Division of

Cancer Treatment

1988 Annual Report
Intramural Activities
Volume I

October 1, 1987
September 30, 1988

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

National
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National
Cancer
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Bethesda,
Maryland 20892

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1987 through September 30, 1988

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

October 1, 1987 through September 30, 1988

The Division of Cancer Treatment (DCT) is the organizational component of the National Cancer Institute (NCI) that is responsible for the identification, development and evaluation of new therapies aimed at the control and cure of cancer. The Division has five major components: Developmental Therapeutics Program (DTP), Cancer Therapy Evaluation Program (CTEP), Radiation Research Program (RRP), Clinical Oncology Program (COP), and the Biologic Response Modifiers Program (BRMP). The research is conducted in intramural laboratories and clinics as well as through grant-, contract-, and cooperative agreement-supported projects throughout the United States and the world. The major emphasis in research efforts of the DCT encompasses chemotherapy, surgery, radiation therapy, immunotherapy, biologic response modifiers, and hyperthermia, used individually and in combination. The conduct of this research follows a stepwise progression and begins in the evaluation of antitumor activity in preclinical tumor systems. Once identified as possessing antitumor activity, the next step is to test and evaluate the safety of the new agent or method of treatment in animals. If the new agent or treatment has an acceptable therapeutic index (i.e., margin of safety between antitumor dose and toxic dose), an investigational new drug (IND) application is submitted to the Food and Drug Administration (FDA). This allows human trials to begin and proceed from Phase I toxicity testing to Phase II antitumor testing and finally to Phase III comparison with existing agents or treatments.

The DCT functions under the scientific and administrative auspices of the Board of Scientific Counselors (Table I). The Board is composed of leading scientists representing the fields of surgical, medical and radiation oncology, hematology, molecular biology and genetics, immunology, and pharmacology. These represent the areas of clinical and basic science particularly relevant to the research mission of the Division. The Division Director relies on the Board of Scientific Counselors for advice on scientific, administrative and fiscal management of the Division. The Board's counsel ensures that the resources allocated to the DCT are utilized in the most optimal fashion.

PERSONNEL AND ORGANIZATION

The DCT is operationally divided into five major components of treatment program priorities. Each program is headed by an Associate Director who is responsible for the overall direction of the science within that program. Personnel changes that have occurred during the past year include the following:

A. Office of the Director (OD)

- Dr. Gregory Curt, Deputy Director, DCT, left to become Director of Medical Education and Chief of Clinical Pharmacology at Roger Williams Hospital, Brown University, in Providence, RI.

- Dr. Marcia Browne, Special Assistant for Clinical Science, left to become Director of Clinical Research, Roger Williams Cancer Ctr., Brown University, in Providence, RI.

TABLE I. DCT BOARD OF SCIENTIFIC COUNSELORS

Name	Affiliation	Term of Appointment
John E. Neiderhuber, M.D. (Chairman)	Johns Hopkins University School of Medicine	1986-1990
Charles M. Balch, M.D.	M.D. Anderson Hospital	1987-1991
Yung-Chi Cheng, Ph.D.	University of North Carolina	1986-1990
James D. Cox, M.D.	M.D. Anderson Cancer Center	1987-1991
Lawrence H. Einhorn, M.D.	Indiana University Medical Ctr.	1985-1989
Emil Frei, III, M.D.	Dana-Farber Cancer Institute	1986-1990
Mark T. Groudine, M.D., Ph.D.	Fred Hutchinson Cancer Res. Ctr.	1986-1990
William R. Hendee, Ph.D.	American Medical Association	1987-1990
Susan B. Horwitz, Ph.D.	Albert Einstein College of Med.	1987-1990
Robert C. Jackson, Ph.D.	Warner-Lambert/Parke Davis	1986-1988
John H. Kersey, M.D.	University of Minnesota Hospitals	1985-1988
John Mendelsohn, M.D.	Memorial Sloan-Kettering Cancer Center	1986-1990
Charles E. Putman, M.D.	Duke University Medical Center	1986-1989
Ralph A. Reisfeld, Ph.D.	Res. Institute of Scripps Clinic	1987-1988
Geraldine Schechter, M.D.	VA Medical Center, Washington, DC	1985-1989
Robert T. Schimke, M.D.	Stanford University	1986-1989
H. Rodney Withers, M.D., D.Sc.	UCLA Center for Health Sciences	1986-1989

- Dr. Eddie Reed, Special Assistant for Preclinical Science, transferred from the OD to the Clinical Pharmacology Branch, COP.

- Ms. Dorothy Tisevich, Deputy Administrative Officer, left to become NIH Analyst, Budget Office, OS, in the Department of Health and Human Services.

- Dr. Mace Rothenberg transferred from the Medicine Branch, COP, to become Special Assistant for Clinical Science, OD.

- Dr. Wyndham Wilson transferred from the Medicine Branch, COP, to become Special Assistant for Preclinical Science, OD.

- Ms. Kathy Russell joined the Administrative Office, OD, as Deputy Administrative Officer; she was formerly the Administrative Officer, COP.

B. Biological Response Modifiers Program (BRMP)

- Dr. Jeffrey Clark resigned as Medical Officer, Clinical Research Branch, to go to Roger Williams Hospital, Brown University.

- Dr. Toby Hecht was recruited as a Microbiologist in the Biological Resources Branch; she was formerly at the University of Maryland.

- Dr. Craig Reynolds transferred from the Laboratory of Experimental Immunology to the Biological Resources Branch.

- Dr. Carl Pinsky resigned as Chief Medical Officer for Extramural Research from the Biological Resources Branch to go to Imre Corp. in New York City.

C. Cancer Therapy Evaluation Program (CTEP)

- Dr. Daniel Hoth, Chief of the Investigational Drug Branch, transferred to the National Institute of Allergy and Infectious Diseases (NIAID) as the Director of the AIDS Program.

- Dr. Susan Ellenberg, Biometrics Research Branch, transferred to the AIDS Program, NIAID, as Chief of their Biometrics Branch.

- Dr. Timothy Moore was recruited for a Senior Investigator position in the Clinical Investigations Branch following a three-year oncology fellowship at the Ohio State University Cancer Center.

- Dr. Paul Hiranaka was recruited from the Food and Drug Administration (FDA) for the AIDS vacancy in the Drug Management and Authorization Section of the Investigational Drug Branch.

D. Clinical Oncology Program (COP)

- Dr. Alfred Chang left the Surgery Branch to become the Chief of Surgical Oncology at the University of Michigan.

- Dr. Robert Young, Chief of the Medicine Branch, left DCT to become Associate Director, Cancer Centers and Community Oncology, for the Division of Cancer Prevention and Control.

- Dr. Jerry Collins left the Clinical Pharmacology Branch to become a member of the staff of the Food and Drug Administration.

- Dr. Daniel Ihde, Deputy Branch Chief, NCI-Navy Medicical Oncology Branch, was also appointed head of the Division of Hematology/Oncology for the Uniformed Services University of the Health Sciences (USUHS).

- Dr. Herb Holmes has become the new Deputy Branch Chief for Operations, NCI-Navy Medical Oncology Branch.

- Dr. Edward Sausville left the NCI-Navy Medicical Oncology Branch to become Associate Professor of Medicine at Georgetown University's Lombardi Cancer Center.

- Dr. James Battey transferred from the NCI-Navy Medical Oncology Branch to the National Institute of Neurological and Communicative Disorders and Stroke.

- Dr. Michael Birrer, Dr. Frederick Kaye, and Dr. Francine Foss left NCI-Navy Medical Oncology Branch to become Assistant Professors of Medicine at the USUHS.

- Dr. Karina Butler joined the staff of the Pediatric Branch as a Senior Staff Fellow; she comes to DCT from the National University of Ireland, Dublin.

E. Developmental Therapeutics Program (DTP)

- Dr. Susan Friedman was recruited to the Laboratory of Medicinal Chemistry from the University of Calgary.

- Dr. Philip Skehan was recruited into the Program Development Research Group from the University of Calgary.

- Dr. John Bader transferred to the Office of the Associate Director, DTP, from the Division of Cancer Etiology.

- Dr. John Cardellina was recruited into the Laboratory of Medicinal Chemistry from Montana State University.

F. Radiation Research Program (RRP)

- Ms. Wendy Fredericks was selected to the Biologist/Physical Scientist position in support of all RRP grants, cooperative groups and contracts; she comes to DCT from the National Institute on Aging.

- Dr. Francis Ruzicka resigned from Federal service in the spring of 1988; Dr. Matti Al-Aish is serving as Acting Chief, Diagnostic Imaging Res. Branch.

PROGRAM REVIEWS

The Division has five scientific programs that are described in detail in their individual sections of this Annual Report. Program highlights are listed below:

Clinical Oncology Program (Associate Director, Samuel Broder)

The Clinical Oncology Program (COP) is the intramural treatment research arm of the National Cancer Institute. The Program conducts basic and clinical research in medicine, surgery, pediatrics, radiotherapy and radiobiology, pharmacology, immunology, genetics and endocrinology in the context of developing curative therapies for cancer. The COP is divided into the Medicine Branch (Chief, Dr. Charles E. Myers), NCI-Navy Medical Oncology Branch (Chief, Dr. John Minna), Surgery Branch (Chief, Dr. Steven A. Rosenberg), Pediatrics Branch (Chief, Dr. Philip Pizzo), and Radiation Oncology Branch (Chief, Dr. Eli Glatstein). A laboratory under the supervision of Dr. Broder operates under the Office of the Associate Director, and is directed at identifying new treatments for AIDS. This office also supports a Biostatistics and Data Management Section, headed by Dr. Seth Steinberg.

Medicine Branch

- After years of laboratory research, the effort to reverse multidrug resistance (resistance to a wide variety of functionally and structurally dissimilar drugs) has moved from the laboratory into the clinic. Two trials have begun that examine the effect of combining a drug (amiodarone or quinidine) known to block the P-170 pump responsible for multidrug resistance with combination chemotherapy in the treatment of colon, adrenal and kidney cancers, tumors known to have high levels of P-170 expression.

- There has been substantial progress in the development of anti-sense oligonucleotides--molecules possessing complementary sequences to specific genetic messages

(mRNAs) in cancer cells. By binding to messages highly expressed in cancer cells, these molecules can selectively inhibit malignant cells and block processes essential for tumor growth. These molecules can be combined with other compounds that could kill those cells while leaving normal cells unharmed.

- Clinical trials with suramin have identified significant antitumor activity against lymphomas and adrenocortical and kidney carcinomas. Laboratory investigation has shown that suramin binds to and inactivates basic fibroblast growth factor (FGF). This is the first drug to be identified as possibly acting through antigrowth factor mechanisms. In conjunction with our increasing understanding of the roles growth factors play in the initiation and progression of cancer, suramin analogs and other growth factor inhibitors are being developed and tested for antitumor activity. This may open the way for a whole new approach to cancer therapy.

- Immunotoxins, molecules that combine monoclonal antibodies with toxin effector moieties for more efficient cell killing, have moved from the laboratory into clinical trial this past year. OVB-3, a monoclonal antibody that binds to virtually all ovarian cancer cells linked to Pseudomonas exotoxin, is being administered intraperitoneally to women with ovarian cancer limited to the peritoneal cavity. This trial has begun accruing patients.

- Results from clinical trials conducted in the Medicine Branch include MB-198, a multimodality therapy for advanced breast cancer that has yielded a complete remission rate of 49 percent and a partial remission rate of 46 percent for women with inflammatory or locally advanced non-inflammatory Stage III breast cancer; numbers that compare quite favorably with existing therapies in this group of patients. A particularly aggressive form of breast cancer, inflammatory breast cancer, appears to be very responsive to this treatment approach. MB-161, a randomized comparison between two combination chemotherapeutic regimens for non-Hodgkin's lymphomas, suggests that the PROMACE-Cytobom combination is more effective than the PROMACE-MOPP combination. MB-133, comparing radiotherapy to chemotherapy for patients with early-stage Hodgkin's disease, indicates an advantage for chemotherapy in disease-free survival, and possibly in overall survival.

- Anti-sense oligonucleotides have been directed at HIV-infected cells, making it potentially useful in the treatment of AIDS. The most promising group of anti-sense compounds in inhibiting HIV is the phosphorothioate oligonucleotides. Although taken up by cells at a slower rate than other kinds of oligonucleotides, they are easier to prepare, and once they are taken up by cells they form strong bonds with complementary mRNAs and are degraded slowly. Phosphorothioate oligonucleotides complementary to the art/trs genes of HIV inhibited the cytopathic effect of the virus by 95 percent. Surprisingly, homopolymers (dA, dC, or dT) also appear to be quite effective at inhibiting HIV transcription. This technology is in the preclinical development stage.

- Further development of dideoxynucleosides and other anti-retroviral compounds useful in the fight against AIDS has continued in Dr. Broder's laboratory and has led to clinical trials at the Clinical Center, NIH. Dideoxycytidine (ddC) is now being given on an alternating schedule with AZT in an attempt to maintain the anti-retroviral effect while reducing the toxicity associated with a regimen of either agent alone. Some patients have been treated with this regimen for over a year without significant toxicities. This pilot study has led to the initiation of a large-scale multicenter study of this combination. Dideoxyadenosine

(ddA) has begun early clinical trial, appears to be quite active in reducing viral protein levels in plasma and in increasing T-cell numbers, and may have the least toxicity of any of the anti-retroviral nucleosides studied. Laboratory efforts have been undertaken to examine the activity of other classes of compounds, and include studies of the anti-sense oligonucleotides mentioned above, acyclic nucleotides, and epoxy analogs.

NCI-Navy Medical Oncology Branch

- A gene known to be missing in a childhood malignancy, retinoblastoma, appears to be missing or present in very reduced levels in 80 percent of patients with small-cell lung cancer or pulmonary carcinoids. Implications of this are that the missing genes possess tumor-suppressing property, the loss of which may be functionally related to the development of certain malignancies.

- Tumors that appear identical to small-cell lung cancer but arise from extrapulmonary sites lack a certain chromosomal deletion ($3p^-$) present on most pulmonary small-cell cancers. This suggests that a different pathogenic process may be responsible for these extrapulmonary small-cell tumors.

- Products of the L-myc oncogene have been identified as short nuclear phosphoroproteins that can transform normal rat embryo cells and nonmalignant cells into malignant cells.

- Gastrin-releasing peptide (GRP) and GRP-associated peptides have been found to have receptors on lung cancer cells and stimulate the growth of these cells _in vitro_. Along with insulin-like growth factor-I, which also stimulates growth of lung cancer cells in the laboratory, GRP and GRP-associated peptides may provide targets for anti-growth factor therapies.

- The intracellular events triggered by GRP have been identified, and involve increases in intracellular calcium and turnover of phosphatidyl inositol. The better understanding of these intracellular events may provide additional targets of antitumor therapy. Cholera toxin and some calcium channel blockers appear to inhibit growth of small-cell lung cancer by disrupting one or another of these pathways.

Surgery Branch

- Work in the field of adoptive immunotherapy has expanded in the Branch and has included the development of tumor infiltrating lymphocytes (TIL) and interleukin-2 (IL-2). When given in conjuction with cyclophosphamide, this treatment approach is capable of curing mice with advanced pulmonary and hepatic tumors not susceptible to other forms of immunotherapy. Pilot clinical trials in patients with advanced malignant melanoma have yielded promising results, with 9 of 14 patients responding to therapy.

- Research into the field of lymphokines has yielded combinations of these peptides that are synergistic in their antitumor activity. The most promising combinations include IL-2 and alpha-interferon, and IL-2 plus tumor necrosis factor. Clinical trials of these combinations have begun in patients with advanced malignancies, and the combination of IL-2 and IFN-α has produced responses in 7 of 15 patients with renal cell carcinoma.

- A new gene has been inserted into the nucleus of TIL cells by a technique called "gene transfection," and is used to make these cells more effective tumor-killing cells. Experiments are being planned to see if this technique is safe and effective in the treatment of cancer patients. This would be the first attempt at medical application of gene transfer in the therapy of human disease.

Pediatric Branch

- Researchers have been examining the patterns of gene rearrangements in acute lymphoblastic leukemia and neuroblastoma as a way of understanding the derangements of normal cell development that lead to neoplasia. Cell lines and tumors have shown distinctive patterns of gene expression suggesting that malignancies may correspond to arrests in cellular maturation. Tumors that appear identical under the microscope have often shown different patterns of molecular markers, indicating that they represent derangements of different stages of cellular development. This observation has been helpful in designing more effective therapies based on molecular markers of differentiation.

- Identifying better ways to treat patients with leukemia that has spread to the CNS ("meningeal leukemia") has been the focus of several laboratory and clinical investigations. From this research, active agents such as 4-hydroperoxycyclophosphamide (a preactivated analog of cyclophosphamide) and immunotoxins (antibodies linked to peptide toxins) have been identified and show promise in laboratory experiments when injected directly into the cerebrospinal fluid (i.e., intrathecal administration). Clinical trials have been conducted with new chemotherapeutic agents, such as diaziquone (AZQ), and with older compounds given in novel ways, such as intrathecal 6-mercaptopurine and intravenous thiotepa.

- The combination of high-dose methotrexate infusion and CHOP chemotherapy has proven very effective in the treatment of high-grade lymphomas (Burkitt's, non-Burkitt's, undifferentiated, and lymphoblastic types). A 90 percent complete remission rate was achieved with this regimen with an overall long-term survival of 60 percent. A surprising finding from this study was the usefulness of serum IL-2 receptor levels as a predictor of response to therapy. This parameter proved more useful than stage of disease, bone marrow involvement, or LDH levels. This finding could help identify patients at high risk of not responding to therapy and who would be good candidates for new therapies.

- Research into optimizing antibiotic therapy for patients with fever and suppressed numbers of infection-fighting white blood cells (granulocytes) has continued in the Branch. The most recent study suggests that single-agent imipenam, a broad-spectrum agent with better group D streptococcal and anaerobic activity than ceftazidime, is as effective as single-agent ceftazidime, thus offering a new option in the treatment of such patients. If fever persists on therapy, patients are being randomized to continue one of these intravenous antibiotics or to switch to ciprofloxacin, a new oral antibiotic. The goal of this study is to simplify the management of cancer patients and to develop methods for treating granulocytopenic patients out of the hospital.

- The number of children with AIDS is increasing at an alarming rate. HIV infection in children is often manifest as encephalopathy with an associated impairment of cognitive development. Through the use of continuous-infusion AZT, researchers in the Branch were able to show dramatic improvement in the cognitive skills and IQ in patients with AIDS and maintenance of this improvement for 6+ months after therapy.

Radiation Oncology Branch

- IUdR has been used as a radiosensitizer for patients with unresectable gliomas and sarcomas. The results seen in gliomas compares favorably with other reports for treatment of this malignancy, with a median survival of 14 months in patients with high-grade tumors. There have been some striking regressions in patients with unresectable sarcomas; over 60 percent of patients have gained local control of their tumors after combined IUdR/radiotherapy despite the fact that the masses are generally considered radioresistant and have typically been huge in size.

- After extensive preclinical study, clinical trials with photodynamic therapy have begun. This technique exploits the selective retention of hematoporphyrin derivatives in tumor cells that kills those cells upon exposure to light of a specific wavelength and leaves surrounding tissues unharmed. This approach seems to be very effective in reopening bronchi that have become obstructed by tumor and in destroying tumor cells that coat the abdominal cavity, as in the case of ovarian cancer.

Radiation Research Program (Associate Director, John Antoine)

- Research into heavy-particle therapy has yielded encouraging results. After ten years of planning and development, clinical trials with neutron-beam therapy have shown significant activity in salivary gland tumors and promising results in some lung, prostate, and head and neck tumors. Proton-beam therapy has proven useful in the treatment of clival chordomas, base of skull chondrosarcomas, and uveal melanomas.

- The rapidly emerging area of medical informatics is being applied to optimize radiation treatment planning. Systems are being developed to extract anatomic features from diagnostic images, define and delineate tumors from normal tissues, define treatment volumes from tumor contours, display three-dimensional images rapidly, and improve simulation techniques. This system would assure optimal treatment planning while minimizing toxicity.

- Positron emission tomography (PET) scanning, which converts metabolic activity of tissues into visual images, has been used to differentiate tumor tissue from normal tissue. This technology has made possible the distinction of normal brain from brain tumors and allows physicians to determine tumor viability following treatment.

