectroscopy to study metabolism and function and to analyze and monitor selected nuclei in cancer patients undergoing therapy. An SBIR project at Medical Advances, Inc., also in Milwaukee, has developed special coils for detection and imaging of breast cancer.

Special coil and instrumentation developments at Johns Hopkins University have aided in the achievement of magnified images (MR microscopy). Another SBIR project with Tecmag, Inc., in Houston is aimed at the development of an MR microscope capable of a spatial resolution of only ten microns.

Excellent progress has been made at the Brigham and Women's Hospital in the development of 2D and 3D MR imaging of brain tumors in conjunction with computer-assisted laser therapy. MRI can thus be used to monitor laser-tissue interactions and separate reversible from irreversible tissue damage. Another advance in 3D imaging of radiation dose distribution has been made in a project at Yale University. These applications demonstrate well the combined uses of MRI for both diagnosis and treatment planning of cancer.

Special MR techniques have been perfected at the University of California at Irvine for measurement of the flow velocity of blood and other body fluids in each voxel of a 3D MR image in the regimes of bulk flow, perfusion, and diffusion. An interesting mathematical improvement in MR image processing has come from the development of "eigenimage filtering" at the Henry Ford Hospital, in which a feature of interest in the image can be enhanced while the background noise is suppressed.

Valuable advances have been made at the University of Utah in the development of highly refined NMR techniques for determining lung water content and distribution in a variety of clinical pathologies. These techniques are providing new avenues for understanding of lung physiology on a microscopic scale at air-water interfaces and the relative role of alveolar recruitment and distention in human adults with lung injury or adult respiratory distress syndrome. These NMR methods, which can detect and characterize pulmonary disease such as pulmonary edema, emphysema, and fibrosis, have the advantage of being noninvasive, relatively rapid, easily reproducible, and less susceptible to motion artifacts caused by respiration, heart motion, or blood flow in the chest cavity than current conventional approaches. Thus they may provide an optimal standard for measuring and monitoring a variety of lung pathologies in the future.

Investigators at Harvard University have been investigating magnetic field effects on iron oxide-loaded lung macrophages in order to understand cytoplasmic viscosity and cell organelle motion at the "microscopic" level. Many subtle rheological, chemical, and physical properties of cell tissues and fluids and their changes have been investigated as a function of temperature and of their mechanical motion measured magnetometrically. Cell activity is maximal around 37 degrees C. Biochemical changes below that temperature are consistent with reversible inhibition of enzymatic processes. Above that temperature the inhibition appears to be irreversible.

The field of MR imaging has advanced to the point of the electron as well as the nuclear signal detection. A new technique for imaging oxygen concentrations in living mouse tissues has been developed at the University of Chicago in a specially designed low frequency electron spin resonance (ESR) spectrometer. The injection of nitroxide spin labels (free radicals) into the tissues enables a sensitive determination and mapping of oxygen concentrations. This technique may eventually be extended to humans and marks one of the earliest practical possibilities for the use of ESR imaging in medicine.

Magnetic Resonance Spectroscopy (MRS) and Multinuclear Studies:

Many investigators are now carrying out in vivo laboratory research in animals using magnetic resonance spectroscopy (MRS) to measure and to follow the concentration of particular magnetic elements (such as hydrogen-1, fluorine-19, sodium-23, and phosphorus-31) which occur naturally in the body or signal from which can be enhanced by administration of contrast agents or treatment pharmaceuticals. Important progress has been made at Wayne State University in analysis of tumor metabolic products by phosphorus-31 MR spectroscopy in order to predict chemotherapeutic response. At Memorial Hospital in New York a number of phosphorus-31 MRS studies have been carried out to follow the metabolism of sarcomas under chemotherapy and the radioresistance and radiosensitivity of tumors under radiation treatment. Fluorine-19 MRS measurements have assisted in the in vivo monitoring of 5-fluorouracil metabolism after methotrexate administration.

With high magnetic fields, it is also possible to obtain MR images of some of the above mentioned magnetic nuclei for research and clinical studies. At the University of Pennsylvania, pharmaceuticals containing fluorine-19 have been used to measure the vascular concentration and distribution of oxygen, and sodium-23 MR 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 prostatic tumors in mice. This work may be extended to all types of tumors.

Pioneering work carried out on sodium-23 MR imaging at Columbia University at high magnetic fields of 2.0 and 3.0 Tesla has been extended to provide a novel methodology for non-invasive differentiation of intracellular sodium and extracellular sodium signals. It has thus become possible to differentiate tumor from normal and edematous tissue, radiation necrosis from tumor recurrence, and to obtain an in vivo index of malignancy. Pre-clinical validation of this technique is being carried out. Preparations are also under way to implement a 5.0 Tesla facility for MR imaging and spectroscopy. This will permit new spectroscopic studies and biologic imaging to be undertaken that heretofore were not possible. These investigators have also reported that recent sodium-23 MR imaging examinations of five patients with AIDS permitted accurate diagnosis and characterization of distinct pathological features of brain lymphoma in all five patients.

Outstanding fundamental research studies have been pursued at Fox Chase Cancer Center using MR phosphorus-31, carbon-13, and proton spectroscopy to elucidate metabolic pathways in malignant solid tumors, especially arterially perfused hepatomas, and to study phospholipid metabolism in transformed cells. This group has used phosphorus-31 NMR to study phosphate metabolites in order to evaluate nutritional repletion in subjects suffering from cancer-induced cachexia. Using the technique of chemical shift imaging (CSI), exceptional progress has been made in developing the first color-coded metabolic NMR mapping of the human brain in vivo and human muscle physiology.

New NMR spectroscopic imaging techniques are being developed at the University of California at San Francisco to measure lactate and other metabolites in evaluating regional ischemia and malignancy. Phosphorus-31 and carbon-13 NMR studies at Evanston Hospital in Illinois are seeking to correlate steady state concentrations of phosphate-containing metabolites with the levels of several proto-oncogenes found in human breast tumor cells, with a particular focus on post-menopausal women with node negative and node positive cancers.

MR Contrast Agents and Pharmacokinetics:

Contrast agents for MRI are of increasing importance not only to the improvement of image quality and the clinical ability to distinguish boundaries and differentiate one type of tissue from another, but with the development of new paramagnetic, superparamagnetic, and ferromagnetic contrast materials, it is becoming increasingly possible to carry out dynamic imaging and vascular and organ function studies using these contrast materials as pharmacokinetic agents. A notable SBIR program at Advanced Magnetics, Inc., in Cambridge, Massachusetts has produced new superparamagnetic iron oxide particles which are more effective as contrast agents than either paramagnetic ions or ferromagnetic particles. They are non-toxic, and the iron is biodegradable in the body. Recent imaging applications include tumors of the brain and gastrointestinal tract, pyogenic liver abscess, and micrometastases of the liver and spleen.

Pharmacokinetic studies and development and evaluation of new contrast agents based on gadolinium and nitroxide free radicals are being carried out at the University of California at San Francisco. The currently FDA approved Gd-DTPA serves as a standard for comparison with new agents. A program at the University of Arizona is concentrating on the development of new liposome-entrapped contrast agents for the detection of hepatic and splenic metastases. These agents look promising in their ability to deliver the desired magnetic contrast material to the

desired sites in the body without prior degradation or dilution. Contrast agents are being used pharmacokinetically at the Pittsburgh NMR Institute to study tissue perfusion in the spleen and placenta of rats and rabbits. Agents employed include Gd-DTPA, Gd-DTPA-albumin, colloidal gadolinium oxide, and magnetite microspheres.

Another study at the University of California at San Francisco has concentrated on the development of iron chelates and their toxicity and clinical effectiveness for many diagnostic uses, including tumor detection and enhancement. A group at the University of Illinois is examining liposomes containing paramagnetic materials as well as nitroxides as agents. Work is continuing on the characterization of various gadolinium complexes at the Brigham and Women's Hospital. Fundamental mechanisms in tissue relaxation properties, which are the major determinants of intrinsic contrast differences in MRI, is the subject of basic research at Yale University.

Dramatic success was achieved recently at the Medical College of Wisconsin at Milwaukee in visualizing the heterogeneity and necrotic areas in tumors in a mouse by obtaining MR images at five minute intervals following injection of Gd-DTPA contrast agent. Assembling these time-lapse images into a cinematic sequence permitted ready tumor visualization and a new understanding of the processes of absorption and elimination of the contrast agent.

MR Bioeffects:

Research has been done on the thermophysiologic effects of Magnetic Resonance Imaging at Cedars-Sinai Medical Center in Los Angeles. Theoretical and experimental studies have focused on the possible slight heating effects that occur in tissues subjected to radiofrequency signals employed in the MR imaging system as well as any effects which might arise from static and gradient magnetic fields. The FDA has also examined this subject continually on all new systems submitted for approval. At present, it appears that no hazardous or adverse biological effects have been observed from present clinical exposure levels. The greatest hazard in present day clinical MRI systems is that of loose flying ferromagnetic objects (such as tools or laboratory hardware, including carts or compressed gas tanks), which may be attracted rapidly into the magnetic field as unintended projectiles. This hazard is easy to control.

IMAGE PERCEPTION

Very interesting results in the area of image perception have been reported by the highly respected research team at the University of Pennsylvania. Eye-fixations have been recorded from radiologists during the search for inconspicuous lung tumors on chest images. The majority of missed lesion receive prolonged visual fixation. An on-line system for coupling the eye-fixations to the displayed image of the chest has been developed. Radiologists can be shown chest regions that received prolonged fixation or no fixation at all. An experimental test of this visual aid to tumor search showed significantly improved tumor detection. This is, to our knowledge, the first demonstration of the use of functional information (eye fixation dwell time) inaccessible to the individual for improving performance on a perceptual task. Studies to elucidate the mechanism of the effect are in progress.

Researchers in image enhancement funded by our program are trying to improve the ability of radiologists to interpret images by training them to recognize and assess the status of specific image features and learn how to combine them for diagnosis. The particular focus has been placed on the differentiation of benign versus malignant lesions in mammography, and this work involves a computer-based tutorial using a library of digitized mammograms. During the past year the DIRB funded investigators have developed a computer interface for presenting radiographs digitally and demonstrated its value to radiologists. Currently, these investigators

are constructing a case library, digitizing and classifying images, and constructing tutorial exercises. The efficacy of the tutorial system will be studied through formative and summative evaluations.