- Boron neutron capture therapy (BNCT) exploits the differential retention of boron in tumor over normal tissues, leading to increased sensitivity of the boron-containing tissues to damage when exposed to low-energy neutron beams. This approach seems to be potentially useful in the treatment of malignant brain tumors. This topic was the subject of an RRP workshop held in May 1988. Ideas presented at this meeting will result in scientific projects that will be announced by future RFAs and RFPs.

Developmental Therapeutics Program (Associate Director, Michael Boyd)

- The Natural Products Branch now has over 4,000 extracts in storage ready to be tested in the human cancer cell line screen. Taxol, isolated from the bark of *Taxus brevifolia*, has shown promise in clinical trials against malignant

melanoma and has a 30-40 percent response rate against ovarian cancer in Phase II trials. Bryostatin, extracted from the marine organism Bagula neritina, is a potent binder to protein kinase C, an enzyme involved in signal transduction in cancer cells. It has also been found to potentiate cytotoxicity of IL-2 and promote proliferation of normal bone marrow elements. Bryostatin has now entered the advanced drug-development stage, and a major collection of 11,000 gallons of the marine organisms has just been completed.

- Over the past year a procedure for in vitro drug screening has been developed. Large-scale feasibility testing is under way and the optimal conditions for the assay are being defined. This screen will include 60-100 human tumor cell lines, thereby maximizing the chance of identifying drugs that may have selective activity against only certain types of cancer. This standardized assay will eventually allow for the rapid, accurate screening of 10,000 compounds per year.

- The testing of anti-AIDS compounds has also been revised in the past year with the addition of a second cell line to the in vitro screening stage of drug discovery. This, too, will decrease the chances of overlooking a compound that possesses anti-retroviral activity. The AIDS screen will be able to analyze over 10,000 compounds per year.

- The Drug Synthesis and Chemistry Branch has made significant contributions in cancer and AIDS drug development through the large-scale synthesis of several drugs with activity against AIDS (ddA, ddC, and penclomedine) and promising antitumor compounds. In addition, a new computer-based model to group and prioritize compounds for screening from the large repository of unique compounds is under development.

Cancer Therapy Evaluation Program (Associate Director, Robert Wittes)

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 R01 and P01 grants relating to cancer treatment. During FY 1988, strategy meetings were held on adjuvant treatment of colon and rectal cancers, melanoma, and adjuvant treatment of breast cancer. These meetings resulted in the formation of large-scale clinical trials in each of these areas. During the next year sessions are planned in esophageal and gastric cancers, brain tumors, non-small-cell lung cancer, the adult acute leukemias, and ovarian cancer.

- In FY 1988 approximately 25,000 new patients were entered on clinical studies (Phases II and III) in CTEP-sponsors trials conducted by the Cooperative Groups. Virtually every type of malignancy is being studied in this collaborative enterprise. Phase III trials of comparative efficacy are the central components of the effort to reduce cancer mortality. In an effort to accelerate accrual onto important clinical trials, studies on four potentially curable malignancies--adjuvant colon, rectal, bladder, and advanced lymphoma--have been designated as "high priority clinical trials."

- In 1988 clinical trials produced positive findings in several large-scale adjuvant therapy trials. The results of the National Surgical Adjuvant Breast Cancer Project (NSABP) studies B-13 and B-14 indicate a significant (30-40%) reduction in disease recurrence at three years post-therapy for women with node-negative

early-stage breast cancer given adjuvant therapy (chemotherapy for those women estrogen-receptor negative, hormonal therapy for women estrogen-receptor positive) over those given no adjuvant therapy. Over 60,000 women per year in the United States could be affected by these findings, prompting the NCI to issue a Clinical Alert to over 12,000 physicians, informing them directly of the results of these studies.

- The benefit of adjuvant therapy in the treatment of colon and rectal cancers was also reported in the results of NSABP studies this year. In the NSABP C-01 study of patients with Dukes B and C colon cancer, the group given combination chemotherapy with 5-FU, methyl-CCNU and vincristine had significantly better five-year disease-free (42% vs. 30%) and overall survival (53% vs. 43%) than the group given no adjuvant therapy. In NSABP R-01, patients with Dukes B and C rectal cancers treated with 5-FU, methyl-CCNU, and vincristine also fared better in terms of disease-free (58% vs. 51%) and overall (67% vs. 59%) survival at five years than their counterparts who did not receive adjuvant treatment.

- A Phase III trial has begun with cisplatin plus WR-1721 in patients with advanced malignant melanoma. Although single-agent cisplatin has been used with only limited success in this disease in the past, the addition of WR-1721 allows administration of higher cisplatin doses by protecting from the toxic side effects of cisplatin. Phase II trials using this combination have achieved a 53 percent response rate in melanoma patients, the highest response rate ever reported for any chemotherapeutic regimen in this disease.

- During the past year the FDA approved CTEP's group C application for ifosfamide for the combination treatment of relapsed or refractory testicular cancer, and is considering CTEP's applications for group C designation for pentostatin in hairy cell leukemia, teniposide (VM-26) in refractory childhood acute lymphoblastic leukemia, methyl-CCNU for combination adjuvant therapy in colorectal carcinoma, and fludarabine for refractory chronic lymphocytic leukemia.

- In a carefully conducted, randomized trial, the Eastern Cooperative Oncology Group (ECOG) has demonstrated the need for post-remission therapy in adults with acute nonlymphocytic leukemia. The study continues, comparing maintenance chemotherapy with a short period of intensification chemotherapy.

- Both alpha- and gamma-interferon have been shown to be active in patients with chronic myelocytic leukemia, and are able to produce complete eradication of the malignant cell line from bone marrow in 25 percent of patients treated with gamma- and 70 percent of patients treated with alpha-interferon. The Cancer and Leukemia Group B (CALGB) and the Southwest Oncology Group (SWOG) are currently exploring the tolerability of various schedules of combinations of these agents in previously untreated patients. An eventual Phase III trial will be developed, although the "standard" therapy arm remains to be determined.

- Colony-stimulating factors (CSFs), a family of molecules that stimulate production and differentiation of blood cells, have now entered clinical trials in an effort to reduce chemotherapy-associated bone marrow suppression. The NCI is supporting trials of CSFs with chemotherapy in breast cancer, lung cancer, ovarian cancer, testicular cancer, multiple myeloma, lymphoma, leukemia, and sarcoma. CSFs have shortened the time to bone marrow recovery in patients receiving high-dose chemotherapy and bone marrow transplantation.

Biological Response Modifiers Program (Associate Director, Dan Longo)

The Biological Response Modifiers Program (BRMP) investigates, develops, and conducts clinical testing of biological therapeutic agents that may alter host defenses or cancer cells in such a way as to lead to the reduction or elimination of the cancer. The Program is composed of extramural and intramural programs conducting clinical and basic research.

- Extramural Phase I-II clinical trials are now under way to study the activity of monoclonal antibodies given alone or with gamma-interferon or interleukin-2, the effect of dose- and schedule-related variations in the effect of IL-2, the effect of I^{131}-conjugated T-101 monoclonal antibody on T-cell lymphoma, and the use of monoclonal antibodies as immunomodulators.

- Discoveries made in the intramural basic research laboratories of the Associate Director of the BRMP have included demonstration that signals that activate normal T-cells--like antigen with self-MHC products, antibodies to the T-cell antigen receptor (T_i), and antibodies to the Thy 1 antigen--can result in the death of malignant T cells both *in vitro* and *in vivo*. This work has led to better understanding of signal transduction in T cells and has identified new targets for antitumor therapy. Other work has focused on how the thymus exerts its influence over the T-cell repertoire for antigen and has led to enormous progress in understanding the structure and function of the gamma-delta receptor on T cells. Three different types of gamma chain and four types of delta chain are used to form the receptor, a diversity that previously had not been appreciated. Work in the intramural laboratories has also examined the roles of IL-2 and IL-2 receptor in the development of T cells. Studies from the laboratory of Dr. Ada Kruisbeek have demonstrated that they are essential for development of mature T cells and that saturation and blockade of IL-2 receptor function in the developing fetus completely blocked the development of normal class I-restricted cytotoxic and class II-restricted helper T cells *in vivo*.

- Molecules secreted by lymphocytes, macrophages and other cells that stimulate the proliferation and activity of a wide variety of cells have been termed "lymphokines" or "cytokines." The interleukins are the largest family of lymphokines identified to date. IL-2 is required for the transformation of lymphocytes into tumor-killing lymphokine-activated killer (LAK) and tumor-infiltrating lymphocyte (TIL) cells. Interleukin-1 is secreted by macrophages to stimulate proliferation of T cells, a process crucial for a normal immune response to occur. Interleukin-3, a molecule that stimulates proliferation of blood cells, may have applications similar to the colony-stimulating factors described above.

- The BRMP's AIDS efforts include elucidating the genetic mechanisms that regulate viral replication in T cells and monocytes, understanding the effect of HIV viral proteins on the cell and on the clinical manifestation of AIDS, the design of novel approaches to attack the virus, and efforts to reconstitute the immune system after viral replication is controlled.

PUBLICATIONS (OD-DCT)

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Curt GA, Chabner BA. Medical oncology: decade of discovery. Internal Medicine for the Specialist 1987;8(no. 8):47-54.

Chabner BA, Browne MJ, Boyd MR. Advances in cancer treatment: the future for chemotherapy. Cancer Nursing 1987;10(suppl 1):40-6.

Chabner BA, Gottesman MM. Commentary. Meeting highlights: William Guy Forbeck Foundation think tank on "multidrug resistance in cancer chemotherapy." JNCI 1988;80:391-4.

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Definitions of Contract Groupings
FY1988 Annual Report

Drug Development

Compound Discovery	-	Preclinical contract program focusing on the discovery and screening of potential anticancer agents. Includes both natural products and synthetics, as well as testing (screening) *in vitro* and *in vivo*.
Compound Development	-	Includes data management, pharmacology and pharmacokinetics testing, formulation and analytical profiles, and toxicological protocols to meet FDA requirements.
Clinical Support	-	Preclinical contracts which provide direct support to the clinical trials program (excluding purchases).

Biologics Development

All non-clinical contracts administered by the Biological Response Modifiers Program, excluding contracts in support of the intramural program.

Diagnostic Imaging

Contracts administered by the Diagnostic Imaging Research Branch, RRP.

Radiation Development

Preclinical radiation contracts, including screening and synthesis of radiosensitizers and radioprotectors, dose calculations, and other preclinical radiotherapy contracts.

Clinical Trials

Drug Evaluation	-	Phase I, II, III drug development contracts administered by the Cancer Therapy Evaluation Program, including foreign clinical contracts in support of FDA requirements.
Biological Evaluation	-	Task order contracts for Phase I/II clinical trials of BRM's.

Radiotherapy	-	All clinical radiotherapy contracts administered by the Radiation Research Program.
Other	-	Contracts which support other research requirements throughout the Division of Cancer Treatment, including program support, data management for extramural contracts, and other technical support. Does not include intramural support contracts.

Support to Intramural

Contracts which directly support intramural research activities in the Developmental Therapeutics Program, the Clinical Oncology Program, and the Biological Response Modifiers Program.

Drug Purchases

The purchase of investigational agents used in DCT-sponsored Phase I/II/III clinical trials, where DCT provides the agents being used in the trial to both extramural and intramural investigators.

Program Management

Includes administration and dissemination of information to the medical and scientific community.

TABLE I

DCT Contract Program for FY 1988
(Dollars in Thousands)

	FY 88 EST.	PERCENT
I. Drug Development	$ 50,711	(67%)
A. Compound Discovery - Subtotal	(18,993)	25%
1. Acquisition	(5,384)	(7%)
a. Natural Products	2,364	3%
b. Synthesis	3,020	4%
2. Screening	(13,609)	(18%)
a. In Vivo	7,411	10%
b. In Vitro	4,509	6%
c. Screening Support	1,689	2%
B. Compound Development	8,941	12%
C. Clinical Support	3,522	5%
D. AIDS Drug Development	19,255	25%
II. Biologics Development	1,367	2%
III. Diagnostic Imaging	0	0%
IV. Radiation Development	1,601	2%
V. Clinical Trials - Subtotal	13,661	18%
A. Drug Evaluation (Phase I/II/III)	3,280	4%
B. Biological Evaluation (Phase I/II)	6,560	9%
C. Radiotherapy	2,571	3%
D. Other	1,250	2%
VI. Support to Intramural	2,655	3%
VII. Drug Purchases	4,100	5%
A. Cancer Drugs	2,100	3%
B. AIDS Drugs	2,000	3%
VIII. Program Mangement	2,465	3%
TOTAL DCT CONTRACTS	$ 76,560	100%

TABLE II

DESCRIPTION OF CONTRACTS

IN THE

DIVISION OF CANCER TREATMENT

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

ALABAMA, UNIVERSITY OF (NO1-CM4-7679)

This Phase Ib study originally proposed the combination of IL-2 and a murine anti-colon monoclonal antibody for patients with Stage IV adenocarcinoma of the colon or rectum. The objectives of the study are to: determine whether different dose levels of IL-2 in combination with monoclonal antibody will enhance antibody dependent cellular cytotoxicity of patients' peripheral blood monocytes and lymphocytes; assess the extent of *in vitro* immune activation by different IL-2 doses as measured by circulating mononuclear cell phenotypes and cytotoxic activity and by serum levels of interferon gamma; determine the effects of IL-2 on the development of human anti-mouse antibody and anti-idiotype responses; and determine toxicity and antitumor effects of the regimen by following the patients and measuring antitumor response. Due to unavailability of the proposed mouse monoclonal antibody, a mouse-human chimeric antibody will be substituted. This contract expires in FY89.

BIORESOURCES LABORATORIES, INC. (N43-CM8-7266) (SMALL BUSINESS INNOVATION RESEARCH Program)

The technology of producing hybridomas which secrete monoclonal antibodies, is now commanding attention in all fields of biology. A particularly important application for monoclonal antibodies is the diagnosis and treatment of human tumors. This necessitates a central information repository to track the results of clinical trials of these immunoreagents. An automated tracking system is mandatory to avoid duplication of effort and to facilitate identification of reagents demonstrating positive results in clinical trials. Optimal conditions for successful therapeutic regimens can be more easily identified through comparison of methods and results on a large-scale made possible by a computerized storage and retrieval system. The proposed project is to create a data bank containing information on monoclonal antibodies used in diagnostic and therapeutic clinical trials. A three-tiered system is proposed: a bibliographic reference file of literature citations and abstracts for monoclonal antibodies with clinical applications; a background file on developers, distributors, availability, and characteristics of the hybridomas and other cloned cell lines producing immunoreagents of clinical significance; and a relational file of data on materials, methods, and results of clinical trials with monoclonal antibodies. The data bank will be designed for easy use on microcomputers and commercially available software by non-computer professionals and it will be widely disseminated to the biomedical community.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7682)

This study will examine the effects, in 15 patients with advanced cancer, of intra-lymphatic injection of IL-2 on the numbers and types of efferent lymphocytes from the thoracic duct. Cells will be harvested, and their activation studied *ex vivo*. When activation conditions have been optimized, the additional step will be taken of reinfusing the cells with IL-2 for cancer

treatment. Patients will be monitored for toxicity, immunological parameters, and tumor response. Preliminary investigations by this group have shown thoracic duct lymphocytes to be excellent sources of lymphokine activated killer cells.

CLEVELAND CLINIC FOUNDATION (NO1-CM4-7673)

This contract supports a Phase Ia/Ib clinical trial of R24 monoclonal antibodies combined with the chemotherapeutic drug DTIC, in patients with malignant melanoma. The rationale for the trial is that both agents have low, but measurable, activity in patients with advanced disease.

A study is designed to explore the optimal schedule for administration of clonally expanded tumor infiltrating lymphocytes with or without recombinant interleukin 2 (rIL2) in patients with metastatic renal cell carcinoma and minimal tumor burden; to study the toxicity of tumor infiltrating lymphocytes and rIL2 given as a constant infusion and compare it with that reported for other types of adoptive immunotherapy, namely, lymphokine activated killer cells and rIL2; and to characterize the effector cell population in clonally expanded tumor infiltrating lymphocytes and compare the cytolytic potency to lymphokine activated killer cells from the peripheral blood. A secondary objective is to determine if objective tumor responses occur in patients receiving adoptive immunotherapy with tumor infiltrating lymphocytes and rIL2.

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CM4-7668)

On this contract, a Phase I clinical trial is being conducted with the anti-melanoma monoclonal, MG21. This monoclonal antibody reacts with the sialoganglioside, GD3, which is found on the cell surface of most melanoma cells. Pharmacokinetics, clinical toxicity, and immunological response are monitored. Patients are treated with MG21 monoclonal antibody for 7 days, then tumor tissue is biopsied for evaluation. Five dose levels of antibody are to be assessed. The highest dose level has not been reached. On March 27, 1987, the Principal Investigator suspended accrual to the trial after 9 patients were treated because of unexpected severe adverse reactions. It will reopen after development of sufficient quantities of MG22, a related and improved monoclonal antibody.

HAZLETON LABORATORIES, INC. (NO1-CM7-3710)

The purpose of this contract is to provide effective inventory, distribution, and quality assurance confirmation for biological response modifiers. The Contractor is responsible for receipt, dispensing, storage, distribution, and inventory control of biological agents. Quality assurance evaluation involves specific assays to confirm sterility and assays to determine pyrogenicity and endotoxin levels. The Contractor performs general safety tests for biological agents intended for clinical use in compliance with Government regulations and helps in the development of master files and investigation new drugs for biologics. Currently, the Contractor provides for storage and distribution of approximately 100 different biologics. The Contractor manages a repository distributing agents to qualified intramural and extramural investigators for preclinical studies. The contract also provides for ascites production and purification of monoclonal antibody and has produced monoclonals specific for melanoma, colon, breast cancers, T-cell receptor, and lymphocyte antigens.

HYBRITECH, INC. (NO1-CM6-7718)

This Contractor performs coupling of chemotherapeutic drugs, toxins, and radioisotopes to monoclonal antibodies directed against specific antigens found on human tumor cells. Appropriate tests are carried out on conjugates to demonstrate that the cytotoxic agent-antibody conjugates

retain antigen-antibody specificity comparable to the unmodified antibody and cytotoxicity in excess of the nonderivatized cytotoxin. The Contractor is required to scale up the appropriate conjugation procedure to provide sufficient quantities of a human use product for preclinical and preliminary clinical trials. The Contractor is conjugating pseudomonas exotoxin to the monoclonal antibody OVB-3 for intramural National Cancer Institute trials. Experiments have been ongoing to couple methotrexate, ricin A chain, Yttrium-90, Indium-111 and Iodine-131 to T101, an antibody directed against a human T-cell differentiation antigen, and 9.2.27, an antibody directed against a human melanoma cell antigen. The Contractor has been supplying T101 conjugated to indium and iodine for biodistribution, imaging and therapy studies. The Contractor continues to refine the T101-methotrexate conjugate, specifically reducing the level of liver localization by conjugating reagents with a lower level of activated chelating agents.

ILLINOIS CANCER COUNCIL (NO1-CM4-7667)

This contract was awarded to conduct a Phase I clinical trial using ^{131}I conjugated to T101, a murine monoclonal antibody directed against an antigen which is expressed on T-lymphocytes, in patients with cutaneous T-cell lymphoma (a rare disease in the United States). The clinical study assesses antibody localization (imaging) in tumor and normal tissues, followed by escalation of the radioisotope dose to assess antitumor effect. Pharmacokinetics, biological effects, and toxicities are being determined. Pharmacokinetic and immunomodulatory data have been reported on all patients. Eligibility was extended to patients with chronic lymphocytic leukemia. Two of seven cutaneous T-cell lymphoma patients have responded; 4 additional cutaneous lymphocytic leukemia patients have been treated but the trial is too early to assess response in this category.

Another phase of this contract is for the performance of a clinical trial seeking to determine the optimal immunomodulatory dose of IL-2 when given as a 24-hour continuous infusion, twice weekly, for a period of 1-year to patients with advanced colon cancer who have undergone surgery but are at high risk of recurrence. The IL-2 dose is 1×10^6 $u/m^2/24$ hrs, which can be administered to outpatients using a portable infusion pump. Long-term toxicities and biological effects are to be monitored during and after each patient's 1-year treatment period. Patient accrual closed in April 1988. In patients treated 3 months or longer, stable increases were seen in lymphocytes bearing leu 19, CD8d and CD16 surface antigens. Patient monitoring and analysis are continuing. This contract expires in FY88.

IMMUNOMEDICS, INC. (N44-CM8-7778) (SMALL BUSINESS INNOVATION RESEARCH Program)

This proposal will study the use of Tc-99m labeled NP-4 murine monoclonal anti-CEA antibody for radioimmunodetection of human colon cancers transplanted into the liver of nude mice. The tumor is a human colonic tumor xenograft (GW-39) which has been serially propagated in the cheek pouch or leg of hamsters. Radioimaging of tumors with antibody labeled with Tc-99m conjugate will be compared to In^{111}-DPTA antibody and I-131-labeled antibody. Methods of optimization for radiolabeling with Tc-99m with high antibody specificity will be developed. The kinetics of tumor localization, imaging quality, and catabolism will be evaluated. Optimization of radioimmunoimaging will also be developed via Tc-99m labeled antibody fragments and via alternative means of linking radiometal to antibodies by a polymer-chelate. It will be useful to develop technetium-99m conjugated monoclonal antibodies for imaging since the capability of performing Tc-99m scans is widely available in nuclear medicine departments.

JEFFERSON MEDICAL COLLEGE (NO1-CM6-7902)

This protocol will test a new approach to producing tumor-specific cytotoxic T lymphocytes ("educated" lymphokine activated killer cells), generated by incubation of apheresed PBLs with autologous tumor and IL-2 ex vivo. Patients will be treated with autologous "educated" lymphocytes, IL-2 and cyclophosphamide to assess toxicity, therapeutic efficacy and modulation of immune parameters. Three populations of lymphocytes, removed from the patient at different points in the therapeutic course, will be compared in immunological function, and ability to be "educated" to react against autologous tumor cells. The clinical study will involve 16+ patients with advanced sarcomas refractory to standard therapy. Cyclophosphamide will be given to reduce host suppressor cells. Preliminary studies include 3 patients treated with cells alone (without cyclophosphamide or IL-2), with one response.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-7665)

A Phase I clinical trial is being done to evaluate the pharmacokinetics, toxicities, biological and antitumor effects of R24 monoclonal antibody and interleukin-2 when used in combination therapy. R24 monoclonal antibody is directed against GD3, a sialoganglioside expressed in large amounts on the surface of most malignant melanoma cells. R24 also cross-reacts with a lymphocyte surface antigen and enhances effector cell function. The clinical study uses a fixed dose level of IL-2, examining four dose levels of R24. A total of 21 patients were treated. One partial response and 2 minor responses were seen. Final analysis shows activation of natural killer, lymphokine activated killer, and R24-mediated antibody dependent cellular cytotoxicity in the majority of patients whose results are available so far. Immunomodulation studies are pending. Dose limiting toxicity was not reached at the highest dose of antibody 12 mg/m^2/day on day 8-12 and IL-2 ($10^6 u/m^2$/day on days 1-5 and days 8-12). This contract expires in FY88.

This contract supports clinical trials to evaluate treatment with different dose levels of R24 F(ab')2 fragments in patients with metastatic melanoma. Toxicity and antitumor effects at different dose levels of R24 F(ab')2 are being monitored and evaluated. Additionally, a variety of parameters are being measured and evaluated during treatment at different dose levels of the R24 F(ab')2. These include the assay of titers of free F(ab')2, and the absolute levels in serum after injection, the evaluation of ADCC in peripheral blood, and the evaluation of anti-mouse immunoglobulin and antidiotype response. The monitoring of the absolute number and percent GD3+ T lymphocyte and other T cell subsets in peripheral blood during treatment, and the monitoring of the activation of T cells before and during treatment is being done.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM6-7891)

This contract will support a Phase Ia/b study of IL-2 with an antibody dependent cellular cytotoxicity-mediating monoclonal antibody. Availability problems have resulted in substituting a B-cell lymphoma antibody for the original antibody proposed.

NEORX CORPORATION (NO1-CM5-7719)

The purpose of this contract is to develop a centralized, coordinated program for uniform preclinical testing and evaluation of monoclonal antibodies and their immunoconjugates prior to entry into clinical trials. The Contractor will test and evaluate monoclonal antibodies and immunoconjugates in several test systems: (1) immunoreactivity against a panel of known tumor cells to define relationships with other monoclonal antibody and establish epitope reactivities by molecular or serologic means; (2) in vitro cytotoxicity assays; (3) virus testing for LCM, retrovirus and MAP test; (4) immunohistologic screening to define antigen positive tissues and

specificity; (5) antitumor effects in the nude mouse model and subrenal capsule assay; and (6) animal toxicity evaluation monoclonal antibody will be evaluated at each level of testing and must exceed pre-established standards of specificity and activity before proceeding to the next level of evaluation. Monoclonal antibodies successfully progressing through the screening process will then be considered for clinical evaluation. Monoclonal antibodies against a 250 kilodalton human melanoma antigen, against human melanoma sialogangliosides and against human colon carcinoma have been evaluated. In addition, monoclonal antibodies conjugated with pseudomonas exotoxin have been investigated for cytotoxicity *in vitro*.

NEORX CORPORATION (N44-CM6-7759) (SMALL BUSINESS INNOVATION RESEARCH Program)

The purpose of this contract proposal is to develop new radioiodine labeled conjugates for monoclonal antibodies that can be used in diagnosis and therapy of cancer. The goal of the proposed research is to investigate a novel method of introducing high specific activity radioiodine into some small molecules that can be conjugated to monoclonal antibodies. The Phase I study demonstrated the feasibility of using organotin intermediates for incorporating no-carrier-added radioiodine into non-activated aromatic compounds, which could subsequently be added to proteins. During the Phase II study, it is planned to optimize the radioiodination reactions and test several different radioiodinated conjugates for *in vitro* and *in vivo* stability. A comparison of a conventional (directly) radioiodinated antibody with the same antibody labeled using the radioiodinated conjugates will be carried out. Studies using an iodine-131 labeled antibody will be conducted (in nude rats) to measure tissue dosimetry. The ultimate goal of the Phase II study is to identify an antibody and a radioiodine conjugate that can become a commercial cancer therapy agent. It is hoped that it will be possible to do the appropriate toxicology studies and file an investigational new drug on this agent near the end of the Phase II funding period.

NEORX CORPORATION (N44-CM6-7760) (SMALL BUSINESS INNOVATION RESEARCH Program)

The objective of the proposed research is to take methodology developed for labeling monoclonal antibodies with radioactive rhenium for cancer therapy through various stages needed to determine biodistribution properties, hence, eventual radioimmunotherapy potential, in a limited number of patients. The results from Phase I of this contract showed that Rhenium-186 can be attached to antibodies under mild conditions and in a stable manner. The proposed study is intended to optimize labeling parameters and specific activity, evaluate methods for stabilization of radiolabeled antibody to radialysis, optimize Re to antibody ratios and tumor targeting in animal models, study therapeutic efficacy in animal tumor models, make radiation dosimetry estimates for humans, and study Re-186 antibody preparations in patients at doses that will provide the biodistribution and pharmacokinetic data needed to justify studies at the therapeutic dose levels. These studies will be carried out with anti-melanoma antibody as a model system with the expectation that the technology of labeling with radioactive rhenium can be demonstrated to be viable as a generally applicable method.

OHIO STATE UNIVERSITY (NO1-CM4-7666)

In this clinical study, the aim is to determine the toxicity of IL-2 and expanded lymphokine activated killer cells in patients with metastatic renal cell cancer. The objective response rate, time to response, and response duration to IL-2 and expanded lymphokine activated killer cells is also being monitored and evaluated. The study is also looking at the correlation of immunologic

parameters, distribution of lymphokine activated killer cells, administered cell numbers, lymphokine activated killer activity against tumor, lymphokine activated killer cell phenotype, and site of disease with response.

PITTSBURGH, UNIVERSITY OF (NO1-CM6-7893)

The protocol will examine the optimal therapeutic and immunomodulatory dose of R24 monoclonal antibody in patients with malignant melanoma. Previous studies have not determined the optimal dose of the antibody, though they have shown promising therapeutic results clinically. The maximum tolerated dose for R24 has not been established. Evaluation of the optimal immunomodulatory dose will include pharmacokinetics of the antibody; in situ antibody localization studies; antibody dependent cellular cytotoxicity function of peripheral blood mononuclear cells; complement dependent cytotoxicity; phenotypic analysis of tumor infiltrating lymphocytes; binding of monoclonal antibody to Leu-11+ circulating cells; and inflammatory response mediators at the tumor site. Baseline and treatment effects on serum Ig levels, complement levels, natural killer function, leukocyte phenotype, anti-Ig levels, and R24-mediated antibody dependent cellular cytotoxicity will be determined. Treatment will include 5 levels/6 patients each receiving a 24-hr infusion of monoclonal antibody on days 1-5 and 8-12 with escalation of dose levels among groups from 8-640 mg/m^2. Antitumor responses will be evaluated monthly.

The clinical investigation uses a new method, developed by this group, of selecting a subpopulation of activated natural killer cells (adherent-LAK or "A-LAK") which they suggest may have higher cytolytic activity per cell than is achieved with unselected lymphokine activated killer cell preparations. This hypothesis will be tested using 6 groups of 6 patients with infusions of different dose levels of A-LAK cells, as "standardized" by activity assays against standard lymphokine activated killer cells. Indium-labelling studies would be performed at the three higher levels. Toxicity and antitumor activity of the therapy will be determined.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM4-7675)

This contract supports studies with the monoclonal antibody LYM-1 in combination with IL-2 for the treatment of B-cell lymphoma. The Contractor will measure monoclonal antibody binding, antigen modulation, pharmacokinetics, immunomodulation, and antitumor effects of the combination.

T & B BIOCLONE, INC. (N44-CM8-7779) (SMALL BUSINESS INNOVATION RESEARCH Program)

An immunoassay developed during the initial period of this contract will be evaluated clinically in the United States and Japan to determine its efficacy as a diagnostic and prognostic indicator of lung cancer. The key element of the assay is the monoclonal antibody, 5E8, which binds a membrane-associated glycoprotein gp160, that is expressed on over 70% of the human lung tumors tested, including squamous cell carcinomas, adenocarcinomas, and large cell tumors. The assay is specific, simple, shelf-stable, requires only five nanograms of antibody per assay, and can be modified to detect either the tumor-associated macromolecule gp160, or anti-gp160 antibodies present in the body fluids of patients. The same antibody, 5E8, is currently being used for the development and testing of immunospecific drug delivery systems. The antibody has also been successfully covalently linked to unilamellar liposomes that contain cytosine arabinoside (AraC) in their aqueous space. Liposomes containing 5E8 (but not a control antibody) bind and deliver the cytotoxic drug to gp160 positive human lung tumors in vitro. Phase II will focus on an in vivo evaluation of immunospecific liposome drug delivery. Human gp160 positive lung tumors proliferate in the lung of severe combined immunodeficient (scid) mice. The therapeutic

effectiveness of the liposomes will be tested in the mouse model. A more direct approach to antibody drug targeting, in which AraC is conjugated directly to the 5E8 antibody, is also being evaluated.

TEXAS, UNIVERSITY OF (NO1-CM6-7899)

This study is a Phase Ib study of patients with measurable Stage III malignant melanoma, to evaluate: (1) the biodistribution and tumor localization of radiolabeled murine monoclonal antibody 96.5, alone or in combination with interferon-alpha; (2) possible alterations in monoclonal antibody pharmacokinetics as a result of escalating dose of monoclonal antibody or concurrent use of interferon-a; (3) monoclonal antibody binding to tumor tissues in vivo; and (4) differential immunomodulatory effects of monoclonal antibody 96.5 alone or in combination with interferon. They will also monitor human anti-mouse antibody production, quantitate tumor infiltrating lymphocytes, assess the induction of 2'-5' as activity in tumor cells in situ, and measure antitumor effects.

WISCONSIN, UNIVERSITY OF (NO1-CM4-7669)

A Phase Ib clinical trial exploring dose and schedule manipulations to determine the optimal immunomodulatory dose and toxicities of IL-2 given over a protracted period to patients with advanced cancer. Accrual was completed after 29 patients with renal cell cancer or malignant melanoma were treated. Two partial responses were seen. Treatment was generally well-tolerated. Final analysis is continuing.

Four sequential Phase I clinical trials are being performed to evaluate the toxicity, biological antitumor effects of 7-day infusions of IL-2 following infusion of autologous lymphokine activated killer cells, allogenic lymphokine activated killer cells, or allogenic cytotoxic T-cells, with or without cyclophosphamide immunosuppression. The trial was closed after 15 patients were treated. No objective antitumor effects were seen. Immunological analysis are pending.

A Phase I clinical trial will determine the optimal immunomodulatory dose of a combination regimen of interferon-beta and interferon-gamma, and evaluate the mechanisms and types of biological response modifications produced by such combination therapy. An initial series of 21 patients completed in September 1986, consisting of patients receiving single doses of interferon-beta and/or interferon-gamma, in which no synergy was demonstrated. A second series was then begun to explore effects of 5-day continuous infusion of interferons and maximum tolerated dose was defined. Patient entry and analysis are continuing to explore optimal biological response modifying dose.

The Phase Ib trial is a combination trial of interferon-gamma and monoclonal antibody B72.3 in ovarian cancer. It will assess the modulation with interferon-gamma of tag-72 antigen reacting with monoclonal antibody B72.3 on ovarian cancer cell in patients with malignant ascites. The protocol is under review and patient treatment is anticipated to start in Summer, 1988.

A study is determining and comparing the immunologic and biologic effects of repetitive 4-day cycles of IL-2 given as a continuous infusion at two dose levels and combined with multiple infusions of autologous lymphokine activated killer cells. The study is also designed to determine the biologic effects of maintenance therapy, clinical toxicities of lymphokine activated killer cells given with escalating doses, the safety factor of IL-2/lymphokine activated killer administration in a non-intensive care unit setting and clinical toxicities of maintenance IL-2.

YALE UNIVERSITY (NO1-CM4-7681)

Phase Ia and Ib clinical trials are underway to define the scope and dose-limiting toxicity of multiple dose level combinations of interferon-alpha and interferon-gamma in patients with renal cell carcinoma. The pharmacokinetics and optimal biological doses of these agents in combination will be defined. Twenty-nine patients have been accrued, doses have reached 10 mu/m^2 interferon-alpha qd x 70 and 1 mg/m^2 of interferon-gamma qd x 5 q 3 weeks. Grade 4 neutropenia has been seen in some patients at doses above 300 mg/m^2 of interferon-gamma, irrespective of the interferon-alpha dose. Three partial responses were seen at various doses without a clear dose response effect. A definite maximum tolerated dose has not been reached.

CANCER THERAPY EVALUATION PROGRAM

CALIFORNIA, UNIVERSITY OF (NO1-CM7-3702)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

CITY OF HOPE NATIONAL MEDICAL CENTER (NO1-CM7-3703)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

EMMES CORPORATION (NO1-CM6-7908)

This contract provides support to CTEP in two areas: a) direct organizational data management and statistical support for specific clinical trials (currently The Testicular Cancer Intergroup, Intergroup Pancreas Study, the Head and Neck Follow-up Efforts and extramural LAK/IL-2 trials); and b) information management assistance to CTEP Professional staff in the analysis of methodology and data emanating from the extramural program. A recent area of involvement has been the CTEP initiated treatment protocols for which EMMES has assisted in protocol and forms development and will serve operations and data management functions.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CM6-7810)

This contract supports the maintenance of the computer aspects of the following CTEP information systems: The CTEP-IS, the Phase II System, and the Drug Distribution and Protocol Monitoring System (DDPMS) with further development of linkages between these systems and with

outside databases. The CTEP-Information System (CTEP-IS) provides computer capabilities to index, track, select, sort, and locate clinical trials. Currently there are nearly 8,000 studies in the system, with 275 trials in the CTEP or PDQ review process at any one time. About 1,000 new studies are added each year with some 1,500 amendments to active studies. The Main System Investigator Directory includes 1,300 investigator addresses and/or phone numbers. The Phase II Results Database (PH II) is a subset of 2,000 studies in the Main System which provides scientific results of Phase II single agent clinical trials primarily for the medical staff and for publication purposes. Users can still access the Phase II system, but it has been phased out and is no longer updated. The Drug Distribution and Protocol Monitoring System (DDPMS) is a computer information system and a support service which is managed by VSE Corporation, subcontractor to IMS, Inc. The Contractor provides system information which allows the Drug Management and Authorization Section, IDB, CTEP to meet the Food and Drug Administration requirements for monitoring investigational drug distribution to clinical investigators. Currently, approximately 500 investigators are registered to receive investigational drugs for use in 3,200 approved protocols. About 140 investigational agents with multiple dosage form are distributed. One hundred clinical drug requests, each with up to eight line items are verified and authorized for shipment daily. The DDPMS consists of more than 100 programs; it is written in PL/I and utilizes the DCRT computer facility.

JOHNS HOPKINS UNIVERSITY (NO1-CM5-7738)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with Oxantrazole, Taxol in leukemia, Dichloromethotrexate, N-Methylformamide (NMF), HMBA/myelodisplastic syndrome, Cytoxan/CBDCA with ABMT, Trimetrexate (abnormal organ function), Ipomeanol and Sulfamic Acid. Pharmacokinetics have been included in the studies of Taxol, Trimetrexate, HMBA, and NMF.

LOYOLA UNIVERSITY (NO1-CM7-3704)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

MARYLAND, UNIVERSITY OF (NO1-CM5-7734)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents (or combination of agents) sponsored by the Division of Cancer Treatment and Phase II/III studies in patients with disseminated solid tumors and leukemia. The Contractor is conducting and/or has completed Phase I studies with AZQ/VP-16 phase I in relapsed leukemia patients, Menogaril, ip DDP/L-PAM, HMBA, Cytoxan/IL-2, 5FU/Uridine and CBDCA/VP-16 leukemia. Pharmacologic studies have been completed on HMBA, Cisplatin/Melphalan and CTX/IL-2. There are currently 13 active Phase II/III studies. These active studies include:

CML-B AZO; Renal-NMF; H/N MTX CBDCA; H/N CBDCA + XRT; Cervical--CBDCA + XRT; Prostate-TMX, Ovarian-TMX; Uterine-TMX; Renal-IL-2; Colon-IL-2; Melanoma-IL-2; LUNG-CBDCA + VP-16 and Pancreas-PALA,5FU.

MAYO FOUNDATION (NO1-CM5-7733)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents (or combination of agents) sponsored by the Division of Cancer Treatment and Phase II/III studies in patients with disseminated solid tumors and leukemia. The Contractor is conducting and/or has completed Phase I studies with 6-Thioguanine, Pibenzimol, Amonafide, Flavone Acetic Acid, Oxantrazole, a pediatric 6-TG study, a leukemia 6-TG study and Phase I studies with salt free Cisplatin. The Contractor is conducting and/or has completed Phase II studies of Menogaril in sarcoma, ovarian cancer, and renal cancer, 5 FU + Folinic Acid in breast cancer, Ifosfamide/VP-16 in sarcoma, 5FU/MeCCNU/VCR/F.A. in a GI pilot study, IV 6TG, IP DHAD, DHAC in melanoma and TNF in melanoma and lung.

MEDICAL LASER RESEARCH AND DEVELOPMENT CORPORATION (N44-CA7-7832)
(SMALL BUSINESS INNOVATION RESEARCH Program)

This contract is to develop a surgical laser system which can remove tumors or other tissues with minimal blood loss, precision, and minimal damage to adjacent tissue. The Phase I program was a demonstration of laser control parameters required for hemostatic action with minimal adjacent tissue damage in a murine model in vivo. Phase II will include demonstration of laser scalpel action, operator interface design, and system integration, providing a prototype surgical laser system. Demonstration of the efficacy of this system will include acute and long-term (wound healing) studies in vivo. Presently available surgical laser systems produce char and hemostasis or no char and no hemostasis. The surgical laser system proposed herein provides hemostasis while making clean sharp cuts with no char formation and minimal adjacent tissue damage. One of the advantages of this system is that the hemostasis laser can be adjusted independently of the cutting laser, thereby providing the opportunity to optimize each system (cutting and hemostatic) independently.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7732)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents (or combination of agents) sponsored by the Division of Cancer Treatment and Phase II/III studies in patients with disseminated solid tumors and leukemia. The Contractor is conducting and/or has completed Phase I studies with PALA/MTX/LEU/5FU, CBDCA+VLB, HMBA, Merbarone and Deoxyspergualin. Phase II/III studies completed and/or ongoing include Carboplatin in pediatric brain tumors and sarcomas and APUD tumors, CBDCA + Vinblastine in esophagus and gastric tumors, NMF in melanoma, Amonafide in lung cancer, Iproplatin in testicular cancer and upper GI tract, Menogaril in mesothelioma, Trimetrexate in prostate tumors, Gallium Nitrate in bladder and small cell lung cancer, DDP + VP-16 vs. CBDCA + VP-16 in testicular cancer, CBDCA + VP-16 + Bleomycin in testicular cancer, 5-FU + DDP vs. 5-FU in colon cancer, PALA + MTX + FU in Adenocarcinomas of Unknown Primary site (ACUP), HMBA in melanoma, HD-MTX in sarcoma, Didemnin in renal cancer, HD M-VAC in bladder cancer and Gallium Nitrate vs. Etritonate in hypercalcemia.