DIGITAL RADIOGRAPHY AND OTHER TECHNOLOGY DEVELOPMENTS

Research in areas of digital electronic communication technology, picture archiving and communication systems (PACS), image acquisition and sorting, and image enhancement has been actively encouraged by the DIRB. Our program has taken a leading role in supporting research in the development and utilization of PACS and other imaging technology.

World leading experts at the University of Chicago developed a new technique based on distance-density curves using angiographic images for real time measurements of average blood flow rates. In their opinion, the proposed method is more accurate than the currently accepted methods. These findings improve interventional radiology procedures and result in better patient management.

Digital radiography is superior to conventional systems in image manipulation, transmission and storage. This technology is entering a new phase in applying Artificial Intelligence techniques for automated detection and characterization of disease. At the University of Chicago, scientists were able to advance digital radiology and computer outputs for detection of clustered microcalcifications in mammograms.

The application of high-speed digital electronics and communication technologies to radiological sciences has been changing the methods of acquiring, storing, viewing, and transmitting diagnostic images. Currently, about 20 percent of radiologic images are acquired digitally. There has been also a trend to replace the remaining 80 percent of images consisting mostly of conventional projection x-ray procedures on film by digital radiography. As a result, a very large volume of digital diagnostic images will be generated. It is therefore essential to investigate methods of compressing these images into a more compact form for storage and transmittal. The research team funded by our program at the University of California in Los Angeles developed a unique approach to compression of digital images. In the past year, this team has successfully completed the compression hardware module for radiologic images, achieved routine compression ratio of 15:1 with a speed of about 4 to 20 seconds per image.

A leading radiology team at the University of California, Los Angeles (UCLA) has also developed a sophisticated, computer-based, picture archiving and communication system (PACS) for the complete acquisition, handling, storage, retrieval, and display of x-ray images and other modality images in digital electronic form. This system has been under evaluation In a variety of clinical settings, including the coronary care unit (CCU) of a 700-bed teaching hospital. Significantly greater use has been made of digital images in this CCU than of film images. In the Pediatric Radiology Department, all image storage, retrieval, analysis, and diagnostic decision-making subsequent to primary acquisition and first reading of the case on film has been converted to the digital radiographic system. This system includes an advanced six-terminal display console previously developed by this UCLA team for simultaneous display of multiple images, comparison of multimodality images, and image processing and analysis.

The main objective of the University of Pittsburgh's project is to assemble and evaluate a multi-port digital radiography system based on two-dimensional solid-state arrays which are fiberoptically coupled to either a fluorescent screen or a scintillating fiberoptic faceplate. The most significant achievements of these internationally known scientists during the last year have been as follows:

1) They have successfully assembled and tested a digital, real-time, multi-port charged coupled device (CCD)-based X-ray camera in which 6 CCD devices are fiberoptically coupled to a fluorescent screen.

2) They have been able to digitally acquire segments of the x-ray image at high speed and to merge them into a single, high quality, high-resolution x-ray image. In their opinion, this is the only operational system of its kind in the world to date.

An SBIR grantee, XData Corporation in Indianapolis, has demonstrated in preliminary form the feasibility of developing a computer-based expert radiology assistant to aid in the diagnosis of breast cancer on the basis of clinical findings, X-ray mammograms, and ultrasound image information. Work is continuing at the University of Michigan on a computer-based system for detecting lesions and microcalcifications in X-ray mammograms. The radiographs are first digitized electronically so that specially designed algorithms can be employed to provide automatic feature discrimi-nation as an aid to the mammographer.

DIAPHANOGRAPHY AND MICROWAVE RADIOMETRY

A program of performance and evaluation of diaphanography on several thousands of women has been carried out over the past four years, principally at the University of Cincinnati and the University of Massachusetts Medical Center at Worcester, with the statistical collaboration of Bolt, Beranek, and Newman of Cambridge, Massa-chusetts. Principal conclusions were based on a 2500 patient subset following evolution and learning experience with the Spectrascan transmission light scanning system employed. The results from light scans alone were compared to those from X-ray mammograms and from physical exams as well as appropriate combinations of two and all three of these modes of diagnosis.

Although light scanning alone is not a highly specific or sensitive modality for the diagnosis and detection of breast cancer, it is clear that strong and meaningful signals are being detected in the light scans for a number of cancers, independent of stage. The overall accuracy of mammography and light scanning used separately for detection is 87% and 60%, respectively. The sensitivity of light scanning alone is the same as for clinical physical examination alone. However, the predictive value that a cancer is truly present when the light scan is positive may be enhanced several times if one of the other modalities (physical exam or mammogram) is also positive. A complex and subtle set of alternative diagnostic decisions and predictive expectations has been examined, given the negative or positve findings from each of the modalities when taken alone or in various combinations, and in terms of the relative indications for biopsy or no biopsy. The consequences of the data are reinforced further and with no surprises by long term followup of one year or more on 2200 women.

Another approach to breast cancer detection has been demonstrated by an SBIR grantee, Microwave Medical Systems, Inc., of Littleton, Massachusetts, in which a multiple antenna microwave radiometry system has been able to detect "hot spots" in a two-layer tissue phantom. Initial trials of this instrument on 183 volunteer women with mammary gland disease at the Nippon Medical School have shown the true negative rate for benign cases at 81.7% and the true positive rate for malignancies at 63.4%.

ULTRASOUND IMAGING , TISSUE CHARACTERIZATION, & BIOEFFECTS

Instrumentation:

Progress continues to be made on the development of a number of advanced ultrasound imaging systems for clinical use on various organ systems of the body. The high speed scanner at Duke

University, by virtue of parallel computer processing of data and linear phased arrays of transducers, can transmit on 32 interlaced channels and receive with dynamic focussing on 16 channels, and can produce real time 3-D ultrasound images. Another highly promising new technical approach at Duke University is the development and study of a real time, adaptive phased array imager, which should generate very high quality images of diffraction-limited resolution, providing the ultimate possible anatomic detail.

A novel reflex transmission imaging system has been developed at SRI International for medical imaging of various body parts, such as the extremities. A high resolution scanner (less than one millimeter spatial resolution) for imaging of abdominal organs and breast tissue is under development by TechniScan, Inc., under an SBIR (small business innovation research) contract at Salt Lake City. The system takes advantage of theoretical and applied research performed under a grant at the University of Utah, which uses very high speed computation to correct errors and artifacts which otherwise would normally occur as ultrasound energy travels through the tissues. The consequent improvement in image quality is dramatic.

Basic Research Studies:

Success in applied imaging system development requires fundamental knowledge about the propagation of ultrasound energy in the body and scattering and absorption phenomena which ultimately affect image quality. To add to this fund of understanding, basic research studies have been carried out for many years at the University of Illinois, Stanford University, Mayo Foundation, SRI International, the University of Wisconsin, and other institutions. Development of an ultrasound anthropomorphic breast tissue phantom at Madison over the past several years has resulted in its adoption by many clinics for use in quality control and training. The same group of investigators have also been developing a tissue-equivalent phantom for general quality control and training use in Magnetic Resonance Imaging systems.

Ultrasound Tissue Characterization:

One of the most active research and development areas in ultrasound imaging today is tissue characterization, where improvements in the understanding of such factors as texture, speckle reduction, angular distribution of reflectivity, speed of sound, attenuation, and other parameters lead to new and improved ways of displaying the image and of determining noninvasively the nature or state of the tissue seen in each area of the image. Highly quantitative studies have been carried out at the University of Texas at Houston on the properties of speed of sound and attenuation of sound (ultrasound) as it applies to clinical examinations of organs such as liver. Quantitative measurements on freshly excised human breast tissue at SRI International have provided most accurate evaluation of interactions of ultrasound energy with breast tissues and improved understanding of how to interpret clinical breast images.

Remarkable progress over many years in high frequency ultrasound research at Riverside Research Institute in New York City, working in collaboration with Columbia and Cornell Universities, has permitted the depiction of dynamic, colored, 3-D images of tumors of the eye and other ocular structures in videotape displays which rotate the image. Measuring three different ultrasound characteristics (or parameters) of each little volume element of tissue permits specific colors to be assigned to different tissues. For example, the distribution of the primary tumor can be mapped in one color and the metastasized tumor in another. They have now been applying the same noninvasive and multiparametric display techniques at lower frequencies to imaging and characterization of liver and spleen tumors.

A novel approach to the detection of tumors based on ultrasound Doppler detection of shifts in the velocity of blood under tumor-induced vascular changes is being pursued with success at Yale University in studies of tumors of pancreas, breast, kidneys, and liver in women.

Contrast Agents:

Good results have been achieved in enhancing the contrast of ultrasound images and the detection and delineation of tumors in the liver by extensive investigations at the University of California at San Diego in animals with perfluorochemical agents, e.g., perfluoro-oxybutylene (PFOB). Suspensions of iodipamide ethyl ester (IDE) particles are being investigated as contrast agents in the livers and spleens of animals at the University of Rochester. This material provides high contrast in ultrasound images because of its large impedance mismatch with water and tissues. At the University of Texas at Houston, the research team has emphasized aqueous chemical mixtures as renal contrast agents. Development studies of microbubbles as potential ultrasound contrast agents were first supported by NIH contract more than ten years ago and will soon be on the market in Europe for human use. Further research on a modified design of the original microbubble approach is being continued under an SBIR grant.

Bioeffects:

Extensive long term research continues to be supported on the potential bioeffects of exposure to ultrasound. It is known that bioeffects can be produced at very high exposure intensities because ultrasound is used regularly to produce high temperatures in tissues (hyperthermia) for cancer treatment. However, it is important to continue to study and monitor the absence of such effects at diagnostic levels. Understanding of the effects of ultrasound energy in different biological tissues continues to increase as the result of research supported at the University of Rochester, the University of Illinois, SRI International, Battelle Northwest Labs, Yale University, and in many other institutions and countries.

Present day research includes work on cells, bacteria, Drosophila, and animals and is concentrated especially on understanding the process called "cavitation" (or the collapse of microbubbles) in fluids. This phenomena has not been observed in mammals at diagnostic levels of ultrasound exposure. It is still possible to say that in over 25 years of ultrasound exposure to millions of patients all over the world, no adverse effects have ever been observed in humans or animals when the exposures are kept below well-defined levels specified for clinical diagnostic use.

IONIZING RADIATION/NUCLEAR MEDICINE SECTION

RADIOLOGIC DIAGNOSTIC ONCOLOGIC GROUP (RDOG)

The Radiologic Diagnostic Oncology Group (RDOG) was established in response to an RFA for National Collaborative Diagnostic Imaging Trial Projects in September, 1987, and patient accrual convened in November, 1987. The group's objective is the timely evaluation of current and emerging imaging modalities in the management of patients with cancer. Furthermore, the development of multi-institutional clinical trial groups allows for rapid patient accrual within a short period of time. This assures rapid evaluation and optimization of imaging techniques for diagnostic staging and serial monitoring of cancer. The results of each study should have direct and immediate impact on patient care and considerable cost saving resulting from the elimination of inappropriate or unnecessary diagnostic studies.

Institutions included in RDOG I are the Cleveland Clinic, Johns Hopkins University Hospital, Memorial Sloan-Kettering Cancer Center, Thomas Jefferson University Hospital, University of California at San Francisco and the University of Michigan at Ann Arbor, with the Operations Control Center managed by the American College of Radiology and the Statistical Center operated by Harvard University. This multi-institutional clinical trials group is currently evaluating carcinomas of the lung and prostate. Semi-annual reports indicate satisfactory progress.

The clinical diagnostic imaging trials of colorectal and pancreatic cancers (RDOG II) have been recently added to the ongoing collaborative group, and several institutions were granted DIRB support [Johns Hopkins, Washington University (St. Louis), University of Washington (Seattle), University of Michigan and New York University]. Thus four institutions joined the six institutions currently participating in the RDOG I clinical trials.

A new RFA (90-CA-05) to establish RDOG III has been issued. The emphasis of RDOG III is on the study of musculoskeletal and head and neck tumors. The closing date was on April 15, 1990. Twenty seven applications have been received and will be reviewed in July, 1990. This new RFA will result in adding five or six new institutions to the existing collaborative group in FY91.

The clinical trials will contribute significantly not only to the optimization of patient care but also to the development of a comprehensive teaching file which should prove to be an excellent resource for Radiology research training and for evaluation of the impact of external factors on radiology interpretations. Moreover, the proposed clinical trials will stimulate spin-off projects addressing some of the questions that are not within the scope of the grant. Potential research projects will involve detailed studies of MR tissue characterization, prognostic factors, impact of work-up bias on test performance, and many other important areas in clinical diagnostic radiology.

PROSTATE EARLY DIAGNOSIS NETWORK

The Prostate Cancer Ultrasonography Network was established in 1988 as a result of an RFA, and three institutions were funded [University of Utah, Baylor College of Medicine, and University Hospital of Cleveland]. These clinical trials deal with early diagnosis of prostate cancer. The Network selected the University of Utah as the headquarters for its management and data analysis center.

Prostate ultrasonography and prostatic specific antigen (PSA) have been utilized in an effort to determine the efficacy of these studies in detecting and staging prostatic carcinoma. Studies assessing patients prior to transurethral prostatic resection for presumed benign disease are in progress. Additionally, patients subjected to radical prostatectomy for prostatic carcinoma and cystoprostatectomy for bladder carcinoma are also being investigated. Further, histological findings are being correlated with ultrasound findings.

Three protocols have been developed by the Network. Patients are being accrued in all institutions. Although data are being accumulated, additional time will be needed for statistical analysis when the number of patients will reach the planned level.

NUCLEAR MEDICINE

Development of new radiolabeled compounds, their biodistribution, toxicity, use of various modalities, such as PET and SPECT, and immunoimaging are just a few examples to illustrate the wide range of the Nuclear Medicine program which comprises a major portion of the traditional research grants (R01) in DIRB.

Program Project Grants (PPG) deal with various aspects of nuclear medical research. The first PPG is focused on the development and evaluation of promising compounds which when radiolabeled have the potential to be used for both the scintigraphic detection and the treatment of cancer. The second PPG continues to explore the improvement of image-derived information by administration of a novel contrast agent. This work has been focused on the improvement of the qualitative and quantitative aspects of imaging by SPECT and the utilization of pharmacoangiography to improve information content and understanding organ physiology. An important goal of the third PPG is to design and develop a scintillation probe for intraoperative tumor detection. By using two detectors, the newly constructed prototype probe effectively discriminates tumor from background noise and improves detection of small metastases when compared to external imaging or other surgical probes. It is expected that similar commercial probe will be constructed for routine use in surgery.

The goal of one of our oldest grantees at the University of Michigan is to develop new chemical compounds for tumor imaging. Last year this group described a new class of tumor imaging agents, the radioiodinated phospholipid ethers (e.d. NM-294). They have now discovered that more chemically simple analogs, such as alkylphosphocholines (e.g. NM-324), possess similar properties. These agents have been found to accumulate in and image all tumors studied to date, including human tumors (small cell carcinoma of the lung, ovarian carcinoma and melanoma) implanted in athymic mice. These new agents have considerable potential for diagnosis and treatment of cancer.

One of the most promising areas in the DIRB-supported research is focused on the metabolism of radiolabeled antibodies which is important for radioimmunoimaging and therapy. Scientists at the University of California at Davis have found that the loss of radioactive Indium [^{111}In] from chelate-antibody conjugates causes problems in imaging and increases radiation dose to normal tissues. They have evaluated the *in vivo* loss of ^{111}In from Lym-1-benzyl-EDTA-^{111}In [antibody conjugated with isothiocyanatobenzyl-EDTA] in normal mice. A monoclonal antibody (CHA 255) that binds to benzyl-EDTA-Indium chelates, but not to other forms of indium, was used to measure the percent of ^{111}In remaining in the chelate. Four days after injection, major portion of the radioactive ^{111}In in the liver, urine and blood was still coupled to the benzyl-EDTA chelate. *In vitro* studies indicate that benzyl-**EDTA**-^{111}In-antibody-chelate conjugate is more stable in human serum than benzyl-**DTPA**-^{111}In conjugate.

Scientists at the University of Washington in Seattle have been improving their ability to interpret images of radiolabeled thymidine in order to measure tumor growth. They have previously used thymidine labeled in the methyl position; however, a synthesis has been recently described for thymidine labeled in the ring-2 position. These investigators have demonstrated that the use of the new method of radiolabeling improves image quality and simplifies kinetic modeling for quantitation. These scientists have also studied the distribution and retention of radiolabeled CO_2, which is the major thymidine metabolite. Their data showed prolonged retention of labeled CO_2 and contradict the notion that the contribution of labeled CO_2 to PET images can be disregarded because of its lung exhalation. Radiolabeled CO_2 is one of the major metabolites of radiolabeled thymidine, glucose, acetate, and fatty acids. Their results indicate that the retention and distribution of labeled CO_2 needs to be taken into account when PET data are interpreted.

Several projects in imaging instrumentation development have brought successful results this year at the University of Arizona, where two SPECT imaging systems based on modular scintillation cameras have been completed. Cardiac imaging device using 16 modular cameras in a ring configuration with a multiple-pinhole coded aperture is operational, and initial phantom images are quite encouraging. Resolution of 5-6 mm has been achieved, and high-quality

tomographic images have been obtained. A hemispherical brain imaging device has been producing good phantom images as well. When complete, this system will use 20 modular cameras. For both of these systems, image reconstruction is accomplished by the specially developed algorithm, which has been now implemented on a parallel computer system designed and constructed by these scientists. For this algorithm, their system is approximately 50 times faster than currently used VAX 8600.

The principal goal of the University of California in San Diego team is to improve the accuracy of early cancer detection. Emulsion particles of the radiopaque fluorocarbon Pefluorooctylbromide (PFOB) given intravenously are taken up by tumor macrophages and liver and spleen macrophages. Structures containing high PFOB concentration can be seen on CT scans. PFOB is also useful in obtaining images with ultrasound and MRI.

Significant progress has been made at Worcester Polytechnic Institute where scientists were able to complete the design of a full-size xenon gas scintillation proportional gamma camera (GSPC) for brain imaging. They recently completed the pressure vessel which has been hydrostatically pressure tested to 80 atmospheres (twice the operating pressure). Pressure induced deflections of the GSPC "entrance window" have been measured and agreed with theoretical calculations. A 37-tube data acquisition system was assembled and tested to 2300 events/second. They have also completed the design of a tungsten grid collimator, specially optimized for the GSPC using the Monte Carlo simulation software developed in their laboratory. These investigators are now working on the final assembly and testing of the GSPC. Such system when completed will improve the spatial and energy resolution of SPECT.

Recent studies at George Washington University have used six different populations of radiolabeled lymphocytes for intravenous injection in C3H/OuJ mice with spontaneous mammary carcinoma. The highest concentration of the labeled lymphocytes in tumor has been observed with tumor infiltrating lymphocytes, either freshly harvested or cultured in interleukin-2. Lower tumor concentrations have been seen with splenic lymphocytes, natural killer (NK) cells and lymphokine activated killer (LAK) cells. Such studies of native and cultured lymphocyte migration will help to explain the variable success of adoptive immunotherapy in the treatment of advanced human malignancies.

MONOCLONAL ANTIBODIES

The number of grants in the area of immunoimaging research supported by our program has been increasing, and this effort has resulted in important publications. The DIRB grantees have investigated several of the most promising monoclonal antibodies (MoAbs) reactive with human colorectal cancer (35,115, B72.3 and 17-1A) to study heterogeneity in binding between and within colorectal tumor masses in human subjects and to evaluate agent biodistribution in athymic nude mice bearing human colorectal tumor xenografts. These studies were designed to look at the differences in antibody binding and localization. The results confirmed the hypothesis predicting significant heterogeneity with respect to absolute tumor uptake. It has been found that human-mouse chimeric variants of murine MoAb 17-1A showed similar binding, tumor uptake and therapeutic efficacy in colon tumor xenografts as compared to murine MoAb. These results suggest that chimeric 17-1A MoAbs may be promising in radioimmunoimaging and radioimmunotherapy (RIT) studies. Estimated four more years are required to develop and standardize these radioimmunoimaging techniques.