MONTEFIORE MEDICAL CENTER (NO1-CM7-3705)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

NEW ENGLAND MEDICAL CENTER HOSPITALS, INC. (NO1-CM7-3706)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM5-7736)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with Amonafide, Flavone Acetic Acid, Merbarone, Ara-AC, Deoxycoformycin, Fostricin, Ipomeanol, BSO/Melphalan and Fazarabine (in leukemia). Extensive pharmacokinetic studies have been conducted.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM7-3701)

The principal objective of this project is the pharmacokinetic analysis of samples from patients with malignant disease accrued to studies with either single or combination of a new and/or established anticancer agent(s). The studies are primarily concerned with the measurement of the agent and/or metabolite levels in plasma, tissue, urine and feces in order to determine the distribution, metabolism and elimination as well as target effect(s) or absorption if the agent is administered orally or by some route other than i.v. The results of these studies are analyzed in particular to help establish the most effective dosage scheduled and to anticipate any situation where altered organ functions could lead to a different pattern of drug distribution, metabolism and elimination resulting in toxicities which would not be expected otherwise. Two agents are evaluated annually.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM2-5606)

This Contractor provides support services for conference management and associated general logistical activities for the Cancer Therapy Evaluation Program. Logistics support includes various technical and clerical tasks ranging from report design and preparation to routine typing. Conference support includes both pre- and post-conference management activities necessary to successfully conduct large as well as small meetings and provide the results thereof to the biomedical research community.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM7-3709)

The contract provides support services for the operations of the Cancer Therapy Evaluation Program, particularly the Investigational Drug Branch, Regulatory Affairs Branch, Biometric Research Branch and the Protocol and Information Office. The Contractor is responsible for the data collection/compilation, technical report preparation, monitoring of clinical activities, administrative coordination, and general logistical support, particularly in the area of investigational drugs which are subject to regulation by the Food and Drug Administration (FDA).

The contract is divided into two principal parts: 1) clinical research/FDA compliance support which includes clinical research support, regulatory affairs support, and drug distribution support; and 2) protocol and information support which includes protocol review and approval, protocol information tracking and dissemination, and protocol results and publications.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7724)

This contract provides a resource to evaluate the antitumor activity and toxicity of antitumor agents using murine leukemia and solid tumor models. After the responsibility for scientific direction and funding for this contract was transferred from DTP to CTEP in 1987, emphasis shifted from the study of congener compounds to the evaluation of antitumor synergy between agents with activity in clinical trials. Although it is planned to conduct some studies using single agents, in general, pairs of agents with demonstrated antitumor activity will be evaluated for the development of combination regimens for clinical trials. Studies to be done under this contract fall into one of the following categories: combinations of cytotoxic agents; combinations of biologic agents; combinations of biologic and cytotoxic agents; single agent antitumor activity of selected biologic agents; and evaluation of biochemical modulation in vivo. The results of these preclinical studies and clinical trials will be compared to determine the utility of this approach in designing combination regimens for clinical trial.

TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER (NO1-CM5-7737)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with Flavone Acetic Acid, Deoxyspergualin, Taxol, TNF, Trimetrexate (leukemias) and I.A. Mitoxantrone. Pharmacokinetic studies have been conducted on all patients accrued.

TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER (NO1-CM7-3707)

This contract is one of a group of Contractors which enter patients onto common protocols. Each Contractor performs clinical evaluations of treatment regimens utilizing activated leukocytes sponsored by the Division of Cancer Treatment under an investigational new drug application (INDA). The objectives of these protocols are to: 1) determine the antitumor activity of activating agents in combination with activated leukocytes in a variety of human cancers in patients with advanced disease; 2) define the toxicities of activating agents administered in combination with activated leukocytes to patients with advanced cancer; and 3) evaluate new methods for activation of leukocytes and determine the antitumor activity of activated leukocytes generated using the new methodology.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-7739)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents (or combination of agents) sponsored by the Division of Cancer Treatment and Phase II/III studies in patients with disseminated solid tumors and leukemia. The Contractor is conducting and/or has completed Phase I studies with Ifosfamide with Mesna, TMTX, Amonafide, Ara-AC, Methyl-CCNU/5-FU, IL-2/gamma interferon, Gallium Nitrate (abnormal organ function), a melanoma monoclonal antibody study, Fostricin and Melphalan/CSF. Phase II studies include: TNP in refractory metastatic breast cancer; Mitoxantrone in breast cancer; CHIP in metatastic breast cancer; high dose ifosfamide with Mesna protection and high dose Vincristine in small cell lung cancer; high dose ifosfamide with Mesna and Methotrexate, VP-16 and Methyl GAG for relapsing or refractory lymphoma; Carboplatin in malignant primary brain tumors; Carboplatin plus cisplatin in recurrent or advanced squamous cell carcinoma of the head and neck; Phase II Pilot study of Carboplatin and continuous infusion Bleomycin in advanced or recurrent squamous cell carcinoma of the head and neck; Trimetrexate (TMTX) in advanced colorectal cancer, metastatic bladder cancer and metastatic or unrestectable primary advanced carcinoma of the uterine cervix; Dihydro-5-Azacytidine in malignant mesothelioma; Gallium Nitrate administered by continuous intravenous infusion in refractory metastatic breast cancer; 2-Fluoro-Ara-Amp in metastatic colorectal cancer; Iproplatin in patients with metastatic gastric adenocarcinoma and advanced, measurable adenocarcinoma of the pancreas; Taxol in patients with metastatic melanoma, non-small cell lung cancer, metastatic breast cancer and colorectal cancer; Didemnin-B in patients with advanced, measurable colorectal cancer, non-small cell lung cancer, small cell lung cancer and metastatic breast cancer; high dose melphalan and total body irradiation with autologous bone marrow rescue as consolidation therapy for multiple myeloma in second remission following VAD or Dexamethasone; intravenous melphalan in patients with advanced, measurable colorectal cancer; pilot study of high dose ifosfamide with Mesna and Methotrexate, VP-16 and Methyl GAG for relapsing or refractory lymphoma; and screening human material for determinants of antineoplastic drug resistance.

THERADEX SYSTEMS, INC. (NO1-CM3-7553)

The objective of this contract is to provide a Clinical Trials Monitoring Service for the Phase I/II CTEP and BRMP investigators and all other investigators using NCI-sponsored investigational drugs. This service has four components--(1) a central data management resource for investigators conducting Phase I clinical trials and Phase II/LAK-IL2 trials; (2) an onsite audit resource for DCT to assure that Phase I/II Contractors are in compliance with Federal regulations; (3) co-site visiting cooperative group members as observers of peer audits; and

(4) onsite auditing of all other individual investigators conducting investigational trials. This contract expired June 29, 1988, and will be succeeded by contract N01-CM8-2708 also to Theradex Systems, Inc.

WARNER-LAMBERT COMPANY (NO1-CM3-7285)

This is a no-cost contract which provides for the development and marketing of diaziquone (AZQ) as an antitumor agent. Its purpose is to facilitate development of the agent through Phase III, prepare the New Drug Application (NDA) for submission to the Food and Drug Administration (FDA) and market the agent following FDA approval of the NDA. Warner-Lambert is collecting the data from their Phase III study of diaziquone and BCNU in primary brain tumors in addition to the data from a study at Duke which compared diaziquone with BCNU in an adjuvant setting. Warner-Lambert will use the data from their study and the Duke study for their NDA submission to FDA.

WISCONSIN, UNIVERSITY OF (NO1-CM5-7735)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with TNF, TNF+gamma interferon, 5-FU/Dipyridamole and SR-2508/CTX. Pharmacologic studies are included for both cytoxic protocols.

CLINICAL ONCOLOGY PROGRAM

BIONETICS RESEARCH, INC. (NO1-CM5-7688)

This contract supports Surgery Branch research by providing human lymphokine activated killer cells for therapeutic trials. This research is directed toward developing new adoptive immunotherapies for the treatment of cancer using lymphoid cells expanded in interleukin-2 or using interleukin-2 directly as an immune adjuvant.

NAVAL HOSPITAL, BETHESDA REGION (YO1-CM8-0159)

The overall objectives and specific accomplishments of this Interagency Agreement between the Naval Hospital, Bethesda (NH-BETH), and the National Cancer Institute (NCI) that we hope to achieve are: (1) Performance of clinical investigations into the diagnosis, staging, and treatment of a variety of malignant diseases through the mechanism of IRB approved clinical protocols. These protocols include studies developed by the NCI-Navy and Naval Hospital, Bethesda, Hematology-Oncology Branches, and the NCI Clinical Center Branches. They represent a collaborative effort between the NH-BETH and the NCI; (2) Integration of the NCI into the patient treatment program of the NH-BETH; and (3) Development of a laboratory program by NCI-Navy Medical Oncology Branch to investigate the biology of human tumors.

ORKAND CORPORATION (NO1-CM6-7716)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the Clinical Branches of the Clinical Oncology Program.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS) (YO1-CM5-0134)

The USUHS will provide one faculty position to support the collaborative efforts for research and educational opportunities between the USUHS, National Cancer Institute (NCI), and Naval Hospital, Bethesda, Maryland (NH-BETH). These collaborative efforts are mutually beneficial to both agencies due to the furthering of both research interests and educational opportunities.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS) (YO1-CM6-0141)

The purpose of this agreement is to document collaborative efforts between the USUHS, National Cancer Institute (NCI), and Naval Hospital, Bethesda, Maryland (NH-BETH). The USUHS will provide positions to support the collaborative efforts for research and educational opportunities between the two agencies.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS) (YO1-CM7-5050)

The purpose of this Memorandum of Understanding (MOU) is to permit collaborative efforts among the USUHS, National Cancer Institute (NCI), and the Naval Hospital, Bethesda, Maryland (NH-BETH) to improve quality of care of patients with cancer and related diseases at NH-BETH. It is recognized by all parties that quality of radiation therapy services is a critical determinant of the quality of a clinical oncology program. All parties recognize that the quality of radiation therapy services are necessary to the research mission of the NCI-NH-BETH medical oncology program and to the undergraduate education mission of USUHS.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS) (YO1-CM8-0158)

The purpose of this agreement is to document collaborative efforts between the USUHS, National Cancer Institute (NCI), and Naval Hospital, Bethesda, Maryland (NH-BETH). These efforts are mutually beneficial to both Institutes due to the furthering of research interests and educational opportunities. The USUHS will provide positions within the Department of Pharmacology. These positions shall be used to employ staff who will work on specific research projects identified by the USUHS and NCI and be responsible for specific duties related to a USUHS faculty appointee.

DEVELOPMENTAL THERAPEUTICS PROGRAM

ABBOTT LABORATORIES (NO1-CM6-7862)

This resource contract provides the Division of Cancer Treatment with facilities and personnel for the development and production of oral dosage forms of investigational anti-AIDS drugs. The dosage forms are manufactured in conformity with U.S. FDA Current Good Manufacturing Practices and in a manner to protect personnel from exposure to potentially toxic agents. The

Contractor is also responsible for all required quality control tests on each agent prepared. These dosage forms are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

AEROJET STRATEGIC PROPULSION COMPANY (NO1-CM8-7225)

This service preparative contract provides for the resynthesis of a variety of compounds required for the clinical evaluation in Phase II and III trials as potential anti-AIDS agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

AERON BIOTECHNOLOGY INC. (N44-CM7-7826) (SMALL BUSINESS INNOVATION RESEARCH Program)

The goal of this project is to develop an in vitro test to improve selection of therapies for individual breast cancer patients by predicting response to methotrexate. The contractor is evaluating a novel assay in which uptake of fluorescein-conjugated methotrexate is measured using a fluorescence activated cell sorter (FACS). The assay does not require clonal growth. Assay conditions are being developed using human breast cancer cell lines. Future correlation and validation studies with fresh tumor specimens are planned. The assay may be useful in determining patient sensitivity and in evaluating mechanisms of resistance to methotrexate.

ALABAMA, UNIVERSITY OF (NO1-CM6-7971)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

ALABAMA, UNIVERSITY OF (NO1-CM8-7267)

This contract is for chemical synthesis and drug design of a variety of compounds for evaluation as potential anti-AIDS agents. The specific objectives of this project are: (a) to synthesize congeners of synthetic compounds with confirmed activity; (b) to design and synthesize prodrugs, and other compounds that possess elements of both congener and prodrug; (c) to synthesize compounds related to products of natural origin and other related heterocycles; and (d) to synthesize anti-sense nucleic acids. These products may include partial structures of analogs and novel heterocycles.

ALDRICH CHEMICAL COMPANY, INC. (NO1-CM6-7771)

The objective of this contract is to synthesize a variety of compounds which have been identified by the program for further development, preclinical toxicology and Phase I clinical trials according to established standards. This contract has recently been transferred to the Pharmaceutical Resources Branch.

ALDRICH CHEMICAL COMPANY, INC. (NO1-CM6-7929)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical evaluation in Phase II and III trials as potential antitumor agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ARIZONA, UNIVERSITY OF (NO1-CM5-7662)

This contract was awarded for the testing of approximately 100 materials in the Human Tumor Colony Forming Assay (HTCFA). Materials found to be inactive in the P388 *in vivo* prescreen are initially tested in the P388 colony forming assay. Those found to be inactive in this assay are then tested in the HTCFA for efficacy. This contract, originally scheduled for termination September 30, 1987, was extended with no additional funds being allocated, until December 31, 1987.

ASH STEVENS, INC. (NO1-CM6-7871)

This service preparative contract provides for the resynthesis of a variety of compounds required for the clinical evaluation in Phase II and III trials as potential anti-AIDS agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ASH STEVENS, INC. (NO1-CM6-7872)

This contract is part of the new Developmental Therapeutics Program (DTP) initiative for AIDS drug development. Chemicals are synthesized on large-scale for toxicology studies and Phase I clinical trial according to GLP and GMP standards. This contract has recently been transferred to the Pharmaceutical Resources Branch.

ASH STEVENS, INC. (NO1-CM6-7927)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical evaluation in Phase II and III trials as potential antitumor agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

AUTOMATED PRECISION, INC. (N43-CM7-3730) (SMALL BUSINESS INNOVATION RESEARCH Program)

This Contractor intends to demonstrate the feasibility of performing solubility determinations automatically. The process depends on measuring the light scattered by a laser beam impinging upon undissolved particles. The DTP currently carries out a considerable number of such

determinations, which are performed manually in the DTP laboratories. The proposed approach is likely to be less expensive, and will lessen exposure to chemicals that often are highly toxic. This is a Phase I project.

BATTELLE MEMORIAL INSTITUTE (NO1-CM1-7365)

This service type Prime Contract with Battelle Memorial Institute is for management of subcontractors carrying out preclinical toxicology studies on potential oncolytic agents, biologic response modifiers and other modalities. Through the Prime Contract mechanism, preclinical toxicology studies of agents under consideration for clinical use are handled under a single management-type contract. The work scope under this contract is comprised of four tasks: Task I - Protocol Studies; Task II - High Priority Studies (i.e., any portion of the protocol of the Toxicology Branch); Task III - Organ Specific Toxicity Testing; and Task IV - Automation of toxicity data, anomaly detection, scheduling of studies, and financial management. This contract expired November 30, 1987.

BATTELLE MEMORIAL INSTITUTE (NO1-CM6-7869)

This contract is for the performance of preclinical toxicology studies of potential therapeutic agents for treating AIDS. These preclinical studies primarily are designed to relate target organ toxicity to plasma drug concentration and in vitro efficacy. Protocols include pharmacokinetic and bioavailability studies in rodents and dogs, cerebrospinal fluid penetration by the drug in dogs, continuous intravenous infusion toxicity studies in dogs and 28-day multiple dose toxicity studies in dogs and rodents. The data from these studies are used to prepare attachment 6a for the INDA. This is the second year of a three year contract.

BEN VENUE LABORATORIES, INC. (NO1-CM6-7865)

This Contractor is required to manufacture dosage forms of compounds with potential activity in AIDS. Compounds are selected and provided by the Government. The Contractor is responsible for manufacturing these dosage forms in conformity to U.S. FDA Current Good Manufacturing Practice regulations. Other responsibilities include quality control testing, packaging, labelling, and distribution of the final product to the National Cancer Institute.

BEN VENUE LABORATORIES, INC. (NO1-CM7-3719) (FORMERLY NO1-CM2-7508)

This resource contract provides for the development and production of parenteral clinical dosage forms of anticancer agents. The Contractor has the capability of preparing production batches of liquid-filled and lyophilized sterile products. Specifically, the Contractor performs the following services: (1) formulation development of parenteral products; (2) production of sterile products; and (3) quality assurance testing of finished products. All products are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

CALIFORNIA, UNIVERSITY OF (NO1-CM5-7710)

This contract was issued for the use of the Human Tumor Colony Forming Assay (HTCFA) in the testing of approximately 75 materials per year. Those materials found to be inactive in both the in vivo P388 assay and the P388 colony forming assay are tested in the HTCFA. This contract was scheduled for termination September 30, 1987, and was extended with no additional funds being allocated until December 31, 1987.

CHARLES RIVER LABORATORIES (NO1-CM3-7526)

This contract provides for the rederivation of approximately 16 mouse and rat strains and two guinea pig strains on an annual basis. Rederived strains will be distributed to genetic centers for expansion and replacement of producing strains. This contract terminated December 31, 1987, and was recompeted.

CHARLES RIVER LABORATORIES (NO1-CM6-7881)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities.

CIVILIZED SOFTWARE (N44-CM7-7811) (SMALL BUSINESS INNOVATION RESEARCH Program)

This Phase II project has as its target product a PC version of the mathematical modelling and curve fitting program MLAB. MLAB currently is written in SAIL and runs on DCRT's DEC10. The new program is to be written in C and is targeted for the IBM AT class PC.

CONNECTICUT, UNIVERSITY OF (NO1-CM5-7692)

This contract furnishes extracts and cryopreserved cultures from 500 strains of unique genera of fungi per year for antitumor and anti-AIDS evaluations. The contract calls for fermentation in three different media, harvest at two stages of the growth cycle and preparation of two extracts, a mycelial extract and a lyophilized broth preparation. Taxonomic distribution is broadly distributed over the Classes Ascomycetes, Basidiomycetes, Deuteromycetes, and Zygomycetes. More than 900 unique organisms have been cultured, over 2,000 extracts from 379 organisms have been delivered for testing and extracts from an additional 300 organisms are awaiting delivery. This contract will expire September, 1988.

CREARE (N44-CM8-7966) (SMALL BUSINESS INNOVATION RESEARCH Program)

This Phase II project, like the Phase II project awarded to Civilized Software, has as its target product a PC version of the mathematical modelling program MLAB. Also to be written in C, this version has as its target computer a 80386 microprocessor based 32 bit machine running under the Xenix operating system.

DARTMOUTH COLLEGE (NO1-CM6-7976)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

ERCI FACILITIES SERVICE CORPORATION (NO1-CM6-7920)

This resource contract provides the Division of Cancer Treatment with storage and distribution capabilities for the large volume of investigational drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. The Contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs, and the prompt removal of expired materials. Further, computerized records are kept of all shipments made and returns received which aids in accountability.

ERCI FACILITIES SERVICE CORPORATION (NO1-CM7-3721)

This Contractor furnishes the National Cancer Institute with facilities and services for the storage and distribution of synthetic chemicals, bulk chemical drugs, and crystalline natural products. Samples are weighed, packaged, and shipped to contract screening laboratories and also to various domestic and foreign research institutions. The contract provides for the maintenance of accurate computerized inventory shipping and distribution records. This is an ongoing operation and supports all the DTP programs.