Monoclonal antibody research at the University of Washington in Seattle has been focused on methods to improve radioiodination of anti-tumor monoclonal antibodies so that deiodination would be decreased while tumor retention of radiolabel would be increased. Coupling of antibodies to radioiodinated tyramine cellobiose has been shown to be the most successful method

to-date. In experimental tumors, this new method has doubled tumor radioiodine retention as compared to standard radioiodination by chloramine T. The same group of investigators have been also developing methods to quantify in-vivo biodistribution of radiolabeled anti-melanoma antibody and to select patients with sufficient tumor concentration for therapy. Successful methods to measure organ and tumor concentrations of antibodies have been developed. Because of the relatively low concentrations achieved in tumor, high dose therapy has been used in combination with simultaneous autologous bone marrow transplant to avoid irreversible marrow aplasia.

At the University of California at Davis, new technology (MRI/MRS) has been used as functional imaging tool to assist in monoclonal antibody research. NMR evaluation of the phosphocreatinine/inorganic phosphate ratio (PCr/P_i) resulted in the establishment of bioenergetic indices for predicting and monitoring tumor response to I-131 Lym-1 radioimmunotherapy. This human anti-lymphoma monoclonal antibody was injected I.V. (200-500 uCi) into athymic mice bearing human lymphoma xenographs, and phosphorus NMR spectroscopy was performed at regular intervals before and after RIT. Thirty six percent of implanted tumors responded to RIT by exhibiting regression or arrested growth, which correlated well with the bioenergetic indices calculated by MRS. These MRS indices may thus prove useful in planning of and predicting RIT response.

DIAGNOSTIC RADIOLOGY COORDINATING COMMITTEE (DRCC)

This committee (DRCC) was created in 1989 to replace the NIH Inter-Institute Diagnostic Imaging Group (IDIG). DRCC was established to promote collaboration among NIH institutes and to facilitate the dissemination of information concerning diagnostic imaging research. There is general agreement that a large amount of information is available concerning NIH resources and activities (both intramural and extramural) that can be shared and disseminated among the various Institutes. The Committee is responsible for the coordination of diagnostic imaging research at NIH, developing a NIH-wide long-range research plan for diagnostic radiology, and reporting on a regular basis to the Director, NIH.

In order to have a focal point to oversee the coordination of NIH-wide imaging activities, the Director of NIH designated the National Cancer Institute to be the lead Institute responsible for the development of the new committee (DRCC). The Radiation Research Program, Division of Cancer Treatment has been identified by the NCI to direct this committee.

The DRCC has representatives from each of the Institutes and other NIH component groups, both intramural and extramural, which have significant interests or programs in diagnostic imaging. The Imaging Planning Panel consisting of leading scientific experts and NIH representatives has been formed to identify the most important directions in radiologic science for the next five years. The first meeting of the panel took place on May 30-31, 1990. The important outcome of this meeting is the preliminary list of important new areas of diagnostic research. The next meeting will take place in November, 1990, 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 corrdinate support of the most important areas of radiologic science; and 2) it will educate and stimulate extramural radiologic scientific community.

FUTURE DIRECTIONS (DIRB)

1. Multi-institutional Imaging Trials for optimization of tumor detection and staging will need to be expanded beyond their current activity. As research in this area expands,

both the malignant disease sites to be studied and the number of institutions participating will be increased. Additional funding is thus necessary. New advances in technology will be clinically tested. RDOG III (Head and Neck and Musculoskeletal tumors) have been approved for funding in FY90. Currently, pediatric solid tumors are planned to be added as RDOG IV in FY91.

2. One of the scientific highlights of the DIRB in FY90 was the recent BSC/DCT approval of the development of in vivo ultrasound microscopic device by the Board of Scientific Counselors (BSC). This concept is planned to be funded in FY91.

3. A DIRB workshop on "Medical Image Processing" was held in February, 1990. This workshop indicated the need to enhance quantitative in vivo characterization of malignant processes. The outcome of this workshop in a form of a new initiative will be presented to BSC in FY91.

4. MRS has important potential for monitoring the effects of radiation therapy, hyperthermia, chemotherapy, and immunotherapy. A workshop held at NIH in September,1989 on "MRS/MRI in Tumor Response to Therapy" enabled members of the NIH Clinical Staff to provide tutorial discussions of current techniques in the treatment of tumors by chemotherapy, immunotherapy, radiation and photodynamic therapy, and hyperthermia. This was followed by papers from physicists, engineers, chemists, and biologists on current laboratory and clinical studies using magnetic resonance spectroscopy (MRS) as a new tool to measure changes in the intensity and distribution of tumor metabolites as a function of time, dosage, pH, temperature, and other biologic variables. Magnetic resonance imaging (MRI) is an indispensable aid to MRS in identifying and localizing the small volume elements within tumors which are being analyzed by MRS. A report is being completed to describe the current progress in applying MRS of protons (hydrogen nuclei) and other magnetic nuclei, such as phosphorus, fluorine, carbon, and sodium, to assess and monitor the state of tumors in research studies aimed at eventual refinement for clinical analytical uses in staging and in prediction of tumor response. Surface tumors are easy to biopsy by surgery. MRS provides important potential for noninvasive assessment of deep-seated tumors, including those of bone and soft tissues. The concept entitled "MRS and Cancer Therapy" is planned to be presented at October, 1990, BSC.

5. New progress in the development of picture archiving and communication systems (PACS) have brought about the need for new software management tools from the field of medical informatics, a new and growing science concerned with the development of decision support tools, data management and physician workstation environments that increase the efficiency and personal productivity of the diagnostic radiologist. New initiatives are expected that will stimulate research and development of knowledge-based systems directed at diagnostic imaging applications. These systems, coupled with PACS networks, will eliminate the need for the patient's traditional x-ray film file which is now tracked by each department care facility. These computer-based systems will improve efficiency, quality control and bring new capabilities to the physician. The first specific DIRB initiative in this area, "Digitization of Chest Radiography for Lung Cancer Detection", has been approved by the BSC in FY90 and expected to be funded in FY91.

6. The "Clinical Applications of Positron Emission Tomography" was explored in the DIRB workshop held September 14-16, 1988. Advances in the use of this diagnostic modality make it possible to study tumor physiology and metabolism in vivo. The new initiatives have been recently approved by the BSC: 1) Clinical Diagnosis of AIDS associated brain

disease using PET and other imaging modalities; and 2) Diagnostic studies of brain tumor metabolism. These initiatives will be funded in FY91.

7. In FY90, several other important areas in radiologic science have been identified by the DIRB and later supported by the BSC. Two new RFA's, "Diagnostic Imaging Studies of Tumor Perfusion" and "Imaging of Bone Marrow Tumors", are currently in preparation.

8. Three-dimensional display and analysis of medical imaging can have significant impact on cancer treatment planning in several areas: 1) accurate 3D display; 2) stereotactic computer-assisted surgery; and 3) tumor volumetric analysis. The workshop entitled "3D Display and Analysis for Cancer Treatment Planning" will be held in July, 1990. At this workshop, the state-of-the-art in the area of 3D imaging research will be evaluated and the future directions prioritized. The most important research directions in 3D imaging requiring DIRB support will be presented to the BSC in October, 1990.

B. RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch (RDB) continues to develop and administer a large program of basic science and clinical research activities related to cancer treatment. The disciplines represented are radiation oncology, radiobiology, radiation chemistry and radiation physics. Research efforts range from investigation of the basic physics and biological effects of radiation to controlled clinical trials for a variety of neoplastic diseases and therapeutic modalities.

Major areas of funding are in particle radiation therapy, radiosensitizers, photodynamic therapy, systemic radiation therapy (SRT), radiation physics, and hyperthermia and its associated biology. An area of increasing interest and importance is boron neutron capture therapy (BNCT).

The following is the RDB budget for FY89 and the estimated budget for FY90.

FY89 and FY90 RDB Budget

			$(thousands)	
	FY89	FY90	FY89	FY90
GRANTS				
Traditional (R01)	153	143	27,263	28,002
Program Projects (P01)	13	15	15,362	17,708
Conference and New Investigator (R13 & 23)	2	6	8	27
First Awards	17	23	1,561	2,040
Merit Awards	10	11	2,834	3,324
Cooperative Agreement (U01)	1	1	1,097	1,383
RFA, 90-CA-07	0	2	0	1,500
RFA, 89-CA-04	2	2	357	335
SBIR	15	14	2,365	3,107
TOTAL GRANTS	213	217	50,847	57,426
CONTRACTS				
Regular	13	10	4,070	3,698
SBIR	4	1	200	250
TOTAL CONTRACTS	17	11	4,270	3,948
TOTAL RDB BUDGET	230	228	55,117	61,374

FY90 Annual Report Summary
Radiotherapy Development Branch

The Radiotherapy Development Branch (RDB) administers a large program of basic, developmental, and clinical research related to cancer treatment utilizing ionizing and nonionizing radiations. Radiation research encompasses a range of scientific disciplines including biology, chemistry, physics and clinical oncology as well as the specialized treatment modalities of photodynamic therapy and hyperthermia. More recently, the role of computer-

based tools for the diagnosis, therapy selection and radiotherapy treatment planning processes have received increased emphasis in the Program. Research efforts range from the investigation of basic mechanisms at the atomic and cellular levels to controlled clinical trials for a multitude of diseases using single or multimodality treatment schemes.

Basic research supported by RDB has generated leads for promising new treatment modalities that are currently being tested in clinical trials. Major areas of funded research include particle radiotherapy, hyperthermia, and general radiobiology. Substantial support is also provided for the development of radiomodifiers, tagged antibody therapy, boron neutron capture therapy, photodynamic therapy, and radiation physics. Radiation modifiers are being explored as protective agents to reduce normal tissue morbidity, and as sensitizers to enhance the effects of radiation on tumors. Advanced treatment planning tools continue to be developed through a series of collaborative working groups that are bringing three-dimensional computer graphics and decision-support tools to the treatment planning process.

PARTICLE RADIOTHERAPY

Radiotherapy with either charged or uncharged particles continues to receive a significant portion of the RDB budget. Neutron therapy Phase III trials compare fast neutrons against best conventional photon therapy for head and neck cancers, prostate and lung tumors, as well as tumors of radioresistant histologies, such as sarcomas of the soft tissue and bone and melanoma. Charged particle therapy with both protons and heavy ions are now successfully treating a variety of tumors in the lung, prostate, 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 tumorcidal doses can be delivered to these lesions which are not attainable with conventional therapies.

The proton beam therapy experience at the Massachusetts General Hospital/Harvard Cyclotron Laboratory, based on the treatment of more than 1950 cancer patients, has confirmed the expectation that higher doses to the target volume and smaller treatment volumes can be used to treat and control selected tumors. Comparing the data to historical controls, proton treatment has resulted in a greater tumor control rate, comparable or lesser morbidity, and no increase in marginal failures. This experience is consistent with the concept that improved radiation dose distribution yields better therapeutic results.

The history of radiation therapy shows clearly that major improvements in dose distribution have yielded clinical gains. Further gains are virtually certain if worthwhile improvements in dose distributions can be realized. The serious issues regarding the use of new techniques which yield further improvements in dose distributions are: the ability to image the target and non-target tissues/structures precisely; the ability to align the target and the beam and to define the uncertainties of that alignment; the ability to deliver the dose to all of the defined target (in 3 dimensions) at each treatment session, and the cost (personnel, equipment, space, etc.). To the extent that gains are demonstrated, the results will prompt efforts to test additional sites for proton beam therapy. Positive results will also support the study of radiation therapy strategies other than protons to improve dose distributions.

Protons may have a place in the armanentarium of cancer therapy. Further research is needed to establish their exact role. Therefore, there is a need for a small number of state-of-the-art, dedicated, hospital-based proton research and treatment facilities in the U.S. The decision by Congress to provide $1.5 million for planning and development of a very limited number of

referral centers for the treatment of tumors with proton beam therapy, comes at a very appropriate time.

NCI plans to issue one or two explanatory grants in FY90 for appropriate planning and developmental activities. Additional proton beam facilities would perform research to:

- Confirm pilot data
- Improve delivery of proton beams
- Determine optimum proton dose
- Study additional tumor types
- Conduct carefully-designed prospective trials to determine if this radiation modality is superior to conventional therapy with respect to morbidity (complication) and outcome (local control and survival.)

Heavy ion radiotherapy continues to progress well at the Lawrence Berkeley Laboratory of the University of California. The Phase III uveal melanoma trial, the chordoma-chondrosarcoma collaborative trial, and accrual of patients in the new randomized helium vs neon protocol for other paraspinal and base of skull tumors, are all ongoing. Accrual will also continue in randomized Phase II and III studies which have been established for locally advanced prostate tumors, sarcoma, paranasal sinus, nasopharynx and "radioresistant" histologies such as melanoma or renal carcinoma. For glioblastoma, a trial has been opened which compares neon ions + chemotherapy with the well-characterized low-LET x-ray + chemotherapy database of the NCOG/UCSF Brain Tumor Research Center studies.

Neutron therapy clinical trials are nearing completion after 10 years of effort. Phase III trials that randomize patients' treatments between neutrons and conventional photon therapy for lung and prostate cancer will reach study goals within a few months. A third randomized effort that is comparing the outcome for head and neck cancer will likely be completed in early 1993. Salivary gland tumors are being referred to the US neutron therapy centers from all over the world after neutrons were determined as the treatment of choice. Three other protocols were closed due to the lack of patient accrual. These studies were designed to explore the efficacy of neutrons in uterine and rectal cancers as well as radioresistant tumors, such as sarcomas and melanomas. Over 1200 patients have been treated on the neutron therapy protocols and approximately 2150 patients have received neutron therapy using the hospital-based neutron therapy generators since 1984.

HYPERTHERMIA

The strong interest in hyperthermia by the research community is shown by the large number of grant applications representing many aspects of both preclinical and clinical studies. Hyperthermia research during the past year has taken two major directions:

1) Elucidation of the molecular or biochemical mechanisms(s) of hyperthermia-induced cytotoxicity and radiosensitization and
2) The development of appropriate devices for the optimum application of hyperthermia to a tumor mass.

Studies have directed attention to the mechanisms of heat damage and the factors which modify this effect. Attempts are underway to model the temperature distribution of tumors and to correlate the temperatures achieved with clinical results. Efforts to develop new and more effective hyperthermia applicators and devices are in progress.

Several studies have attempted to gain an understanding of the cellular lesions induced by hyperthermia that kill mammalian cells. At Thomas Jefferson University, investigators approached the problem by comparing the response of heat-induced structural lesions with cytotoxicity in synchronous CHO cells treated with a variety of agents. Heat-induced cytotoxicity was modified with local anesthetics, acrylamide, caffeine, and H-PLDR during this past year. The death of heated (-/+ acrylamide) G1 populations of cells correlated well with the inability of the cytoskeleton to reform within 24 hr after heating, while death of heated S-phase cells does not. Studies of acrylamide sensitization using time-lapse cinematography indicated that this drug enhanced lesions responsible for lysis and not lesions resulting in irregular divisions. The effectiveness of local anesthetics as heat sensitizers was shown to be dramatically influenced by external pH. Caffeine was shown to be a heat sensitizer and its sensitization was shown to occur independent of the drug-induced influx of extracellular calcium. Using modified extraction procedures for studying nuclear matrices and nucleoli from heated cells using resinless electron microscopy. These investigators showed that DNA (as well as RNA) had a structural role in the architecture of the nuclear matrix and that heat modifies the architecture. Numerous studies were also performed defining the optimal conditions for repair of heat induced, potentially lethal damage.

At the University of California in San Francisco, researchers have studied the mechanism of cell death as caused by hyperthermia. They reported three major findings: 1) heated mammalian cells maintain their ion gradients, and then either die within a few days without recovery of RNA or protein synthesis, or they recover macromolecular synthesis and then die as a result of aberrant divisions, 2) to obtain the same amount of killing or modification of the radiation response when cells are heated in the G_1 or S phase, the duration of heating must be decreased two-fold for a 1° increase in temperature between 43°C and 57°C (previously shown for temperatures between 43°C and 47°C), 3) cells can be induced to become resistant or thermally tolerant to heat effects that are either irreversible or slowly reversible and probably cause aggregation of proteins.

In order to determine whether hyperthermic temperature exposure radiosensitizes cells by inhibiting the repair of DNA dsb, investigators at the University of Utah studied the rejoining of radiation-induced DNA lesions at temperatures of 25°, at which less cytotoxicity occurs than at 37°C, and at 41°, where enhanced cytotoxicity occurs. DNA dsb rejoining was somewhat slower at 25°, and somewhat faster at 41°, than at 37°C. However, by 5 hr the same fraction of DNA dsb persisted in the cell's genomic DNA. Thus, they reported that inhibition of DNA dsb repair at hyperthermic temperatures does not account for the observed enhancement of radiation-induced cytotoxicity at hyperthermic temperatures.

Memorial Sloan-Kettering investigators studied the effect of 43° hyperthermia on radiation given at low dose rates (LDR) to provide a scientific basis for ongoing brachytherapy clinical studies at the University of California in San Francisco. To simulate clinical hyperthermia, a treatment of 43°C for 1 hr was given before or after the LDR radiation delivery, or in two fractions of o.5 hr each, before and after. Pre-heating and post-heating yield thermal enhancement ratios (TER) or 4.9 and 3.0 respectively. Two treatments of 0.5 h each, bracketing the LDR irradiation, give a TER or 4.3. The same heat dose, in conjunction with ADR irradiation, yields a TER or 2.9, independent of the sequence of treatment and the fractionation of heat dose. The results show that (1) hyperthermia enhances radioresponse at low dose rate more so than at acute dose rate, and (2) that pre-heating is better than post-heating for brachytherapy.

One of the major physiological factors which modify the effects of hyperthermia is blood flow. Several research groups have directed their attention to this important area. At the University of Minnesota, the heat-induced changes in blood flow in tumor and normal tissues is being

studied. Human skin blood flow was observed to increase as much as 20-fold upon heating at 42-43°C. In rats, the response of blood circulation to heat appears to vary considerably in different organs. It was found that the blood vessels in tumors and normal tissues become rather thermotolerant after heating. The implications of thermotolerance on the response of human tumors to fractionated heating are being investigated. It was also found that intracellular pH can be effectively reduced by ionopheres such as amiloride and nigericin and thus can markedly increase the thermal killing of tumor cells.

At Case Western Reserve, researchers are exploiting the fact that the average extracellular pH of tumors is lower than the normal physiological pH by using the pH differential between tumor and normal cells to selectively improve response of tumor cells to radiation and heat therapy. They have obtained evidence that agents which disrupt the ability of cells to maintain their intracellular pH gradient against an acidic environment are able to potentiate radiation and heat response in a pH-dependent manner. The K^+H^+ ionophore nigericin, when added to cells after a radiation dose of 9-10 Gy, prevents recovery from potentially lethal radiation damage when pH is 6.7 to 6.8, and potentiates radiation damage when pH is 6.6 or lower. Addition of non-toxic amounts of nigericin to cells undergoing heat treatment at 42°C for 10 to 30 minutes greatly increases the response to heat, reducing cell survival to less than 1% of controls under conditions where heat alone is only slightly toxic. Nigericin itself has limited potential for in vivo use. However, knowledge of the biochemical mechanisms by which nigericin potentiates radiation and heat damage under acidic environmental conditions will lead to development or identification of clinically useful response modifiers.

A glycoprotein (GP50) has been identified by investigators at the University of Utah who showed that GP50 synthesis is increased during thermotolerance development in a variety of mammalian cell lines and that it correlates with thermotolerance expression in a series of thermotolerance-deficient mutants. They characterized heat shock protein and GP50 synthesis under conditions of stepdown heating and showed that the enzymes involved in 0-linked, but not N-linked glycosylation, are also significantly increased prior to and during thermotolerance development. In separate experiments, they showed that microinjection of poly(A)$^+$RNA, isolated from thermotolerant cells conferred thermotolerance to recipient cells. This finding clears the way for the eventual identification of specific genes involved in the expression of thermotolerance and will be used to determine the relative capacity of heat shock proteins vs. glycoproteins, such as GP50, in mediating cellular heat resistance. Understanding the biochemical and genetic basis of thermotolerance should lead not only to improved applications of hyperthermia in cancer therapy, but also applications in other areas of stress physiology, e.g., agriculture, with the eventual production of more heat-resistant crops and livestock.