FEIN-MARQUART ASSOCIATES, INC. (NO1-CM7-3727)

This service contract provides for the maintenance of the Drug Information System (DIS) used by the DTP staff, DTP contractors, and DTP suppliers. The DIS is a computer-based system, containing information on the nearly half million chemicals that have been tested by the DTP. The information includes test results, sources, physical data, shipping histories, graphical chemical structures, etc. The system generates off line reports, is accessible online for querying, and is used for monitoring as well. The Contractor maintains the files, takes care of software problems as they occur, and responds to DTP requirements for alterations and improvements of the computer programs.

FEIN-MARQUART ASSOCIATES, INC. (N44-CM6-7813) (SMALL BUSINESS INNOVATION RESEARCH Program)

This SBIR Phase II contract will provide the Pharmaceutical Resources Branch with a computerized pharmaceutical tracking system for investigational drug development. The tracking system will provide computer support for monitoring bulk chemicals and chemical analyses, dosage form development, pharmaceutical production, inventory and distribution control, and shelf-life studies.

GEORGIA, UNIVERSITY OF (NO1-CM7-3712)

This contract, with the University of Georgia Research and Education Foundation, has the responsibility of performing shelf life evaluation of clinical drugs. The Contractor monitors the stability of dosage forms at several storage temperatures. The testing involves the use of multiple analytical methods. The method most frequently used for assay of the stability samples is high performance liquid chromatography (HPLC). The data that is developed is used to verify the stability of NCI's investigational drugs during the clinical trials and is supplied to the U.S. Food and Drug Administration in support of NCI's IND filings. This Contractor also has the responsibility of conducting reserve sample inspections as required by the FDA Current Good Manufacturing Practices.

GEORGIA TECH RESEARCH CORPORATION (NO1-CM8-7269)

This contract is for chemical synthesis and drug design of a variety of compounds for evaluation as potential anti-AIDS agents. The specific objectives of this project are: (a) to synthesize congeners of synthetic compounds with confirmed activity; (b) to design and synthesize prodrugs and other compounds that possess elements of both congener and prodrug; (c) to synthesize compounds related to products of natural origin and other related heterocycles; and (d) to synthesize anti-sense nucleic acids. These products may include partial structures of analogs and novel heterocycles.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM2-3911)

This contract operates the Animal Production Area at the Frederick Cancer Research Facility (FCRF). The contract operates as a Primary Genetic Center, Rederivation Center, and Embryo Freezing Center. Strains are received from the NIH Repository for use at the FCRF and distribution to other NCI contract activities. The bulk of the production on this contract is for supplying the animal needs of the researchers located at the FCRF. Animals are also sent from FCRF to other NCI funded research activities. This contract was recompeted and Harlan/Sprague Dawley was awarded a seven year extension of this same contract.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM6-7918)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for the many NCI research activities.

HAUSER CHEMICAL RESEARCH, INC. (NO1-CM8-7221)

This Master Agreement for Master Agreement Orders in the large-scale isolation of antitumor agents from natural sources was established to permit the isolation and purification of active materials from organisms, such as plants and marine invertebrates, for use in preclinical development and clinical trials. This Master Agreement Order mechanism enables the prompt solicitation and award of projects to be conducted in this area from a pool of qualified contractors. As yet, no project has been assigned to the Contractor.

HAWAII, UNIVERSITY OF (NO1-CM6-7745)

This contract furnishes extracts and preserved cultures from 180 unique strains of blue-green algae (cyanobacteria) per year for antitumor and anti-AIDS evaluations. During the first 18 months of the contract a total of 680 extracts from 340 organisms were delivered and 168 preserved, viable cultures were deposited. Biological testing of the extracts has shown several novel bioactive materials which are under investigation as potential anti-AIDS chemotherapeutic agents. A total of 650 cultures have been cultivated already over a broad taxonomic distribution within the Orders Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales, and Stigonematales.

HAZLETON LABORATORIES AMERICA (NO1-CM6-7931)

This contract is for the performance of preclinical toxicology studies of potential therapeutic agents for treating AIDS. These preclinical studies primarily are designed to relate target organ toxicity to plasma drug concentration and in vitro efficacy. Protocols include pharmacokinetic and bioavailability studies in rodents and dogs, cerebrospinal fluid penetration by the drug in dogs, continuous intravenous infusion toxicity studies in dogs and 28-day multiple dose toxicity studies in dogs and rodents. The data from these studies are used to prepare attachment 6a for the INDA. This is the second year of a three year contract.

H.G. PARS PHARMACEUTICAL LABS., INC. (NO1-CM6-7972)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

HIPPLE CANCER RESEARCH CORPORATION (N43-CM5-7831) (SMALL BUSINESS INNOVATION RESEARCH Program)

The objectives of this contract are to standardize in vitro human murine and canine bone marrow progenitor assays for use in quantitative and qualitative measurements of the toxic effects of known and developing anticancer drugs. The toxicities found in bone marrow culture will be correlated with clinical hematotoxicity. In vitro studies of drug induced myelotoxicity have largely used mouse bone marrow. However, because of the differences in murine and human pharmacokinetics, drug metabolism and drug sensitivity of hematopoietic precursor cells, such murine studies often have not provided useful quantitative data on levels of clinical hematotoxicity in humans. Comparative murine versus canine versus human myelotoxicities will enhance dosing and scheduling efficiency in the clinic when considered in light of preclinical findings in rodents and dogs.

HIPPLE CANCER RESEARCH CORPORATION (N44-CM7-7829) (SMALL BUSINESS INNOVATION RESEARCH Program)

Having demonstrated the feasibility of growing human tumor cell colonies in glass microcapillaries during a Phase I SBIR contract, a Phase II contract was awarded. In this first year, of the two year contract, improvements to instrumentation are to be made, optimal assay conditions will be established, and studies involving colony growth kinetics and the in vitro stability of anti-cancer agents in this system will be initiated.

ILLINOIS, UNIVERSITY OF (NO1-CM6-7705)

The objective of this contract is to perform a survey of the literature published worldwide on natural products and related fields, and to provide the Project Officer pertinent information required for the evaluation and acquisition of new and novel compounds that may have biological activity useful in the treatment of cancer. Reports of biological activity of extracts of plants, animals, bacteria, fungi, and marine organisms are also provided. This is a key contract for the acquisition of new agents for the DTP screening program. This contract has been awarded for a three-year period ending December 30, 1988.

ILLINOIS, UNIVERSITY OF (NO1-CM6-7925)

This Contractor is undertaking plant collections in South East Asia. The objective is to collect 1,500 plant samples per year for submission to NCI for extraction and screening against a panel of human cancer cell lines. Collections are currently ongoing in the Philippines, Malaysia, and Thailand. A number of medicinal plants have been collected in Nepal, and negotiations to collect in Indonesia are being finalized. Over 2,500 samples have been submitted to the Natural Products Repository at the Frederick Cancer Research Facility in Frederick, MD.

INSTITUT JULES BORDET (NO1-CM5-7645)

Primarily, in vivo testing of materials collected in Europe is conducted at this laboratory. On occasion materials that originated in the U.S. or other countries are tested. In vivo assays, at a level of approximately 10,000 L1210 equivalents, are scheduled. Testing, in depth, is scheduled upon request of the Project Officer. This contract is scheduled for termination September 30, 1988.

INTEGRATED GENETICS, INC. (N44-CM8-3717) (SMALL BUSINESS INNOVATION RESEARCH Program)

The objective of this contract is to develop a panel of well-characterized human tumor sublines that express the MDR (multidrug resistance) phenotype for use in large-scale, disease-oriented anticancer drug screening programs. The sublines will be obtained by selection in drug-containing media or by gene transfer techniques. The Contractor will characterize the sublines for multidrug resistance, mdr gene copy number, etc. These sublines should provide a valuable resource for study of the molecular mechanisms contributing to the MDR phenotype and should be useful in identifying new anticancer agents for the treatment of drug-resistant neoplasms.

IOWA, UNIVERSITY OF (NO1-CM4-7594)

This resource contract provides the Division of Cancer Treatment with facilities and personnel for development and production of oral dosage forms of investigational anti-cancer drugs. The dosage forms are manufactured in conformity with U.S. Current Good Manufacturing Practices and in a manner to protect personnel from exposure to potentially toxic agents. The Contractor is also responsible for all required quality control tests on each product prepared. These dosage forms are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

IOWA, UNIVERSITY OF (NO1-CM6-7916)

This contract provides services involving dosage form development and manufacture of investigational drugs for subsequent clinical evaluation. Compounds to be formulated are selected and provided by the National Cancer Institute. The Contractor has primarily developed and produced sterile freeze-dried injectable products under this contract. However, this Contractor has the capability to produce a wide variety of pharmaceutical dosage forms. The Contractor is also responsible for completing all required quality control tests on each lot of drug. All products are packaged, labeled, and shipped to the National Cancer Institute for redistribution to clinical investigators.

IOWA, UNIVERSITY OF (NO1-CM6-7979)

This resource contract provides the Division of Cancer Treatment with facilities and personnel for the development and production of oral dosage forms of investigational anti-AIDS drugs. The dosage forms are manufactured in conformity with U.S. FDA Current Good Manufacturing Practices and in a manner to protect personnel from exposure to potentially toxic agents. The Contractor is also responsible for all required quality control tests on each agent prepared. These dosage forms are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

KANSAS, UNIVERSITY OF (NO1-CM6-7912)

This contract investigates approaches to resolve difficult dosage form development problems not amenable to usual solubilization or stabilization methods. This Contractor has considerable expertise in the application of molecular complexes and reversible derivatives to improve solubility. The Contractor also is responsible for pilot scale preparation and chemical analysis of the formulations developed under this contract.

MAYO FOUNDATION (NO1-CM6-7904)

Preclinical pharmacology studies of antitumor agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data as well as the efficiency of Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to preclinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of antitumor agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because of the increasing emphasis on in vitro screens which is contemplated in the next several years. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed preclinically, the Phase I clinician is alerted to their potential human significance. This is a Task Order Managed contract.

MIAMI, UNIVERSITY OF (NO1-CM6-7877)

This contract provides for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility or a contract research laboratory. All testing is scheduled by the Project Offcier.

MICROBIOLOGICAL ASSOCIATES (NO1-CM7-3728)

This contract functions in four major areas: 1) to operate and maintain a virus serum diagnostic laboratory. Serum samples are submitted from contract animal suppliers and testing laboratories; 2) to test experimental tumors (animal and human) for viral contaminants; 3) to perform ELISA

tests annually for the detection of mouse hepatitis virus (MHV); and 4) to produce vaccinia virus which is used for immunizing mice against infectious ectromelia. This contract terminated February 29, 1988.

MIDWEST RESEARCH INSTITUTE (NO1-CM6-7932)

This contract is for the performance of preclinical toxicology studies of potential therapeutic agents for treating AIDS. These preclinical studies primarily are designed to relate target organ toxicity to plasma drug concentration and in vitro efficacy. Protocols include pharmacokinetic and bioavailability studies in rodents and dogs, cerebrospinal fluid penetration by the drug in dogs, continuous intravenous infusion toxicity studies in dogs and 28-day multiple dose toxicity studies in dogs and rodents. The data from these studies are used to prepare attachment 6a for the INDA. This is the second year of a three year contract.

MIDWEST RESEARCH INSTITUTE (NO1-CM7-3713)

Midwest Research Institute is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program as potential antitumor agents. The Contractor determines identity and purity of the compounds by appropriate methods. The Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (thin layer, gas liquid and high performance liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the Contractor provide the data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

MIDWEST RESEARCH INSTITUTE (NO1-CM8-7202)

This is a contract for the preclinical toxicological evaluation of potential antineoplastic drugs. Studies are performed in both rodents and dogs in order to determine target organ toxicity and its reversibility and to recommend safe clinical starting doses. The types of studies performed are: single and multiple dose toxicity; toxicity after continuous administration of up to 120 hours; and pharmacokinetics. The data from these studies are used to prepare attachment 6a for the INDA. This is the first year of a five-year contract.

MIDWEST RESEARCH INSTITUTE (NO1-CM8-7228)

Midwest Research Institute is one of the two contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program as potential anti-AIDS agents. The Contractor determines identity and purity of the compounds by appropriate methods. The Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (thin layer, gas liquid and high performance liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the Contractor provide the data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

MISSOURI, UNIVERSITY OF (NO1-CM6-7723)

This contract provides for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility in a contract research facility. All testing is scheduled by the Project Officer.

MISSOURI BOTANICAL GARDEN (NO1-CM6-7923)

This Contractor is undertaking plant collections in Madagascar and adjacent islands, and tropical and subtropical areas of Africa. The objective is to collect 1,500 plant samples per year for submission to NCI for extraction and screening against a panel of human cancer cell lines. Collections are currently ongoing in Cameroon and the Central African Republic, and over 2,000 samples have been submitted to the Natural Products Repository at the Frederick Cancer Research Facility in Frederick, MD. Collections are currently being initiated in Tanzania.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-7644)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals, standards for nomenclature used to identify stocks and strains of laboratory animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by an Ad Hoc committee whose membership represents commercial animal production colonies, governmental and academic institutions, and non-profit research institutions.

NEW MEXICO STATE UNIVERSITY (NO1-CM6-7974)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

NEW YORK BOTANICAL GARDEN (NO1-CM6-7924)

This Contractor is undertaking plant collections in Central and South America with emphasis on the tropical rain forest areas. The objective is to collect 1,500 plant samples per year for submission to NCI for extraction and screening against a panel of human cancer cell lines. Collections are currently ongoing in twelve Central and South American countries, and over 2,400 samples have been collected, including over 200 medicinal plant samples from Belize.

NORTHWESTERN UNIVERSITY (NO1-CM8-7257) (Formerly NO1-CM3-7538)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM6-7903)

Preclinical pharmacology studies of antitumor agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data as well as the efficiency of the Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to preclinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of antitumor agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because of the increasing emphasis on in vitro screens which is contemplated in the next several years. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed preclinically, the Phase I clinician is alerted to their potential human significance. This is a Task Order Managed contract.

ORI, INC. (NO1-CM6-7909)

This is a "fast response" service contract, intended to provide computer programming support for the various and often urgent needs arising from the DTP drug testing program. Accordingly, in the past, the Contractor has created software to enhance a chemical-structure input program, to handle robot-generated data, to monitor DTP computer utilization, to accept new databases within the DIS, to display, in graphic form, the results of in vitro tests and so on. The contract is operated through a task-order mechanism.

PATHOLOGY ASSOCIATES, INC. (NO1-CM8-7258)

This contract provides pathology and veterinary services to the Toxicology Branch to support the preclinical toxicological evaluation of drugs for cancer and AIDS. In addition to a pathology materials repository, this contract is utilized to perform pathology quality assurance review of completed studies; pathology support in the form of site visits, slide preparation, performing necropsies, and histopathologic diagnosis of lesions; veterinary support such as site visits, the development of special surgical procedures, and instruction in these procedures; and equipment storage, maintenance and shipment. This is the first year of a five year contract.

PHARM-ECO LABORATORIES, INC. (NO1-CM6-7928)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical evaluation in Phase II and III trials as potential antitumor agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

PHARM-ECO LABORATORIES, INC. (NO1-CM6-7933)

This service preparative contract provides for the resynthesis of a variety of compounds required for the clinical evaluation in Phase II and III trials as potential anti-AIDS agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

POLYSCIENCES, INC. (NO1-CM6-7707)

This Master Agreement for Master Agreement Orders in the large-scale isolation of antitumor agents from natural sources was established to permit the isolation and purification of active materials from organisms, such as plants and marine invertebrates, for use in preclinical development and clinical trials. This Master Agreement Order mechanism enables the prompt solicitation and award of projects to be conducted in this area from a pool of qualified contractors. The Contractor is currently working on a task to isolate Taxol, NSC 125973, from the bark of *Taxus brevifolia*.

POLYSCIENCES, INC. (NO1-CM6-7977)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

PROGRAM RESOURCES, INC. (NO1-C07-4102)

This Contractor is located at the Frederick Cancer Research Facility in Frederick, Maryland, and is divided into the following segments:

Biological Testing Branch (BTB)

Provides for two Information Specialists and one Administrative Specialist in the Office of the BTB.

Biological Training Program

Support is provided for students from Frederick Community College (FCC) who work a minimum of 20 hours per week for DTP projects. Support includes tuition, books, guest lecturer fees, and some laboratory expenses when a project is not clearly defined.

DTP Chemical Prep Lab

This project is responsible for the appropriate weighing, solubilization, partial chemical characterization and the preparation of multiple aliquots of natural product and synthetic materials prior to their testing by project 76622 for their ability to inhibit growth of any class of a wide variety human tumor cell lines *in vitro* and *in vivo* when the latter systems are established.

Genetic Monitoring

Monitors all of BTB contract rat colonies for genetic purity. In addition, monitors the starts received from VRB-NIH, both pre- and post-rederivation.

Gut Flora Monitoring

Receives thirty animals weekly from one of our Primary Genetic Centers. The animals originate from our isolator colonies at the Genetic Centers, and this contract monitors their animals for gut flora and possible virus contamination. The animals are sent weekly on a schedule made up by BTB.

In Vitro Cell Line Screening

In Vitro Cell Line Screening Program consists of four projects:

In Vitro Cell Line Screen

This project supports equipment, media, serum procurement, and other activities that cut across or support all of the following three projects.

IVCLSP 560 - Primary Drug Evaluation Laboratory

This project supports that part of the IVCLSP program that serves as the operational center for the primary (first stage) *in vitro* screening of unknown agents against multiple human tumor cell lines in an attempt to discover new anti-cancer drugs. The objective is to screen 10,000 substances against 100 well characterized human cell lines annually.

IVCLSP 539 - In Vitro Drug Investigations Laboratory

This project provides the program with a research capacity for developing additional assay techniques appropriate for use in the Primary Drug Evaluation Laboratory and focuses on microtiter assays suitable for high-volume, semi-automated testing. In addition, this project will support the development of secondary and/or confirmatory screening techniques as a follow-on for those substances found to have anticancer activity in the primary screening assay.

IVCLSP 434 - In Vitro Cell Systems Research and Development Laboratory

This project supports efforts to develop suitable cell lines for use in the primary assays from a wide variety of human tumors. The work involves establishing tissue cultures from direct tumor tissue or xenografts, establishing their identity and freedom from adventitious agents, and characterizing those biological and biochemical attributes known or suspected of being important in the primary assay.

In Vivo Model Development

This activity is closely related to the *in vitro* cell line screening project described above. Selected human tumor cell lines are being developed for use as models for *in vivo* drug evaluations.

Partial Support for Harlan/Sprague Dawley

Provides Work Orders and Shared Service type functions for the Harlan/Sprague Dawley Animal Production contract at Frederick. It serves as a method for paying PRI for services performed at the Animal Production Area at Frederick.

PDRG Laboratory Support

Provides service support, including materials, supplies, and equipment purchases for the PDRG intramural laboratory.

Rodent Serology Monitoring

Receives serum samples from animal contractors and research laboratories to test for viral contaminants monthly. The scheduling of the serum samples is done by the BTB Project Officer.

Tumor Bank

This segment has as its major goal the maintenance of approximately 20,000 frozen tumor vials. This Contractor furnishes needed tumors to the various DTP laboratories, as well as to other research institutions, both domestic and foreign. The tumors are supplied both in vivo and in vitro.

Tumor Procurement and Preparation

Tumors will be procured from various surgical sites. Initial procurement efforts are being undertaken at Johns Hopkins University. The tumors will be adapted for the in vitro/in vivo DTP screening effort.

AIDS Chemical Prep Laboratory

This project is responsible for the appropriate weighing, solubilization, partial chemical characterization and the preparation of multiple aliquots of synthetic and natural product materials prior to their testing by project 76612 for ability to inhibit the growth of HIV in vitro, as well as in vivo when the assay procedures (for the latter) are established.

AIDS In Vitro Drug Screen Lab

This project provides research and development capacity, the facilities and the staff to develop the necessary technology, and implement the screening of large numbers of anti-HIV compounds annually in a safe, semi-automated system. Additionally, it serves as a conduit for the transfer of screening technologies to other laboratories involved in anti-HIV screening.

AIDS In Vivo Drug Screen Lab

This project supports a research and development effort (currently conducted primarily through PDRG) to establish systems whereby various drugs and natural products could be tested in vivo for their anti-HIV activity. This is envisioned as a follow-on assay for compounds subsequent to having been shown as active in vitro.