Researchers at the M.D. Anderson Cancer Center have shown that cell lines overexpressing calcium-binding proteins had reduced rates of synthesis of heat stress protein (HSP) 26. They loaded the parent cell line, which has normal levels of calcium-binding proteins, with the calcium chelators BAPTA or quin 2 and showed that rates of synthesis of hsp 26 are again reduced. These observations were consistent with their hypothesis that the signal controlling the rate of synthesis of the hsp 26 family is strongly affected by intracellular calcium. They have synthesized oligonucleotide probes they believe will hybridize to the hsp 26 mRNA and will attempt to determine if this altered synthesis rate is being regulated at the mRNA level.

Cellular survival patterns and the development of thermal tolerance after heat stress have been examined in cultured mammalian cells in relation to the uptake, exchange and procession of cellular lipids by researchers at the Oregon Health Sciences University. Studies using tracers such as radiolabeled cholesterol and fluorescent lipids in combination with flow cytometry and other techniques have shown that heat causes significant alterations in subcellular organelles such as the golgi apparatus which are involved in lipid processing and also that, at the time of

thermal tolerance development, there are appreciable changes in cellular lipid status. In bacterial systems, radiolabeling studies with amino acids have indicated that heat stresses result in a cellular response involving a translocation of newly synthesized proteins to the cell membrane. Further investigations using ^{31}P NMR analyses of membranes from bacterial auxotrophs grown under different regimes of fatty acid supplementation show alterations in signal at those temperatures at which this protein translocation occurs. Other NMR studies have also indicated an important role of pH in determining membrane lipid ordering which relates to bacterial survivals after hyperthermia.

Studies were conducted at the University of Kentucky on radiation and thermal sensitivity of murine fibrosarcoma (FSa-II) cells which recurred after radiation. Nine recurrent clones each were obtained by in vivo and in vitro clonings. Their response to radiation and hyperthermia was obtained by in vitro colony formation assays. Four in vivo recurrent clones were more sensitive and the other four were more resistant to radiation compared to original cells. Seven in vitro recurrent clones were more sensitive to radiation than the parent line and none of them were more resistant. Thermal sensitivity of both in vitro and in vivo recurrent clones widely varied. Nine clones were more resistant to heat (44°C) than the original parental cells while others showed identical or reduced sensitivity. Two common features of recurrent clones were low plating efficiency and prolonged doubling times.

The pO_2 profile along various points within the vascular bed of tumors growing in dorsal flap window chambers was studied at Duke University. These studies were performed using Whelan-style microelectrodes, placed immediately adjacent to microvessels, as visualized through the window under transmitted light, magnified @ 200x. It was found that a significant percentage of flowing vessels in the center of these tumors had pO_2's less than 5 mmHg. In fact, some even had pO_2's of 0mm. In addition, there was a very sharp declining gradient in pO_2 moving from the tumor periphery toward the center. These results have important implications regarding the uptake and metabolism of oxygen within tumors.

Hyperthermia instrument development has been focused on systems that have the potential for heating tumors at depth in the body. At this time, the technology is such that ultrasound devices appear most likely to achieve this goal. At the University of Illinois, novel ultrasound phased array applicators have been designed via extensive computer simulation and implemented in prototype form. A 32-array prototype constructed on a section of spherical focusing shell, called the sector-vortex array, was implemented and shown to provide field patterns remarkably similar to theoretical numerically simulated predictions. A 64-element spherical section array prototype was also designed and tested with experimental results agreeing remarkably well with theory. Electronic systems and an entirely new theory for computing the optimum phase distributions necessary to drive these arrays have also been designed and implemented. These new phased array applicators should give precise spatial and temporal control of energy deposition patterns for tumor heating at depth in the body.

At Dartmouth, two improvements have been made in an interstitial microwave array hyperthermia system air cooling and phase control to individual antennas. These developments permit fewer antenna implants to be used to heat larger tumor volumes, result in more homogeneous heating patterns, and provide for shaping of the temperature pattern to adjust for regions of high or low bloodflow. An improved microwave applicator has been developed for treating benign prostate hyperplasia.

A dynamic phantom for ultrasound hyperthermia has been developed at SRI International. A perfused ultrasound phantom with capability to scan temperature profiles in a 15 x 15 x 7.5 cm volume of tissue-mimicking gel was fabricated. The phantom incorporates 96 themocouples, mounted on a rigid carriage, which translates them within oil-filled tubules

embedded in the gel. Perfusing liquid, which can be blood-mimicking, can be circulated through 90 pairs of tubules oriented orthogonally to the above-mentioned tubules. Paired tubules permit unidirectional or countercurrent flow of perfusate for comparison with either assumption in a numerical model. A significant aspect of this phantom is that clinical ultrasound applicators can soon be evaluated under a variety of controllable conditions and assumptions concerning the state of perfusion of the phantom. Furthermore, comparison of spatial and temporal temperature profiles in the phantom with predictions of numerical models under the same initial conditions will permit the numerical models to be used with greater confidence under other initial conditions that may be impractical to simulate physically.

Small business firms are also interested in developing hyperthermia systems capable of heating deep tumors. Labthermics Technologies, Inc. is developing a novel ultrasound frequency modification to electronically focus a three dimensional ultrasonic beam. Criteria for specifying the path and dwell time of the beam focal region to produce a uniformly heated volume will be defined, and a strategy for the placement of temperature monitoring probes to act as control points will be developed. Enhanced methods for therapy planning using various imaging and temperature probe insertions and localization techniques will be investigated to provide the therapy preparation methodologies necessary for practical clinical implementation of the system. The commercial potential of the proposed work is based upon the demonstrated effectiveness of hyperthermia for cancer therapy, and the present lack of sophisticated heating systems capable of three-dimensional control of a heating beam deep within the body. The proposed system is designed to heat deeply-seated tumors while sparing intervening and surrounding normal tissue. Such devices are not at present commercially available, though their need in the clinical environment is well known. Instrumentation is also being developed to improve temperature measurement and thermal mapping that is necessary during clinical hyperthermia treatments.

Phoenix 14, a small business grantee, is exploring a new approach and system for lung cancer hyperthermia based on propagating focused ultrasound (US) through lung tissue which has been temporarily filled with non-toxic liquids, termed "perfluorocarbons" (PFC). These liquids provide the acoustic propagation medium required to transmit the ultrasound.. The procedure will be done in a manner which continually sustains patient respiration. In addition to improving lung cancer therapy, the techniques envisioned have the potential to benefit lung cancer diagnostic procedures. The commercialization of such a system, one with unprecedented efficacy and safety, is expected to find a wide and available market.

At the University of Arizona, hyperthermia by surgically placed ferromagnetic implants is undergoing a clinical trial. Forty patients have been treated with this modality. Half of these patients represent Grade III and Grade IV brain tumors. Mean survival is 12 months with three (2 AA, 1 GBM) now alive at two years from diagnosis. Glioma research is carried out in collaboration with the Barrows Neurological Institute, Phoenix. In addition, these investigators developed a new implant configuration which is far more efficient in absorbing power for a given diameter of afterloaded catheter. Newer, more efficient coils have been implemented. Strict, innovative quality assurance procedures based upon calorimetry have been implemented, and three additional institutions, UCSF, University of Wisconsin and City of Hope Medical Center will be clinically activated. These institutions will increase accrual of patients and help to shake out problems of disseminating this new technology

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT), as a potential treatment modality for solid malignancies, 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 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 with PDT.

Several new classes of photosensitizers have been synthesized and characterized spectrally. These classes include purpurins, phthalocyanines, bacteriochlorophylls, napthalocyanines, benzoporphrins, chlorins, the far red absorbing iso-BOSiNC and metal complexes of octabatoxphtholocyanine. All of these classes appear to be potent photogenerators of singlet oxygen. The pharmacokinetic studies of iso-BOSiNC conducted on normal and tumor-bearing rats and mice have shown ten times more iso-BOSiNC in tumorous than in normal tissue at 24 hours post injection. Phototherapy of the tumor-bearing animals resulted in cure rates exceeding 80 percent.

The success of phototherapy depends on getting the photosensitizers to the tumor tissue. One investigator has found the tumor localizing components of hematoporphrin derivative to be a series of porphyrin esters and ethers. Studies with pure ether dimers indicate that formation of this linkage results in a shift of dye hydrophobicity so that hydrophilic monomers become more resulting structures have the appropriate properties which lead to affinity for circulating plasma lipoproteins which, in turn, mediate tumor localization. This investigator also partially characterized three new classes of sensitizers: purpurins, benzoporphyrins and chlorins. The first two classes localizing as does HPD, but the chlorins bind mainly to plasma HDL and are representatives of a different localization mode. The use of specific drug delivery systems (Cremophor, cyclodextrins, liposomes) can modify the dye: lipoprotein affinity. Formulation of a sensitizer in Cremophor, for example, targets LDL for binding. It may, therefore, be feasible to change the affinity of dyes varying widely in hydrophobicity by varying the delivery system.

PDT predominantly targets either the tumor cells or the tumor vasculature. Investigators are exploring the basic cellular mechanisms associated with their destruction. One investigator demonstrated that PDT mediated by Photofrin II can induce members of several stress related genes. The glucose regulated protein (GRP-78), metallothionein, and heme oxygenase have all been documented to be activated following photodynamic therapy. In addition, the proto-oncogene, c-fos is also induced by photodynamic therapy. Subsequent studies have shown that the stress protein (GRP-78) can play a protective role against the cytotoxic actions of photodynamic therapy. Transfection studies are currently being performed in order to further evaluate this phenomenon.

Another investigator has shown that various tumor cells macrophages and endothelial cells respond to PDT in vitro by releasing eicosanoids, the major components of which have been identified as PGE_2 and 6-keto-$F_{1\ alpha}$. Their role in tumor following PDT is being studied further.

It was recently reported that hematoporphyrin derivative may exert some of its antitumor activity through inhibition of a form of mammalian DNA polymerase delta. Recent results demonstrate that the complement of mammalian DNA polymerases is more complex than originally thought. It was thought that there was one polymerase delta. Now, three polymerases delta, epsilon and epsilon star have been identified. Photo-crosslinking studies strongly indicate that each is a distinct enzyme with an active site subunit of characteristic molecular weight. Using inhibitors, the investigators are attempting to distinguish differences in their properties that could suggest their cellular roles.

RADIATION MODIFIERS: SENSITIZERS AND PROTECTORS

During the past year, both the pre-clinical and clinical areas associated with radiation modifiers continued to be active. The emphasis of the pre-clinical studies is on chemopotentiation by radiosensitizers. Clinically, the evaluation of etanidazole (SR-2508) in a Phase III trial is continuing.

Several studies have been investigating the potential of radiosensitizers as chemosensitizers. At the University of Wisconsin, investigators focused on the development of chemosensitizing agents. They synthesized mixed-function compounds consisting of a 2-nitroimidazole functionality covalently linked to a chloroethylating species resulted in compounds with preferential hypoxic toxicity. Structure-activity studies with these compounds has provided leads for the synthesis of the next generation of compounds which could have significantly improved anti-tumor selectivity. In collaboration with chemists at the University of Toronto, they have started to examine the molecular mechanism of chemosensitization by determining the biological properties of individual reductive metabolites of a model chemosensitizing 2-nitroimidazole. These experiments indicate that the one electron reduction intermediate (nitroso-) is a potent cytotoxic agent, reacts with and depletes cellular thiols, induces significant DNA damage and enhances the toxicity of the chemotherapeutic drug, melphalan. Further studies along these lines should help elucidate the active intermediates responsible for the biological effects of reduced nitroimidazole sensitizers.

Wake Forest University researchers reported that when BCNU was administered at various times after unclamping misonidazole containing s.c.9L tumors, it took 20-30 min before chemopotentiation was completely eliminated. This data supported the idea that hypoxic metabolism of the 2-nitroimidazole continues after re-oxygenation. When misonidazole was administered and the tumor clamped 5-120 min prior to administering BCNU, a small amount of potentiation occurred after 5 min of clamping. This same amount of potentiation was obtained until the tumor had been clamped for 60-90 min after which a tremendous increase in the potentiation of the cell kill occurred. These data suggest that misonidazole must be metabolized under hypoxic conditions for a prolonged period of time before the maximum chemopotentiation can be obtained. The scheduling of the two agents in a clinical chemopotentiation trial appears to be critical, if the maximum potentiation is to be obtained.

At Dartmouth College, investigators have developed a new approach for delivering high local doses of cisplatin to tumors using a biodegradable open-cell polylactic acid matrix. This delivery system has been evaluated in dogs undergoing limb sparing therapy and dogs with transplantable murine tumors. In the latter model, the higher levels of cisplatin measured in the tumors without increased normal tissue toxicity resulted in therapeutic potentiation when combined with radiation therapy superior to that attainable using systemic administration of cisplatin. Both radiosensitization of hypoxic cells and radiopotentiation, by carboplatin pre- and post-irradiation, respectively, correlated with an increased production of DNA single strand breaks as measured using alkaline elution and DNA unwinding (FADU). However, there was no evidence for increased production of DNA double strand breaks in these protocols using neutral elution.

Harvard Medical School researchers have conducted studies using etanidazole to sensitize cell killing by melphalan. They have demonstrated that intermittent hypoxia alone is sufficient to produce chemomodification, a process felt to require continuous hypoxia. However, the type of hypoxia that is relevant to clinical tumor resistance remains to be defined. These findings

indicate that chronic hypoxia may not be necessary to produce the biochemical effects ascribed to it. Rather, intermittent hypoxia can cause these effects.

Yale University investigators are examining the ability of perfluorochemical emulsions to modulate tumor oxygenation. In one series of experiments, BA 1112 tumors in rats were irradiated locally with brachytherapy while a single, clinically-usable dose (15 ml/kg) of a clinically-approved perfluorochemical emulsion (Fluosol-DA, 20%) and carbogen was administrated throughout irradiation. The radiation plus radiosensitizer produced a significant increase in tumor cell kill than did radiation alone. These studies provide a firm scientific basis for recommending clinical trials testing Fluosol-DA as an adjunct to brachytherapy in the treatment of cancer.

At the Cleveland Clinic Foundation, the radiosensitization of RIF-1 tumors by Fluosol-DA (20%) and carbogen was studied by ^{31}P and ^{19}F nuclear magnetic resonance spectroscopy (MRS). Of 60 C3H mice implanted s.c. with RIF-1 tumors, one-third were injected i.v. with Fluosol-DA (20%) under a carbogen atmosphere and an hour later irradiated with a single x-ray dose of 40 Gy. Another third were just put in a carbogen atmosphere for an hour and then irradiated. The final third were controls with no carbogen before or during irradiation. ^{31}P MR spectra were acquired shortly before and 2, 12, 24, 48, 72, 120, 168, and 216 h after irradiation. As monitored by ^{19}F MRS, Fluosol reached equilibrium level in tumors within 5 min after i.v. injection and remained in the tumors for more than 2 weeks. Each treatment group showed a reduction in ß-nucleoside triphosphate to P_i ratio (referred to as ATP/P_i ratio) at 2 and 12 h after irradiation and subsequent increases in ATP/P_i ratio and MRS-measured pH. The time development of neither these nor alternative ^{31}P MRS indices distinguished tumors with different tumor growth delays (TGD). Neither TGD nor MRS detected significant therapeutic improvement in mice pre-treated with Fluosol-DA(20%) and carbogen. However, a drop in the ATP/P_i ratio at 24 hours to less than 25% of the initial ATP/P_i value, is a good predictor of drastic tumor mass loss (50% or more reduction) within the first week (sensitivity of this test is 88% and its specificity is 92%).

The major highlight of this past year is the demonstration at Stanford University that the newly discovered bioreductive agent, SR 4233 (3-amino-1, 2, 4-benzotriazine 1,4-dioxide), which has a highly selective toxicity for hypoxic cells, is also an extremely effective selective radiosensitizer of a variety of mouse tumors in protocols with fractionated radiations similar to those used clinically. SR-4233 sensitizers both hypoxic and aerobic cells if they are exposed to the drug under hypoxic conditions either before or after irradiation. The drug has to be activated by the fluctuating hypoxia in the tumors. No radiosensitization of normal tissues is seen resulting in therapeutic gain factors of ~2.0 for these fractionated protocols. As the drug doses are highly tolerated (even giving 5 x/week for 6 weeks), this drug appears to be ideal for clinical trials with radiotherapy. Further development of this agent will be done by Sterling Drug, Inc. the pharmaceutical company who recently licensed this agent.

At Harvard University, investigators have reported that in their Phase I trial of continuous infusion etanidazole, there have been some marked changes in brain tumors following brachytherapy plus sensitizer. These changes need to be evaluated prospectively. In their Phase II trial for patients with locally advanced prostate cancer, the complete response rate at 3 months post irradiation is 70%, about three times higher than historical control Both studies need to have these early results documented.

RADIOLABELED ANTIBODY DIAGNOSIS AND THERAPY

Radiolabeled monoclonal and polyclonal antibodies and their fragments directed against tumor cell surface antigens have shown promise as both diagnostic and therapeutic agents in-vitro and in human tumor implants in animals. These observations have led to research in supporting this technology that is only now receiving significant federal funding. There are at least 4 foci of interest in this NCI research area; Radiation Research Program(RRP), Radiation Oncology Branch(ROB) and Biological Response Modifiers Program(BRMP) in Division of Cancer Treatment(DCT), and the Cancer Immunology Branch(CIB), Division of Cancer Biology and Detection(DCBD). Research is ongoing in the development of radionuclides for imaging and therapy. For imaging tumors, a low energy gamma emitter of about 150 KeV is optimum. For therapy a medium energy beta or high energy alpha emitter is necessary to deposit the energy to kill tumor cells. Investigations are studying the chemistry of linking the radionuclides and antibodies for greatest stability in in vivo.

Clinical trials are being carried out at a small number of research centers. The Radiation Therapy Oncology Group (RTOG) is conducting a Phase I/II study in the treatment of Hodgkin's disease with Yttrium-90 labeled antiferritin IgG. A Phase III trial of antiferritin IgG labeled with Iodine-131 to treat malignant hepatoma is also being performed.

RDB is supporting of a Dosimetry Center, through RTOG, at Johns-Hopkins University. The Center is training other RTOG members in the computerized calculations of tumor volume dosimetry. These dosimetric calculations will be useful in evaluating the effects of any type of cancer therapy on neoplastic masses.

As more is understood about the inhomogeneous distribution of radiolabeled antibodies, it is apparent more research is needed on dosimetry. Two investigator-initiated grants are currently funded in this area and two additional grants were funded in FY89 in response to an RFA.

Strong central coordination of all of these research areas is necessary to thoroughly explore radiolabeled ligands/conjugates for the diagnosis and therapy of cancer.

RADIOTHERAPY TREATMENT PLANNING

Three institutions are funded as a Collaborative Working Group to develop portable and transportable software to attack the time-consuming and labor-intensive tasks of 3-dimensional radiation therapy treatment planning. The first year was devoted to identifying resources in other scientific disciplines that may have applicability to the specific tasks to be addressed and criteria for evaluating their usefulness to radiotherapy problems. New software and documentation standards are emerging from the group which will yield software tools that can be exchanged and used at different institutions, irrespective of the hardware, operating system and computer architecture of the different facilities. These contributions will have important consequences for the future development of sophisticated software that can then be adapted to community-based health care centers. Working Group investigators and scientists from the NASA space agency have met to explore the possibility of a technology transfer so that image management tools developed for NASA can be applied to the radiotherapy community medical imaging problems. It is likely that some of the space-agency software can be adapted to the image-processing problems of the radiological sciences.