PURDUE RESEARCH FOUNDATION (NO1-CM6-7699)

The objectives of this contract are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; and (2) prodrugs that are chemically altered transport forms of the lead compound. The chemical modifications will aim at improving biological and pharmaceutical properties including (a) water-solubility; (b) hydrolytic stability; and (c) spectrum of activity and specificity. In addition, the contract provides for the modification of compounds of natural origin and synthesis of heterocycles with improved antitumor activity and reduced toxicity. These modifications may range from partial structures to structural analogs.

PURDUE RESEARCH FOUNDATION (NO1-CM8-7268)

This contract is for chemical synthesis and drug design of a variety of compounds for evaluation as potential anti-AIDS agents. The specific objectives of this project are: (a) to synthesize congeners of synthetic compounds with confirmed activity; (b) to design and synthesize prodrugs, and other compounds that possess elements of both congener and prodrug; (c) to synthesize compounds related to products of natural origin and other related heterocycles; and (d) to synthesize anti-sense nucleic acids. These products may include partial structures of analogs and novel heterocycles.

RAYLO CHEMICALS, LTD. (NO1-CM6-7866)

This contract is part of the new Developmental Therapeutics Program initiative for AIDS drug development. Chemicals are synthesized on large-scale for toxicology studies and Phase I clinical trial according to GLP and GMP standards. This contract has recently been transferred to the Pharmaceutical Resources Branch.

RAYLO CHEMICALS, LTD. (NO1-CM6-7975)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

This contract is part of the new Developmental Therapeutics Program initiative for AIDS drug development. Chemicals are synthesized on large-scale for toxicology studies and Phase I clinical trial according to GLP and GMP standards. This contract has recently been transferred to the Pharmaceutical Resources Branch.

RESEARCH TRIANGLE INSTITUTE (NO1-CM6-7703)

This contract provides for the synthesis of radiolabeled anticancer chemicals and drugs for use in preclinical pharmacological and clinical studies. The materials prepared are not available from commercial sources. All materials are analyzed for purity and identity by autography assay, etc. This contract also provides storage facilities for labeled materials and distributes labeled compounds as directed by the National Cancer Institute staff.

RESEARCH TRIANGLE INSTITUTE (NO1-CM6-7970)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

RESEARCH TRIANGLE INSTITUTE (NO1-CM7-3714)

Research Triangle Institute is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program as potential antitumor agents. The Contractor determines identity and purity of the compounds by appropriate methods. The Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (thin layer, gas liquid and high performance liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the Contractor provide the data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

RESEARCH TRIANGLE INSTITUTE (NO1-CM8-7227)

This contract provides for the synthesis of radiolabeled anti-AIDS compounds for use in preclinical pharmacological and clinical studies. The materials prepared are not available from commercial sources. All materials are analyzed for purity and identity by autography assay, etc. This contract also provides storage facilities for labeled materials and distributes labeled compounds as directed.

SEAPHARM, INC. (NO1-CM6-7919)

This contract was intended to furnish approximately 1,000 deep water (30-830 m) marine organisms per year for antitumor and anti-AIDS evaluations. During the first eighteen months a total of 581 samples were delivered. The project was not meeting its goals and together with difficulties in scheduling ship-time and increased collection costs, a decision was made to terminate the contract in February, 1988 for the convenience of the Government.

SEAPHARM, INC. (NO1-CM6-7967)

This contract furnishes approximately 1,000 shallow-water, marine organisms per year for antitumor and anti-AIDS evaluations. The organisms are selected to represent the greatest possible taxonomic and ecological diversity over a broad region of the Indo-Pacific area. During the first 18 months of the contract, 1,486 samples were delivered and an additional 378 samples are awaiting shipment. The contract also allows for bulk recollections of organisms of biological interest.

SIMONSEN LABORATORIES (NO1-CM6-7917)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities.

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM6-7973)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7646)

This contract is for *in vivo* testing at a level of 24,000 L1210 equivalents. The majority of testing is conducted utilizing tumor systems and protocols of the secondary tumor panel. Minimum testing is conducted in the P388 prescreen, which is being phased out. In depth *in vivo* studies for the characterization of potential new *in vivo* tumor models are conducted at the request of the Project Officer. This contract utilizes both conventional and nude mice. This contract is scheduled for termination September 30, 1988.

SOUTHERN RESEARCH INSTITUTE (YO1-CM6-0147)

This is a joint Department of Defense and National Cancer Institute contract for the testing of agents against the HTLV-III and related retroviruses. Approximately 160 assays are conducted per week. This contract is scheduled for termination July 14, 1988, and is being recompeted.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7905)

Preclinical pharmacology studies of antitumor/anti-AIDS agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data, as well as the efficiency of the Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to pre-clinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because of the increasing emphasis on *in vitro* screens. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed preclinically, the Phase I clinician is alerted to their potential human significance. This is a Task Order Managed contract.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7911)

In order to assist the Biological Testing Branch in the monitoring and quality control of existing human and murine tumor lines, as well as the host animals, this contract was awarded. The development of new human tumor lines, parallel to the disease-oriented in vitro prescreen, is conducted under this contract. Suggestions regarding the refinement of existing protocols are made as deemed necessary, and the cell kinetics for current tumor models are elucidated.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7968)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

SOUTHERN RESEARCH INSTITUTE (NO1-CM7-3726)

The major objective of this contract is to optimize the antitumor activity of agents identified by primary screens. To meet this objective, studies using various in vivo experimental tumor models are conducted in which drug concentration and exposure time of the tumor cells and host to the drug are varied. Results are interrelated with pharmacokinetic, toxicologic and biochemical information to devise and recommend treatment strategies for clinical trial. Other objectives are to explore new therapeutic approaches, and to more fully assess the therapeutic potential of a new drug by conducting experiments against advanced staged tumors and tumors at different sites, and by determining drug-resistance profiles.

SOUTHERN RESEARCH INSTITUTE (NO1-CM8-7232)

This is a new contract awarded to Southern Research Institute in May 1988. The objectives of the project are to optimize the in vivo antiviral activity and evaluate the therapeutic potential of compounds that are known to inhibit the growth and/or cytopathic effects of the human immunodeficiency virus (HIV). These objectives will be achieved by conducting a series of specialized in vitro and in vivo experiments using murine retroviral models which possess one or more properties of the syndrome produced by HIV infection. Tasks will include evaluation of the influence of drug schedule and route of administration on antiviral activity and determination of anti-viral activity of formulated products. Results from this project will be interrelated with pharmacologic and toxicologic information to devise and recommend treatment strategies for clinical trial.

SOUTHERN RESEARCH INSTITUTE (NO1-CM8-7259)

This is a contract for the preclinical toxicological evaluation of potential antineoplastic drugs. Studies are performed in both rodents and dogs in order to determine target organ toxicity and its reversibility and to recommend safe clinical starting doses. The types of studies performed are: single and multiple dose toxicity; toxicity after continuous administration of up to 120 hours; and pharmacokinetics. The data from these studies are used to prepare attachment 6a for the INDA. This is the first year of a five year contract.

SPRINGBORN LIFE SCIENCES, INC. (NO1-CM8-7256)

This is a contract for the preclinical toxicological evaluation of potential antineoplastic drugs. Studies are performed in both rodents and dogs in order to determine target organ toxicity and its reversibility and to recommend safe clinical starting doses. The types of studies performed are: single and multiple dose toxicity; toxicity after continuous administration of up to 120 hours; and pharmacokinetics. The data from these studies are used to prepare attachment 6a for the INDA. This is the first year of a five year contract.

SRI INTERNATIONAL (NO1-CM6-7864)

SRI International is one of the two contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program as potential anti-AIDS agents. The Contractor determines identity and purity of the compounds by appropriate methods. The Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (thin layer, gas liquid and high performance liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the Contractor provide the data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

SRI INTERNATIONAL (NO1-CM6-7969)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature with reported biological activity which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

SRI INTERNATIONAL (NO1-CM7-3715)

SRI International is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program as potential anti-tumor agents. The Contractor determines identity and purity of the compounds by appropriate methods. The Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (thin layer, gas liquid and high performance liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the Contractor provide the data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

STARKS ASSOCIATES (NO1-CM6-7798)

The objective of this contract is to synthesize a variety of compounds which have been identified by the program for further development, preclinical toxicology and Phase I clinical trials according to established standards. This contract has recently been transferred to the Pharmaceutical Resources Branch.

STARKS ASSOCIATES, INC. (NO1-CM6-7926)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical evaluation in phase II and III trials as potential antitumor agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

STARKS ASSOCIATES, INC. (NO1-CM6-7934)

This service preparative contract provides for the resynthesis of a variety of compounds required for the clinical evaluation in phase II and III trials as potential anti-AIDS agents. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. The major thrust of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

STARKS ASSOCIATES (NO1-CM6-7978)

This is one of the 11 Master Agreement Contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds from the literature, with reported biological activity and which cannot be obtained in sufficient quantities from the original investigators are also synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of compounds of interest to intramural scientists, and potential radiosensitizers and radioprotectors.

STARKS C.P. (NO1-CM4-7608)

This contract is in support of the Drug Synthesis and Chemistry Branch's fundamental responsibility to acquire selected novel synthetic compounds for evaluation as potential anti-cancer agents - the initial step in the National Cancer Institute's anticancer drug development program. The major focus of this contract is the active solicitation, acquisition, documentation and management of the flow of approximately 10,000 compounds per year of diverse structural and biological types. These compounds are selected by the Drug Synthesis and Chemistry Branch from a much larger pool of compounds provided through this contract in quantities adequate for the primary anticancer screen. This contract also acquires a significant proportion of the larger samples needed for secondary screening of the many new leads that are identified. This contract has also taken on the responsibility for the acquisition of compounds for AIDS screening.

STATE UNIVERSITY OF NEW YORK RESEARCH FOUNDATION (NO1-CM6-7698)

The objectives of this contract are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the anti-tumor spectrum; and (2) prodrugs that are chemically altered transport forms of the lead compound. The chemical modifications will aim at improving biological and pharmaceutical properties including (a) water-solubility; (b) hydrolytic stability; and (c) spectrum of activity and specificity. In addition, the contract provides for the modification of compounds of natural origin and synthesis of heterocycles with improved antitumor activity and reduced toxicity. These modifications may range from partial structures to structural analogs.

STATE UNIVERSITY OF NEW YORK RESEARCH FOUNDATION (NO1-CM8-7216)

This contract is for chemical synthesis and drug design of a variety of compounds for evaluation as potential anti-AIDS agents. The specific objectives of this project are: (a) to synthesize congeners of synthetic compounds with confirmed activity; (b) to design and synthesize prodrugs, and other compounds that possess elements of both congener and prodrug; (c) to synthesize compounds related to products of natural origin and other related heterocycles; and (d) to synthesize anti-sense nucleic acids. These products may include partial structures of analogs and novel heterocycles.

TACONIC FARMS (NO1-CM5-7730)

This Rodent Production Center contract produces athymic nude mice under maximum barrier conditions.

TECHNICAL RESOURCES, INC. (NO1-CM6-7907)

This Developmental Therapeutics Program contract provides a Program-wide resource for support services to the extramural preclinical anticancer and anti-AIDS drug discovery and development efforts. The services include: (1) support to the functions of decision-point committees; (2) planning and logistical management for DTP-sponsored conferences, seminars and workshops, including preparation of proceedings; (3) maintenance of files for the grants, contracts and NCDDG programs; (4) special reports and other Program-related documents; (5) graphics, slides, and prints on a rapid turnaround basis; and (6) a variety of miscellaneous tasks related to the planning and operational phases of the total DTP effort.

TEXAS A&M RESEARCH FOUNDATION (NO1-CM8-7211)

This contract monitors the genetic purity of the strains produced at the Genetic Centers and Rodent Production Centers. The testing is done by checking biochemical markers, and animals are sent for monitoring on a weekly basis scheduled by the Project Officer.

UTAH, UNIVERSITY OF (NO1-CM6-7863)

This contract carries out dosage form development studies leading to an acceptable injectable dosage form on compounds with activity versus HIV. These studies involve solubility assessments, determination of pH versus stability profiles, preparation of pilot scale batches, and evaluation of the stability of the product under simulated use conditions. The Contractor has experience with several methods of improving drug solubility including complexation and preparation of prodrugs that is being applied to resolve difficult formulation problems.

VSE CORPORATION (NO1-CM5-7654)

This service contract provides for operation and maintenance of the DTP Biological Data Processing System. The system provides support for acquisition of biological testing data produced by DTP's anti-cancer and anti-viral drug screens. Both of these activities currently rely on automated in vitro test systems which are being developed to allow screening of many thousand samples per year. This contract provides software support at the laboratory level for data acquisition, as well as support at the mainframe level for maintenance of data files. Support is also provided for acquisition and maintenance of in vivo drug testing data.

Z, INC. (NO1-CM7-3720)

The objective of this small business contract is to perform a variety of computer searches such as full structure searches, substructure searches and data item searches in support of the DTP program. The Contractor utilizes several data bases such as DIS, DARC, Questel, NLM, and Dialog. Another task under this contract is the development of chemical names for compounds of interest.

OFFICE OF THE DIRECTOR

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM3-6011)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo to provide up-to-date information services in support of our cancer treatment program, both preclinical and clinical. This is a cost-sharing contract and is strongly supported by the Japanese Foundation for Cancer Research and the Japan Society for the Promotion of Science.

TECHNICAL RESOURCES, INC. (NO1-CM5-7658)

This contract provides technical support services to the Office of the Director, DCT, as well as to the program areas of DCT in the performance of the planning and analytical tasks and general logistical support in the development of related or otherwise required documentation and conference support activities of the Division. This contract was awarded in April 1985 for a five-year period.

RADIATION RESEARCH PROGRAM

ALLEGHENY-SINGER RESEARCH CORPORATION (NO1-CM3-7512)

This Contractor is expected to develop the criteria, guidelines and procedures for the proper use of the equipment representing the major heat generating modalities (radiofrequency, microwave and ultrasound) and the ancillary equipment necessary for the treatment of cancer with heat. The criteria and guidelines developed will be utilized to initiate a quality assurance and assessment program in hyperthermia. This Contractor is expected to implement and conduct such a program in the latter years of the contract. This contract is being recompeted.

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM4-7564)

This is one of five contracts awarded in May 1984 (a) to develop protocols for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, lung cancer, liver metastases, and congenital heart disease. They have also provided coordination for the CWG of the development of the lung cancer protocol. Patient imaging has been completed for these studies, and data analysis and documentation are continuing.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7684)

This is one of five contracts awarded in May 1984 (a) to develop protocols for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, lung cancer, brain neoplasms, and congenital heart disease. They have also provided coordination for the CWG of the development of the uterine neoplasms and congenital heart disease protocols. Patient imaging has been completed for these studies, and data analysis and documentation are continuing.

CALIFORNIA, UNIVERSITY OF (NO1-CM5-7708)

This Contractor is a member of the Interstitial Collaborative Working Group, a consortium of three institutions, funded to develop recommendations and guidelines for a program in interstitial radiotherapy. The group's recommendations include guidelines for calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and after loading procedures where appropriate.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7315)

The University of California at Los Angeles is a member of the Neutron Therapy Collaborative Working Group, a consortium of several institutions charged with carrying out neutron therapy clinical trials. The Contractor provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support the clinical research program. The Working Group is investigating the efficacy of neutron therapy when compared to conventional photon treatments. Phase III protocols now open for randomized studies to compare neutrons with photons include: 1) Stages B_2, C and D_1 adenocarcinoma of the prostate gland, 2) squamous cell carcinoma of the upper aerodigestive tract, 3) regional non-small cell lung cancer, and 4) radioresistant histotypes, including soft tissue and bone sarcomas, melanoma, thyroid and renal cell cancers. Neutrons have been selected as the treatment of choice for salivary gland tumors.

CHEUNG LABORATORIES, INC. (N44-CM7-7838) (SMALL BUSINESS INNOVATION RESEARCH Program)

In a Phase I study this Contractor demonstrated the feasibility of using phase and amplitude controlled multiple microwave applicators for improving the power disposition patterns, i.e., the specific absorption rate (SAR), when inducing hyperthermia in deep seated tumors by electromagnetic means. In the Phase II study, the Contractor will perform dosimetry studies using his microstrip applicators and tissue equivalent phantoms. Heating patterns will be measured in the phantoms at various frequencies. The power generating system will be automated to control the phase and amplitude of the applied power to each applicator to achieve optimal heating patterns in desired locations. Software used will be developed and integrated into the system and the system tested in the clinical setting.

CLEVELAND CLINIC (NO1-CM4-7685)

This is one of five contracts awarded in May 1984 (a) to develop protocols for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, lung cancer, brain neoplasms, and congenital heart disease. They have also provided coordination for the CWG of the development of the cervical myelopathies protocol and for the continuing coordination of the brain neoplasms protocol. Patient imaging has been completed for these studies, and data analysis and documentation are continuing.

DOTY SCIENTIFIC, INC. (N44-CM7-7804) (SMALL BUSINESS INNOVATION RESEARCH Program)

This Contractor is developing low noise, low cost broadband class AB radiofrequency pulse amplifiers for use in magnetic resonance imaging (MRI) systems operating from 5MHz to 140MHz; non-saturating 200W bipolar power circuits; and high density, high efficiency, 12KW 80KHz switching power supplies. Following system integration and prototype manufacturing, class AB pulse amplifiers should be available, operating at pulse powers up to 5KW, at greatly reduced first cost and operating costs over presently available commercial products, while still maintaining superior performance.

DUKE UNIVERSITY (NO1-CM4-7686)

This is one of five contracts awarded in May 1984 (a) to develop protocols for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, brain neoplasms, lung cancer, and congenital

heart disease. They have also provided coordination for the CWG of the initial development of the brain neoplasms protocol. Patient imaging has been completed for these studies, and data analysis and documentation are continuing.

LASERGUIDE, INC. (N43-CM8-7261) (SMALL BUSINESS INNOVATION RESEARCH Program)

A NdYag (Neodymium-Yitrium-Aluminum-Garnet) laser based system for interstitial and external beam hyperthermia for the treatment of cancer will be developed. Such a system could provide a very cost effective method which does not have the problems of the present microwave and radiofrequency systems. It would provide local hyperthermic heating which would not have constructive/destructive interference problems and which can be monitored and controlled by the use of an inexpensive microthermal couple system.

Phase I studies will include (1) determination of the optimum NdYag wavelength to be used (1.06 or 1.32 microns), (2) what is the actual delivered power required to raise a given volume of tissue to $<45^{o}$? (3) what is thermal distribution generated in vivo (rat model)? and (4) evaluation of thermal couple monitoring and feedback control mechanism for the system. The information obtained in Phase I will be used to develop a more complete system in Phase II aimed at more advanced animal and clinical testing.

LUXTRON CORPORATION (N44-CM7-7841) (SMALL BUSINESS INNOVATION RESEARCH Program)

The overall objective of this project is to develop a system for fast, accurate, on-line measurement and control of tissue temperature during microwave-induced interstitial hyperthermia as used in cancer therapy. Phase I has demonstrated the feasibility of instrumenting an interstitial catheter with multiple rf-immune fiberoptic sensors located in the wall of the catheter without significantly increasing catheter size. This design leaves the bore free for insertion of the microwave antenna, places the sensors in better thermal contact with the tissue being heated, and makes possible an on-line assessment of the temperature distribution along the length of the antenna during treatment. Phase II has two major subgoals, namely: (1) further improvement of the catheter and sensor materials and design to allow a cost-effective volume fabrication and assembly; and (2) evaluation and preliminary development of a cost-effective instrument capable of supporting a large number of multisensor catheters. Prototype catheter evaluation will also include extensive animal and clinical testing.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM4-7616)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation. This contract was completed 12/15/87.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM4-7687)

This is one of five contracts awarded in May 1984 (a) to develop protocols for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out

performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, and brain neoplasms. They have also provided coordination for the CWG of the development of the musculoskeletal tumor and liver metastases protocols. Patient imaging has been completed for these studies, and data analysis and documentation are continuing.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-7695)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation. This contract was completed 12/15/87.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7776)

This Contractor is a member of the Interstitial Collaborative Working Group, a consortium of three institutions, funded to develop recommendations and guidelines for a program in interstitial radiotherapy. The group's recommendations include guidelines for calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and after loading procedures where appropriate.

MICHIGAN, UNIVERSITY OF (NO1-CM6-7913)

The objective of this collaborative effort is to evaluate the capability of improving electron beam dose distributions with presently available beam delivery systems, imaging systems, and computerized treatment planning systems. Evaluation of the role of tissue inhomogeneity corrections, error analysis, advanced calculations using new physics models, and new treatment techniques such as dynamic treatment shall also be made. Recommendations and guidelines shall be developed for the application of the findings of this study to the practice of electron beam radiotherapy. Scheduled to be completed by 7/31/89.

MICROWAVE MEDICAL SYSTEMS, INC. (N44-CM7-7821) (SMALL BUSINESS INNOVATION RESEARCH Program)

The goal of this SBIR contract is to develop a device for early detection of extravasation of intravenously administered cytoxic drugs using non-invasive microwave radiometry to monitor the fluid temperature at the injection site. Phase II of this contract began 15 September 1987 and is scheduled for two years. Progress in the first six months of the contract is on schedule and satisfactory.

NATIONAL BUREAU OF STANDARDS (YO1-CM6-0129)

The National Bureau of Standards (NBS) is developing national dosimetry standards for neutron dose deposition in tissue and tissue-equivalent materials. This work will improve the accuracy and consistency of measurements of absorbed dose for neutron radiation therapy. The work supports facilities at the NBS for the calibration of instruments used in neutron therapy.

NATIONAL BUREAU OF STANDARDS (YO1-CM6-0140)

This Interagency Agreement supports the development of two temperature fixed-triple point standards in cells appropriately designed such that the calibrations of various hyperthermia temperature measuring devices may be accurately and easily checked. The fixed-point standards will be chosen to bracket the hyperthermia temperature range of greatest interest, i.e., 41-44°C.

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM6-7868)

The capability for evaluating chemical compounds for radiation sensitizing and/or radiation protective properties is provided by this resource. Various physico-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radiosensitizing or radioprotective characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiosensitization using mammalian cell cultures. Potential radiosensitizing compounds which appear to be superior to the standard--misonidazole--will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radiosensitizers), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All radioprotective compounds tested will be compared with the standard - WR-2721. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds.

This contract should provide new radiosensitizers and radioprotectors or leads in developing new types (classes) of radiation modifying compounds.

RADIATION MONITORING DEVICES, INC. (N44-CM7-7842) (SMALL BUSINESS INNOVATION RESEARCH Program)

Under a Phase II project, this Contractor will develop a clinical working prototype of a computer-controlled multi-leaf collimator which can shape the radiation port to conform to an irregularly-shaped treatment field. The company proposes a model which can fit into the brackets currently used to hold the treatment field blocks used in existing machines. The new system will be well suited to dynamic radiation therapy; treatment plans which require a number of different treatment portals each day; and portal-shaping during arc therapy.

RADIATION MONITORING DEVICES, INC. (N44-CM6-7807) SMALL BUSINESS INNOVATION RESEARCH Program)

The Radiation Monitoring Devices (RMD) continue to develop and improve the photovoltaic CdTe radiation sensor. The hardware and software for the detector were developed and fully operational. An operational prototype system was constructed and is being tested with clinical phantoms in collaboration with their medical and industrial consultants. The contractor will

continue to: a) establish improved crystal growth techniques for producing photovoltaic CdTe detectors; b) conduct further research on better detector fabrication procedures to enhance sensitivity, speed of response and noise characteristics; c) develop the hardware and software needed to implement this concept into an all solid state, x-ray exposure controller; and d) design and build a full scale, portable laboratory prototype and evaluate its performance during the terminal year of this contract. Contract will terminate in September, 1988.

SRI INTERNATIONAL (NO1-CM7-3708)

The objective of this contract between NCI, SRI International and Stanford University is the design, synthesis and biological evaluation of novel radiosensitizers. The primary focus of the work is the identification of leads other than electron affinic-2-nitroimidazoles. Other types of compounds that are being investigated have different modes of action. These include inhibitors of the repair of potential lethal damage, shoulder modifiers, and glutathione depleters.

TEXAS, UNIVERSITY OF (NO1-CM6-7914)

The objective of this collaborative effort is to evaluate the capability of improving electron beam dose distributions with presently available beam delivery systems, imaging systems, and computerized treatment planning systems. Evaluation of the role of tissue inhomogeneity corrections, error analysis, advanced calculations using new physics models, and new treatment techniques such as dynamic treatment shall also be made. Recommendations and guidelines shall be developed for the application of the findings of this study to the practice of electron beam radiotherapy.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-7775)

M.D. Anderson Hospital at the University of Texas is a member of the Neutron Therapy Collaborative Working Group, a consortium of several institutions charged with carrying out neutron therapy clinical trials. The Contractor provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support the clinical research program. The Working Group is investigating the efficacy of neutron therapy when compared to conventional photon treatments. Phase III protocols now open for randomized studies to compare neutrons with photons include: a) Stages B_2, C and D_1 adenocarcinoma of the prostate gland; b) squamous cell carcinoma of the upper aerodigestive tract; c) regional non-small cell lung cancer; and d) radioresistant histotypes, including soft tissue and bone sarcomas, melanoma, thyroid and renal cell cancers. Neutrons have been selected as the treatment of choice for salivary gland tumors.

THERMAL TECHNOLOGIES, INC. (N44-CM7-7855) (SMALL BUSINESS INNOVATION RESEARCH Program)

In the Phase I study, this Contractor combined two separate thermal probe methods to produce a simultaneous, integrated methodology for the local transient quantification of tissue thermal conductivity, diffusivity, and levels of tissue perfusion by a single measurement, eliminating the requirement for a no-flow calibration procedure. In Phase II, the Contractor plans to initiate a program of instrument development and *in vitro* verification. In collaboration with other participating institutions, this thermally-based measurement instrument will be introduced into the clinical environment for a critical and timely evaluation of its usefulness in hyperthermia therapy.

WASHINGTON, UNIVERSITY OF (NO1-CM9-7282)

The University of Washington is a member of the Neutron Therapy Collaborative Working Group, a consortium of several institutions charged with carrying out neutron therapy clinical trials. The Contractor provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support the clinical research program. The Working Group is investigating the efficacy of neutron therapy when compared to conventional photon treatments. Phase III protocols now open for randomized studies to compare neutrons with photons include: a) Stages B_2, C and D_1 adenocarcinoma of the prostate gland; b) squamous cell carcinoma of the upper aerodigestive tract; c) regional non-small cell lung cancer; and d) radioresistant histotypes, including soft tissue and bone sarcomas, melanoma, thyroid and renal cell cancers. Neutrons have been selected as the treatment of choice for salivary gland tumors.

WASHINGTON UNIVERSITY (NO1-CM4-7696)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation. This contract was completed 12/15/87.

WASHINGTON UNIVERSITY (NO1-CM6-7915)

The objective of this collaborative effort is to evaluate the capability of improving electron beam dose distributions with presently available beam delivery systems, imaging systems, and computerized treatment planning systems. Evaluation of the role of tissue inhomogeneity corrections, error analysis, advanced calculations using new physics models, and new treatment techniques such as dynamic treatment shall also be made. Recommendations and guidelines shall be developed for the application of the findings of this study to the practice of electron beam radiotherapy. This contract is scheduled to be completed by 7/31/89.

YALE UNIVERSITY (NO1-CM5-7777)

This Contractor is a member of the Interstitial Collaborative Working Group, a consortium of three institutions, funded to develop recommendations and guidelines for a program in interstitial radiotherapy. The group's recommendations include guidelines for calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and after loading procedures where appropriate.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1987 - September 30, 1988

I. Introduction and General Organization

The Developmental Therapeutics Program (DTP) is a comprehensive program of the Division of Cancer Treatment charged with the discovery and preclinical development of new anticancer and anti-HIV agents for introduction into clinical trials. The DTP utilizes both intramural and extramural components to accomplish its research oriented and investigative mission. The overall Program is managed by the Office of the Associate Director. The progress of potential clinical candidates through the Decision Network (Drug Development) process is summarized in Table 1.

The Program is comprised of three separate components. The drug discovery component is represented by the Natural Products Branch, Drug Synthesis and Chemistry Branch, Biological Testing Branch, and Information Technology Branch. The drug development component is made up of the Pharmaceutical Resources Branch, Pharmacology Branch, Toxicology Branch, and Grants and Contracts Operations Branch. The third arm of the Program, the Intramural component, consists of four laboratories: Laboratory of Biological Chemistry, Laboratory of Biochemical Pharmacology, Laboratory of Medicinal Chemistry, and Laboratory of Molecular Pharmacology. A highly skilled research and resource group, the Program Development Research Group, completes the intramural component of the DTP.

II. Program Accomplishments

A. Extramural Program

1. Natural Products Branch

The major responsibilities of the Natural Products Branch (NPB) are: (1) acquisition of crude biological materials of plant, marine, and microbial origin for the DTP screening program; (2) contract research directed toward isolation of new agents from active extracts; (3) world-wide literature surveillance, and acquisition of natural products with demonstrated biological activity or novel structural types for evaluation; (4) procurement and preparation of large quantities of active agents for drug formulation, advanced biological testing, toxicology and clinical studies.

The development of new anticancer and antiviral screens in DTP has regenerated a high level of interest in identifying new natural product leads. A major new program effort in the collection of natural products leads from a wide variety of sources, including terrestrial plants, marine invertebrates, algae and marine microorganisms has been initiated. Several new contracts have been awarded. The NPB has devoted a good deal of effort towards development of the necessary procedures, operations, and data support for the repository, extraction, drug preparation, and screening laboratories relevant to natural products.

During the past year, the NPB made significant contribution in several areas. In the fermentation area, NPB has initiated programs utilizing novel source organisms. Plant collections are in progress in the tropical rain forest regions of Africa, Central and South America, and South East Asia, and shallow waters marine organisms are being collected in the Indo Pacific region. Plant and marine organism collections are extracted at the Frederick Cancer Research Facility (FCRF) and over 4,000 extracts are currently in storage at 20°C awaiting testing in the human cancer cell line and HIV screens. Literature surveillance and contact with scientists worldwide has resulted in the acquisition of 292 pure natural pruducts for anticancer screening and 166 for anti-AIDS screening. Taxol, isolated from the bark of _Taxus brevifolia_, has shown some promise in clinical trials against melanoma, and is showing a 30% response rate in an ongoing Phase II trial against ovarian carcinoma. A 60,000 lb collection of the dried bark of _T. brevifolia_ is due for completion in 1988, and further collections are envisaged in 1989. Bryostatin I has been selected for advanced development and a major collection of 11,000 gallons of the source organism, _Bugula neritina_ (a marine animal in the Bryozoa) is underway as is methods development for large-scale extraction and isolation. Bryostatins are members of a totally new chemical class, the bryopyrans, and have extremely potent biological activities as activators of protein kinase C. They have shown extremely good selectivity in the disease oriented screen and in addition stimulate growth of granulocyte-macrophage colony forming units and show potentiation of IL-2 cytotoxicity. There is very high interest in DCT in the development of the bryostatins.

2. _Drug Synthesis and Chemistry Branch_

The fundamental responsibility of the Drug Synthesis and Chemistry Branch (DS&CB) is the discovery of novel synthetic anticancer and anti-AIDS leads through the

acquisition, synthesis structure-activity optimization and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents in the DTP. The DS&CB achieves its objectives by engaging in a variety of Program activities, namely, acquisition of a large number of synthetic compounds of diverse biological and structural types through the development and maintenance of an extensive net-work of scientific liaison on a worldwide basis, structure-activity optimization through the synthesis of congeners and prodrugs, radiolabelled syntheses, task order resyntheses, storage, inventory and distribution and computer-assisted structure-activity analysis. Collaborative programs are also under way with the Natural Products Branch and intramural laboratories. The DS&CB played a central role in organizing the Acquisition Input Committee (AIC) of DTP which is now functioning effectively.

During the past year, the DS&CB made significant contributions in several areas. Within a very short period of the operation of the new AIDS screen, NSC 614846 (Carbovir) was identified as an interesting anti-AIDS lead with good potential. A novel carbinolamine derivative, NSC 602668, developed in one of the drug design contracts, with excellent anti-tumor activity against a broad spectrum of tumors, is being rapidly developed further. The large-scale synthesis of several compounds has been accomplished, for example, DDA (NSC 98700), DDC (NSC 612049) and Penclomedine (NSC 338720). The synthesis of several radio-labelled compounds for preclinical and clinical studies were completed; for example Bayer compound (NSC 320846), AZT, DDI (NSC 612049) and DDC (NSC 606170). The master resynthesis contracts have provided a variety of compounds of program interest that are not available in sufficient quantity from the original suppliers (approximately 60 compounds) in a cost-effective way. The storage and distribution contractor has organized, repackaged and reshelved more than 112,000 compounds. The contractor has shipped approximately 8,000 compounds to foreign and domestic investigators. Several contracts, for resynthesis, radiolabelled synthesis, and congener and pro-drug synthesis in support of the AIDS program have been established. The syntheses of Castanospermine analogs and the Cyanobacteria anti-AIDS lead have been initiated. Collaborative programs with the intramural groups resulted in the synthesis of a variety of compounds, e.g., several fatty acid derivatives of Coenzyme-A, indanone mustards, B-methylene TAD and cyanotyrosines. A new computer-based clustering model to group and prioritize compounds for screening from our large repository of unique compounds is under development.

3. Biological Testing Branch

The Biological Testing Branch (BTB) is responsible for the development and implementation of a disease-oriented *in vitro* screening program for a large number of candidate cancer chemotherapy compounds, and for follow-up *in vivo* testing of selected agents. The BTB is also responsible for developing an AIDS testing program which includes the establishment and implementation of an anti-HIV screen (*in vitro*) with follow-up *in vivo* testing capabilities. The BTB manages a large resource for the production, quality control, and distribution of genetically and biologically defined rodents. These disease free experimental animals are distributed to investigators at NIH, FCRF, and other investigators on a cost reimbursement basis. The BTB maintains a large repository of experimental animal and human tumor lines for usage by DCT and other NCI programs. Tumors are also distributed to qualified cancer research investigators on a cost reimbursement basis.

In the cancer area during the past year, feasibility studies were accomplished to assure the capability of testing 10,000 compounds annually in the *in vitro* screen against a large number of human tumor cell lines. Screening protocols are in the final stages of testing prior to their adoption. Limited *in vivo* model development studies have continued. In the absence of quantitative data for potential evaluation models, it has been decided that the subcutaneous model which provides significant tumor inhibi-tion/cell kill information, and which has been studied extensively in murine models will be utilized. Comparative *in vivo* model development studies will continue.

In the AIDS area the *in vitro* screen has been implemented to the extent that testing has exceeded the 10,000 compound level on an annualized basis. For the *in vivo* screen initial studies have been conducted with a micro-encapsulation model using the AIDS virus. Additional studies will be performed and implemented as appropriate with feline and/or bovine lentivirus models and other retroviruses.

Quality standards for animal production have been maintained. Adjustments have been made in animal production to reflect changes in DTP objectives including more reliance on athymic mice and less overall volume of usage. The payback system has continued to work extremely well in making the animal production system cost effective.

The tumor bank has expanded its capacity to accommodate a number of the cell lines utilized in the disease-oriented screening program. Steps have been taken to enhance the acquisition of cell lines from both the cancer and AIDS testing programs and to make these lines available for distribution.

4. Pharmacology Branch

The Pharmacology Branch (PB) is involved with two basic aspects in the preclinical drug development program: detailed therapeutic studies and pharmacokinetic studies on candidate agents. The Branch conducts special studies in exploration of the therapeutic efficacy of new drugs particularly as related to treatment regimens such as continuous infusion, etc. Of particular importance is the evaluation of agents versus advanced stage tumors and metastatic disease. The effects of particular agents against drug resistant tumors is another prime interest.

Over the past year, nine new drugs were evaluated against one or more of the above systems including two for which INDAs have been filed. A special effort has been expended on Taxol, a clinically significant new agent, to evaluate various prodrugs enabling newer formulations to be created obviating the need for the more cumbersome and sometimes toxic standard product. Pharmacokinetic profiles have been initiated in mice on eight antineoplastic agents and one promising new anti-HIV drug.

Two major initiatives are worthy of mention. Firstly, the Branch designed and performed studies to answer questions and concerns raised in the early clinical trials with Flavone Acetic Acid. The data demonstrated that urine alkalinization shifted the toxicity and therapeutic response curve to an equal extent while increasing drug clearance from the plasma. Secondly, the Branch is involved in the initial design of projects to study the antiviral effects of new drugs *in vivo*. Drugs found active in the *in vitro* screen are intended to be studied for route and schedule dependency in retrovirus infected mice. Additionally, a screen is being developed for antifols active versus the dihydrofolate reductase and p-aminobenzoic acid analogues active against dihydropteroyate synthetase from *P. carinii* and *T. gondii*.

5. Information Technology Branch

The Information Technology Branch (ITB) provides data processing and data management support for the DTP. The

Branch supports new initiatives and ongoing activities including the disease-oriented screens for active anticancer and anti-HIV agents. Large databases supporting the chemical, biological and administrative activities of the Program over the past 30 years are also maintained. A wide variety of interactive, specialized programs have been written by Branch staff to support individual staff needs.

This year special emphasis is being placed on data collection and analysis from the new anticancer and anti-HIV screens. The Biological Data Processing System is the key support system for the acquisition and processing of anticancer and anti-HIV screening data. Basically the system is comprised of laboratory microcomputer systems interfaced to microtiter plate readers for data collection and linked to mainframe computers at the Division of Computer Research and Technology for data storage and review by staff scientists. Over 3,000 materials have been screened for anti-HIV activity for which the data has been collected, evaluated and archived. The Drug Information System (DIS) is a database used to store information on chemical structures, inventory information and shipping information. This database also stores screening information for a subset of compounds screened by the Program.

The ITB staff has embarked on an interesting and unusual special project concerning the use of robotics in the repetitious, labor intensive aspects of the drug screening laboratories. The developed robot weighs bottles containing drug samples to be tested. In the future, we anticipate use of robotics for sample addition to the test plates, a task requiring absolute accuracy and reliability.

Major deficiencies have been identified this past year with the laboratory support system as the screening mode has been scaled up. The scheduling program (AVAIL and ASGN) has not functioned as expected, the *in vitro* database contains some errors which have proven difficult to correct, and chemical information processing has been hampered by the unreliability of the DEC 10 system. An expert consultant group will be established to assist the Program in devising a state-of-the-art solution to these information technology problems. Additional innovative activities are now in place which will alleviate some of the more troublesome problems, for example, management of the *in vitro* database is being transferred to a commercial system to enhance the fidelity of data collection. Moreover, the DIS and other systems currently running on the DEC 10

system will be transferred through 36 to 32 bit conversion to a dedicated DTP support system and connected to the laboratories via Ethernet.

6. <u>Pharmaceutical Resources Branch</u>

The Pharmaceutical Resources Branch (PRB) provides comprehensive pharmaceutical support to the Division of Cancer Treatment. High quality drugs and formulated products for investigational use are supplied for preclinical and clinical investigations on new agents for the treatment of cancer and HIV infections. The Branch consists of four functional areas through which its major functions are performed.

The Chemical Resources area provides for the acquisition/synthesis of large-scale lots of new compounds. During the past year, over 25 compounds exceeding 180 kilograms were produced. Bulk drug was produced for Dideoxyadenosine, Dideoxyinosine, Dideoxycytidine, Merbarone and HMBA among others. The Analytical Resource is charged with the complete characterization of new investigational agents and assessment of formulated products. In excess of 100 lots of chemicals and formulated drugs were assayed this year. Several plant extracts were assayed for the promising antimitotic, Taxol.

The Pharmaceutical Development Resource converts bulk chemicals into viable pharmaceutical products for clinical use. This portion of the Branch emphasizes the newer approaches to drug formulation since both the physical and chemical characteristics of the discovered compounds require significant research prior to production of a satisfactory clinical product. Dosage development was completed on eight new agents and three new delivery systems were investigated, namely, emulsions, liposomes and microencapsulation. The last Resource in the Branch is the Clinical Product Service. This component is responsible for the storage and distribution of the drugs and shelf-life storage determinations. $2.5 million were spent this year in the purchase of drugs. Clinical Products is also responsible for producing the Pharmaceutical data sheet and handling specifications for each new agent.

This year an emergency drug distribution system was installed to satisfy off-hours drug requests for the treatment of HIV. Additionally, the receipt and distribution of biological products increased significantly over past years. It is noteworthy that the Branch designed and implemented a special dose-pak for a five arm blinded study with AZT and placebo. Over

the next year the Branch will continue to concentrate on evaluating and producing new parenteral drug delivery systems such as emulsions, liposomes and micronized particles.