PATTERNS OF CARE

Evaluation of treatment outcome from patients treated with radiation therapy for breast, cervix, prostate, recto-sigmoid cancer and Hodgkin's disease will yield important data on how the type, kind and location of cancer treatment facilities can affect the patients' treatment outcome and survival. First, 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 how type of facility, i.e., academic institution or satellite facility, will be used to determine how these factors influence the survival and complication rates of radiotherapy patients. Previous Patterns of Care Studies have been carried out on patients treated in 1973, 1978 and 1983 and yielded important information about how variables such as these affect survival and outcome. The current study will explore these issues with more recently treated patients, as well as study the effect of how different patterns of fractionation, or the dose administration as a function of time, affect patient outcome. Results of the effort will then be communicated to the radiotherapy community through newsletters, education symposia and professional meetings to improve the quality of patient care in radiotherapy.

RADIATION BIOLOGY

The NCI, primarily through the Radiotherapy Development Branch, RRP, DCT, continues to support a major portion of radiation biology research in the United States. This research is dedicated to improving radiation therapy as a treatment modality. Tumor and normal tissue radiobiology at the molecular, cellular and animal levels continues to be vigorously researched.

The following examples illustrate the breadth and diversity of this program.

(1) Investigations are underway at UCLA to monitor changes in the concentration of myelin basic protein (MBP) and 2'3-cyclic nucleotide 3'-phosphohydralose (CNPase) in the spinal cord and cerebrospinal fluid of irradiated guinea pigs to ascertain whether changes in these biochemicals are related to the onset of progressive radiation-induced myelopathy (paralysis). The tentative conclusion is that progression and perhaps prediction of radiation myelopathy may be monitored by immunohistochemical methods.

(2) Recently, at the University of Chicago, it was discovered that a potentially mutagenic cytokine is transcriptionally induced by x-rays and may contribute to cancer induction. Tumor necrosis factor-alpha mRNA was found to be increased after treatment with x-rays in certain human sarcoma cells. This is accompanied by the increased production of TNF-alpha protein. This, in turn, enhances radiation lethality in both TNF-alpha-producing and non-producing tumor cells. These data suggest that, in addition to the direct cytotoxic effects of x-rays, production of TNF-alpha may add to radiation lethality through autocrine and paracrine mechanisms.

(3) In research at the University of Utah, it has been found that DNA double strand break (dsb) induction occurs, in hamster cells, as an exponential (single hit) function of radiation dose. Up to 90% of DNA dsb induction is attributable to radical (presumably hydroxyl radical) attack on the DNA fiber. While highly condensed chromatin structure very successfully protects DNA from radical attack, DNA dsb induction is equivalent in "naked" DNA and DNA containing nucleosomes as relaxed chromatin. Thus, the presence of histone proteins on the DNA fiber, per se, does not protect DNA from dsb induction.

(4) The use of ^{31}P-magnetic resonance spectroscopy (MRS) offers considerable hope as a predictive indicator of cancer treatment outcome with ionizing radiation. At the Cleveland Clinic, it has been shown that serial, long-term ^{31}P-MRS monitoring of patients with a variety of tumor types suggests that tumors which respond and achieve local control show an early decrease in either PME/ATP-beta or PDE/ATP-beta ratios, accompanied by minor changes in the high-energy metabolites. On the other hand, many non-responding tumors demonstrated either approximately constant PME and PDE to ATP-beta ratios on an increase in these ratios. These trends appear non-specific to tumor histologies and sites. Similar work is ongoing at Massachusetts General Hospital and the University of California (San Francisco). It is important to realize that this MRS testing is non-invasive.

(5) A major problem with using radiolabeled antibodies for cancer therapy is the low level of radioactivity deposited in the tumor. Various groups including ones at Duke University and the University of Pittsburgh are attempting to improve the chemistry of radionuclide attachment. The Duke group was the first to report labeling on antibody fragment with astatine-211 (^{211}At), which emits densely damaging alpha particles. The antibody fragment retained its immunoreactivity and tumor localizing capacity. The Pittsburgh group is endeavoring to utilize the Auger effect of ^{125}I on the alpha emissions of ^{211}At coupled to steroid hormones or peptide hormones to target the therapeutic effects of radioisotopes to receptor enriched tumor cells.

(6) Slow but steady progress continues in the search for suitable compounds for boron neutron capture therapy (BNCT). At the University of California(San Francisco) research is underway to utilize low-density lipoproteins (LDL) to transport and microinject boron compounds into cancer cells. Uptake and biological efficacy studies in vitro are consistent with receptor-mediated endocytosis of the boronated LDL, and boron remains firmly bound in (cell culture) despite repeated washing and suspension in boron-free medium. The boron distribution is intracellular, with a biological efficacy indicative of a cytoplasmic location. At Brookhaven National Laboratory, investigations continue into the use of the boron-containing amino acid analog, p-boronophenylalanine (BPA). Though originally envisioned as a melanoma-specific boron delivery agent, BPA has been shown to selectively deliver therapeutically useful amounts of boron to tumors other than melanoma, specifically a murine mammary tumor and rat glioma. Thus BPA may have broad utility as a boron delivery agent for BNCT

SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

In FY 1990, the RDB funded 1 SBIR contract (Phase II) and 14 SBIR grants (4 Phase I, and 10 Phase II). Funded research areas included photodynamic therapy, fast neutron therapy, boron neutron capture therapy, photon therapy, and ultrasonic hyperthermia, expert systems for Radiation Oncology real time portal scanning for Radiation Oncology, variable collimation, microdosimetry, laser interstitial therapy and beta dosimetry.

WORKSHOPS

Radiation Resistance - September 1990

This workshop scheduled for the second half of September 1990 will convene a panel of experts to address the simple yet profound question of why there is such a variation in response to radiation at the cellular, sub-cellular, and molecular biological level. It is expected that recent

advances in molecular biology can shed considerable light on this area. It is anticipated that one or more RFA's may result.

Normal Tissue Tolerance - September 1990

Scheduled for September 5-7, 1990, this workshop will address the issue of documentation of normal tissue response to radiation. Current data is sketchy, fragmented and not collected in a standardized manner for use in treatment planning optimization algorithms which select optimal therapy on the basis of minimizing complications to normal organs and tissues. Comprehensive data on partial and whole volume irradiation of critical organs and structures is needed. One or more RFA's is anticipated.

FUTURE DIRECTIONS (RDB)

The Radiotherapy Development Branch will continue to stimulate, develop and administer clinical research and basic science research in radiation biology, chemistry and physics and support the development of advanced computer-based tools that improve the treatment planning and delivery of radiation therapy. The particle radiation therapy program, using both charged and uncharged particles, will continue to be a high priority research area for the near future.

The use of Hyperthermia as a treatment modality continues to interest the medical community. However, further research needs to be performed in the development of deep heating units and of non-invasive thermometry. Hyperthermia as an effective adjunct to radiation and chemotherapy needs to be confirmed by standardized, randomized clinical trials for specific disease processes and anatomic sites.

Photodynamic therapy (PDT) as a treatment modality is less well developed than the traditional discipline of radiation oncology but because of promise in this area further research efforts will be stimulated. The present chemical compounds used for light-stimulated radiation treatments probably are not the optimal drugs for PDT. Newer photosensitizing drugs will require clinical testing. Further development of light producing lasers and light delivery systems will be encouraged.

Further research and development of radiolabeled immunoconjugates and cell specific receptors, a form of systemic radiation therapy (SRT), is necessary to explore the possibility of cellular radiotherapy. The dosimetry of these radionuclide tagged compounds is an important research area of this rapidly developing therapeutic approach and requires further support. Radiolabeled immunoconjugates for therapy and diagnosis will continue to be a high priority research area of the Radiation Research Program.

As research advances unfold in the chemistry and biology of boron containing compounds which preferentially concentrate in tumors, the interest in boron neutron capture therapy (BNCT) should increase. Irradiation of a boron compound with low energy neutrons causes emission of a short range alpha particle, which deposits an intense radiation dose at the cellular level. This is an additional example of possible cellular radiotherapy. The RDB anticipates an increasing role in this research area.

Radiosensitizers have demonstrated an ability to increase the sensitivity of neoplastic tissue to radiation and attempts will continue to improve the efficacy of these agents and to decrease their toxicity. Many of these radiosensitizers also have chemosensitizing activity. New compounds need to be developed. This development is dependent on the capability to screen a large number

of compounds for radiosensitizing activity. A more rapid screening system with greater capacity has received approval and will be activated in the coming year..

Medical informatics is a new and emerging medical discipline concerned with the development of knowledge-based systems that are used to develop decision support tools, data management systems and physician workstation environments that increase the efficiency and personal productivity of the radiation oncologist. The Patient Data Query (PDQ) system developed and supported by the NCI is a first step in creating systems that can take advantage of new technological applications that are available through the use of medical informatics techniques. Decision systems are needed to 1) support physician evaluation in selecting optimal therapy; 2) to follow patients placed on protocol to assure that clinical trials are conducted in an efficient and cost-effective manner; 3) to provide connections to clinical databases and laboratory test results used in the patient evaluation and diagnostic processes; and 4) tutorial expert systems for resident teaching and for continuing medical education in radiation oncology. It is essential that the physician be involved in the development and implementation of physician workstations to make certain that the scientific and clinical data is handled in a manner that enhances and improves patient care, but at the same time, supports the physician efficiency in the busy clinical environment.

Research supported by NCI over the last decade has shown that the development of sophisticated computer-based treatment planning tools is essential to the routine use of three-dimensional planning and treatment in the clinic. New computer tools are needed that 1) support the management of medical images through computer networks, 2) develop decision-support aids for the radiotherapist in the therapy selection, tumor definition and treatment planning processes; and 3) interface the computerized medical record with intelligent databases. Radiotherapy is the most computer-intensive discipline in medicine, primarily because of the anatomic information required to define the tumor and treatment volume, and the calculations that are needed to characterize the radiation dose to the tumor and the normal tissues at risk. Development of new computer-based systems that support the physician in all aspects of radiation therapy planning and delivery will result in better care and management for the cancer patient.

Dynamic conformal radiotherapy using conventional photon and/or electron beam accelerators is a new and exciting research area in which complex treatment plans are developed and implemented that conform precisely to the tumor treatment region, resulting in greater sparing of normal tissues. These developments will require advances in three-dimensional treatment planning, robotic vision techniques, expert knowledge systems and digital imaging verification systems. Technology transfer from the artificial intelligence community and medical informatics will greatly assist in this effort.

☆U.S. GOVERNMENT PRINTING OFFICE: 1991-288-230