7. Toxicology Branch

The Toxicology Branch (TB) is primarily responsible for conducting studies to determine the potential hazards of new anticancer and anti-HIV agents to primary organ systems. These experimental observations are done in rodents and dogs from which the starting dose for clinical trial is estimated and the dose escalation scheme is determined. During the past year, the Branch completed final reports on six new agents for inclusion as attachment 6a in new Investigational New Drug Applications. Two of the agents, Dideoxyadenosine and Dideoxyinosine, are new front line treatment for HIV infections. Preliminary toxicity studies have been initiated on five new agents including Carbovir, a promising new agent for the treatment of AIDS.

The Branch initiated two new projects in the last year designed to provide more definitive information on human toxicity of investigational agents. These projects include studies on the *in vitro* assessment of toxicity in bone marrow committed stem cell compartments yielding a comparative hazard profile across species lines, including human. Another important project is the study of comparative metabolism of anti-HIV agents in lymphocytes from experimental species and humans. As these special studies are just being initiated and the project highly investigational, little comparative information has come available. However, the Branch has found that human lymphocytes phosphorylate dideoxynucleosides at a rate 10-80 faster than those cells from dogs. Importantly, there appears to be little difference in the comparative toxic effect of these compounds on bone marrow stem cells.

8. Grants and Contracts Operations Branch

The Grants and Contracts Operations Branch (G&COB) is responsible for administrative and managerial support for the Program-sponsored extramural activities. These activities encompass all grants, contracts and cooperative agreements.

At the end of FY 1987, 321 grants totalling $47,124,000 were administered by the Branch. The subject matter of the grants ranges over the entire drug development spectrum. Likewise, the Program awarded 21 contracts supporting the development of antitumor agents and 23

contracts supporting the development of anti-HIV agents. In all, the Program awarded $125,455,599 worth of contracts this year as a total value over the next three to five years.

The Branch also manages and administers the National Cooperative Drug Discovery Group Program through a series of cooperative agreements. This program was established in 1983 to exploit exciting new developments in biomedical research into new and more effective anticancer treatments. Seven agreements involving 37 laboratories in 25 institutions are now operational at an annual cost of $3,600,000. The seven groups are involved in research on several molecular events including polyamine synthesis, oncogene expression, growth factor binding and topoisomerase inhibition. Additionally, one group is focusing on new techniques to cure lung cancer. Thirty-three new applications to the program were reviewed this year in an effort to expand the program into three conceptually new areas: general mechanism of action, disease-oriented strategy, and novel model development for the prediction of efficacy. Awards will be made in the not to distant future.

B. Intramural Program

1. Laboratory of Biological Chemistry

The Laboratory of Biological Chemistry (LBC) is responsible for identifying as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. Agents are designed to interact with these targets and are evaluated for biochemical and antitumor effectiveness. An important aspect of this mission is to develop appropriate in vivo systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler in vitro model systems. Accordingly, the LBC is involved in identifying endogenous factors present in vivo that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. Approximately 75% of the LBC's resources is applied to non-traditional targets for antitumor drug design and study. These non-traditional targets include early key biochemical events signaling cell proliferation or differentiation. The other 25% of LBC resources is applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated projects to develop new chemotherapeutic agents to block the action of growth factors. Non-traditional targets selected for drug design and study include: second messengers inositol triphosphate diacylglycerol; myristoylation of cellular oncogene products; protein kinase C; and selected G-proteins.

The products of two distinct oncogenes (src and ros) are associated with increased levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. Test systems have been developed to identify drugs that inhibit phosphatidylinositol turnover or synthesis. Studies of the activation of phospholipase C demonstrated that this activation is attenuated by pretreatment with agents that activate protein kinase C such as phorbol esters and diacylglycerols.

A series of analogs of _myo_-inositol was synthesized and evaluated. The 5-deoxy-5-fluoro-inositol analogue is incorporated into cellular phospholipid and phosphorylated to a compound similar to PIP, but not further phosphorylated to the corresponding PIP2 derivative.

It may be possible to alter the activity of an oncogene product by interfering with its localization in the plasma membrane. Myristoylation has been shown to be critical for the membrane localization and cellular transforming activity of $p60^{src}$ and has been implicated for other transforming proteins. N-Myristoyl transferase was partially purified from bovine brain. Several potential inhibitors of the enzyme were synthesized and are being tested for their _in vitro_ effects on the purified enzyme.

Investigations were continued in three interrelated projects that center around the role of protein phosphorylation in various regulatory systems. The first project deals with the role of the proto-oncogene tyrosine protein kinase, c-_fes_, in myeloid cell

differentiation. The c-fes kinase was purified and characterized from HL-60 cells expressing the granulocyte phenotype as a result of treatment with DMSO. The genomic DNA encoding c-fes was transfected into HL-60, KG-1a (GM-CSF-independent), and K562 (resistant to differentiation) cells to study the role of the c-fes protein in the ability of these cells to differentiate along the myeloid or monocytic pathway. The second project deals with the role of calcium- and phospholipid-dependent protein kinase (protein kinase C)in multidrug (mdr) resistant cells. We found that not only is there an overabundance of protein kinase C in mdr cells, but that the isoform pattern for this family of kinases (seven at last count) also differs from their sensitive counterparts. The other facet of this project is to determine the endogenous protein substrates for these protein kinases. The third project addresses the role of protein phosphorylation in the regulation of replication of the human immunodeficiency virus (HIV) which causes AIDS. It is known that the HIV does not replicate when the sor gene is deleted, but replicates at a faster than normal rate when the 3'-orf gene is deleted. Thus, the products of these two genes impose negative and positive regulatory functions, respectively. We found that lysates of HIV contain a unique Mn^{2+}-dependent protein kinase activity that is not present in lysates of the ribosome fraction from uninfected cells. We are presently trying to determine the open reading frame in HIV encoding this protein kinase using both viral lysates and systems employing the cloned sor and 3'-orf genes.

Studies were extended on ARF, a recently characterized G-protein. Three phenotypes resulting from disruption of ARF1 have been defined: slow growth at 30#C, cold sensitivity, and supersensitivity to fluoride ion. Indirect immunofluorescent staining of yeast cells with affinity purified ARF antibodies suggest that ARF may be localized or concentrated in microtubules. These results are preliminary but point to a new site of action of regulatory G-proteins.

Although many biological effects of retinoic acid have been described, the mechanism for these actions is unknown. We have now discovered that in the human acute myeloblastoid cell line, HL-60, a covalent bond is formed between retinoic acid and protein. Based on sensitivity to hydrolysis with hydroxylamine, about 70% of the retinoic acid moiety is linked to protein *via* either an oxygen-ester or a thio-ester bond.

A method has been developed for the fixation and permeabilization of HL-60 cells so that intracellular antigens can be detected with the use of a fluorescence activated cell sorter. This method has been applied in a study of the changes in the level of c-*myc* oncogene protein during differentiation of HL-60. Our results indicate that c-*myc* protein decreased during differentiation at a much slower rate than would be expected from the decreases in c-*myc* mRNA levels under the same conditions.

Multidrug-resistance is a well documented phenomenon that limits the chemotherapeutic effectiveness of many traditional antitumor agents. An understanding of the physiologic function of proteins associated with multidrug-resistance could lead to the design of more effective chemotherapeutic strategies with existing agents or the associated proteins may be considered as targets for drug design, in which case a new class of antitumor agents might arise. A radioactive photoactive analog of vinblastine was used to identify a specific vinblastine binding p-gp in multidrug-resistant cell lines. P-gp vinblastine photolabeling was blocked by a number of indole alkaloids previously shown to increase anticancer drug cytotoxicity and increase drug retained by cancer cells. It was also found that there is a correlation between the level of vinblastine photolabeling of P-gp and the cellular collateral sensitivity of CEM/VLB cells to verapamil up to about 40-fold vinblastine resistance.

The mixed disulfide of methyl mercaptan and L-homocysteine, S-(methylthio)-L-homocysteine (L-SMETH), inhibits the growth of L1210 leukemia cells in culture at micromolar concentrations. The inhibition is markedly promoted by added cupric ion, but not by ions of other metals, is steriospecific, and is competitive with glutamine. The inhibition is also completely relieved by cytidine in a non-competitive manner, but not by guanosine or uridine, indicating that the principal damage to the cellular economy resides in the amination of uridine to cytidine.

A project involving traditional targets for drug design and study is a continuing project to determine the relative dependency of host and tumorous tissue on *de novo* *vs* salvage pathways for the synthesis of pyrimidine and purine nucleotides *in vivo*. Early comparative data indicate that uridine salvage makes a substantial contribution to the pyrimidine nucleotide pools of mouse liver, intestine, and kidney in the

intact animal. Activation of uridine salvage is an early event in the mitogenic response, occurring within minutes of serum stimulation of quiescent fibroblasts.

2. Laboratory of Biochemical Pharmacology

The Laboratory of Biochemical Pharmacology (LBP) is responsible for the mode of action of new anitumor drugs. The LBP studies new agents which have originated within the DTP and also agents derived from extramural sources in whose preclinical development the Program is playing a major role. Over the past two years, the LBP has also participated actively in elucidation of the cellular pharmacology of compounds with anti-HIV activity, currently under development within the Program.

Significant progress has been made on the elucidation of the cellular and molecular pharmacology of anti-HIV drugs of the 2',3'-dideoxynucleoside (ddN) type. A complete study of seven compounds of this class has allowed the development of a general hypothesis to explain the extremely wide variation (three orders of magnitude) in the antiretroviral activity of this group. The most important factor appears to be the susceptibility of the individual ddN's to phosphorylation to the 5'-triphosphate level. It should be noted however, that most of the compounds are similar in therapeutic index, although AZT and ddCyd are less favorable in this respect than some of the other nucleosides: an increase in retroviral potency within this class is also accompanied by a parallel increase in cytotoxicity toward host cells (e.g. T-cells and monocytes/macrophages).

Substantial further progress has been made on the difficult technical problem of the measurement of intracellular levels of ^{3}H-ddATP seen after exposure of human T-cells to ^{3}H-ddAdo. It has now been definitely established that ddAdo and ddIno both lead to the same anabolic end-product, ddATP, a result of considerable practical importance in view of the Phase I/II clinical trials of ddAdo and ddIno. In another area of ddAdo pharmacology we have made the empirical observation that the formation of ddATP in T-cells is enhanced several-fold by the co-administration of the deoxynucleosides 2'-deoxyadenosine and 2'-deoxyinosine, and also by low levels of the adenosine deaminase inhibitors 2'-deoxycoformycin and EHNA.

Studies continued on the biochemistry and pharmacology of tubulin, a protein critical for cell division which is the site of action of many antitumor agents.

Copolymerization of tubulin-GDP and tubulin-GTP was examined to define their relative efficiencies in microtubule elongation and the minimum concentration of tubulin-GTP required for the initiation of microtubule assembly. Nucleotide concentration and composition markedly affects microtubule stability, and possible explanations for these properties were evaluated. Work continued on developing methods to separate the tubulin subunits preparatively, and on reconstitution of active tubulin following denaturation. Purification continued on two microtubule-associated proteins. One causes the formation of massive bundles of microtubules, the other degradation of GDP to GMP.

Structure-activity studies with the potent antimitotic drugs derived from the South African tree Combretum caffrum were completed, defining two agents (combretastatin A-2 and combretastatin A-4) as exceptionally promising drugs based on excellent cytotoxicity, potent antitubulin activity in vitro, and retained activity against multidrug resistant cell lines. Work was initiated with highly cytotoxic peptides derived from a marine organism, Dolabella auricularia, which potently inhibit microtubule assembly.

3. Laboratory of Medicinal Chemistry

The Laboratory of Medicinal Chemistry (LMC) was established to give increased intramural emphasis to (1) the discovery and identification of new anticancer and antiviral drugs from both synthetic and natural sources and (2) the development of analytical methodology appropriate for the quantitation of new drugs in biological fluids and the identification of metabolites. Essentially all projects are collaborative in nature, either among the synthetic and analytical chemists within the LMC or between the LMC and other NIH or academic laboratories.

Arabinosyl-5-azacytosine (ara-AC), a compound synthesized a number of years ago in this laboratory, started Phase I clinical trials in three institutions including the NIH Clinical Center. The LMC has developed an analytical method for the quantitation of ara-AC in human body fluids and is applying the technique to pharmacokinetic studies in the COP clinical trial. Plasma, pleural and CSF samples from 20 patients have been analyzed.

LMC interest in the antitumor and antiviral activity of cyclopentenyl nucleosides continues to be high based on the DCT Decision Network Committee selection of one of

our analogues, cyclopentenyl cytosine (CPE-C), as a potential clinical candidate. CPE-C is very active against a number of NCI in vivo tumor models as well as both RNA and DNA viruses. An HPLC assay suitable for use in both preclinical and clinical CPE-C pharmacokinetic studies was developed using isocarbodine as an internal standard. With a limit of quantitation of 25 ng/ml, this method was used to show that the compound was stable for 24 hours in human plasma and that < 5% protein binding occurred. The terminal half-life in the rat was 62 minutes.

3-Deazaneplanocin A (3DN), a compound synthesized by the LMC, is the most potent known inhibitor of the enzyme S-adenosylhomocysteine hydrolase. It has been found to have excellent antiviral activity against vesicular stomatitis virus, parainfluenza-3 and vaccinia virus. In vivo, 3DN is more active than ara-A against the murine vaccinia tail pox model. Because it is poorly phosphorylated, 3DN is much less cytotoxic than its parent compound, the fermentation product neplanocin A.

A phosphoramidite synthon was synthesized and used in an automated DNA synthesizer to prepare decamers containing dihydro-5-azacytidine and 5-azacytidine at defined nucleotide sites. These compounds will be used to investigate the role of 5-azacytosine-containing oligomers on the inhibition of DNA methylase and the consequent effects on gene expression.

The initial compounds have been prepared on a new project to synthesize protein kinase C inhibitors. These materials feature a rigid glycerol backbone and are designed to define the critical spatial relationships which exist between the polar and non-polar groups in activators of this enzyme.

The oral bioavailability of 5-fluoro-2',3'-dideoxycytidine (5-F-ddC) was determined to be 70%, the same as the parent compound, ddC. In anti-HIV testing at FCRF, both 5-F and 5-Br-ddC were determined to be active.

2'-Fluoro-2',3'-dideoxyarabinosyl adenine (2'-F-dd-ara-A) has been shown to be just as active and potent as ddA but completely acid stable - a desirable property for an orally administered product. Its parent compound, ddA, is very unstable under acidic conditions. This difference was exemplified by 2'-F-dd-ara-A and ddA oral bioavailability values of 80% and 30%, respectively, when the compounds were administered in saline to dogs.

Two synthetic analogues of the unusual anti-HIV fermentation product, oxetanocin, were prepared for evaluation. The first was inactive and the second is currently on test.

CSF/plasma ratios were determined for the anti-AIDS drug ddC and its metabolite, ddU, in rhesus monkeys. The ratio for ddU (15%) was five times that for ddC and both ratios appear to be influx rather than efflux related.

4. Laboratory of Molecular Pharmacology

The major goal of the Laboratory of Molecular Pharmacology (LMP) is to obtain basic knowledge that could be applicable to the development of new strategies for the selective killing of human tumor cell types.

In order to determine the role of topoisomerases in drug sensitivity, we are studying an unusual topoisomerase which we have found in a drug-resistant cell line. Although the cells are resistant to topo II inhibitors, topo II is present at near normal levels. Instead we find the presence of a large amount of an unusual form of topo I. We have purified the enzyme to homogeneity and are investigating its properties. Evidence was obtained for the existence of a form of topo II-DNA complex that is tight but mobile and non-covalent. We refer to these as "bracelet complexes" because of the apparent ability of the topo II in such complexes to glide along the DNA and to dissociate at strand ends. If the DNA is circular (relaxed circles) and has no ends, then the complex has increased stability. The polyamines, spermine and spermidine, increase the level of such complexes, perhaps by blocking the migration of the bracelet complexes towards dissociation sites along the DNA.

Evidence was obtained in support of the hypothesis that the cytotoxic effect of drug-stabilized topo II-DNA complexes may be due to irreversible consequences which may occur when a drug-stabilized complex is encountered by a replication fork. It was found that if the movement of replication forks is inhibited with aphidicolin, cell survival is increased, even though the level of drug-stabilized complexes remains unaltered.

We have now developed hypotheses using computer aided molecular modelling to account for the observed specific sequence preferences of uracil mustard and quinacrine mustard, and we have begun to investigate

compounds designed by molecular modelling to test these hypotheses. Methods are also being established to investigate preferential reactivities in intact cells and to relate them to drug sensitivity. Previous work had shown that mammalian cells have the capability to repair DNA selectively in regions that are transcriptionally active, and that this capability helps the cells to survive DNA damaging treatments.

A significant step was taken towards an understanding of the factors which govern the repair of DNA lesions preferentially in essential genes. DNA repair deficient Chinese hamster cells were transfected with a prokaryotic or a human repair gene in order to determine whether DNA repair function introduced by these genes would discriminate between active and inactive genes. Studies are in progress to determine whether restored repair capacity is preferential for active gene regions.

An understanding of the abnormalities in the regulation of cell proliferation in malignant cells may open new possibilities for therapy. Our Laboratory is concentrating on the regulation of histone variant genes. Although the synthesis of the major nucleosomal histones is regulated in synchrony with DNA synthesis, certain variant histones are regulated differently. Previous work in our Laboratory identified and characterized a minor histone variant, which we designated H2A.z, and showed that synthesis of this protein is regulated according to the proliferative state of the cells. We have cloned and sequenced the cDNA of H2A.z genes from human, cow and rat, and found the gene to be highly conserved in these species. The cDNA sequences are being used to isolate and investigate the genomic sequences so as to disclose the regulatory regions of the gene. Aspects of the sequence structure are consistent with the possibility that the differences in regulation could in part be due to differences in message stability.

Since DNA replication in mammalian cells can be limited by the availability of histones, our Laboratory has been investigating the factors affecting the kinetics of histone biosynthesis, degradation and incorporation into chromatin. The previous work led to the hypothesis that the levels of soluble histones regulate the concentration of histone mRNA by stabilizing or destabilizing the mRNA, and in particular that there is a phenomenon of chromosome cycle compensation in which histone mRNA levels rise to maintain the rate of histone synthesis when overall protein synthesis is partially inhibited. The hypothesis accounts for the

observed effects of inhibitors of protein or DNA synthesis on the level of histone mRNA and on histone pool kinetics in proliferating cells. The different histone classes may have individual influences on the state of chromatin.

The events during the transition from cell proliferation to quiescence are being investigated. Failure to carry out this transition normally, may be essentially what makes cells malignant. It was found in non-malignant cells that when the cells are put into serum-free medium, which causes the cells to become quiescent after completing the current S-phase, histone synthesis remains undiminished even though overall protein synthesis immediately drops to the level characteristic of quiescent cells.

Table 1

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/87 - 3/31/88)

NSC Number	Name	Compound Type*
	Decision Network IIA	
	Antineoplastics	
284751	8-chloro-cAMP	S
354646/357704	Morpholino anthracyclines	S
602668	Carmethizole	S
339555	Bryostatin 1	NP
366140	Pyrazoloacridine	S
375575	Cyclopentenyl cytosine	S
	Anti-HIV	
614635D	Discreet	S
614846	Carbovir	S
613671/613672	Oligophosphorothioate Nucleotides	S
614552	Castanospermine	NP
	Decision Network IIB	
	Anti-HIV	
612049	Dideoxyinosine (7/17/87)	S
	Decision Network III	
	Antineoplastics	
326231	L-buthionine sulfoximine	S
329680	Hepsulfam	S
339638	Fostriecin	NP
361456	Pyrazine diazohydroxide	S
150014	Hydrazine sulfate	S
	Anti-HIV	
098700	Dideoxyadenosine	S
612049	Dideoxinosine (1/27/88)	S

*S = synthetic
NP = natural product

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06191-01 OAD

PERIOD COVERED
October 1, 1987 through September 30, 1988

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

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

	Name	Title	Affiliation
PIs:	M. Alley, Ph.D.	Pharmacologist	OAD, NCI
	M. Boyd, M.D., Ph.D.	Associate Director	OAD, NCI
	J. Cardellina, Ph.D.	Suprv. Res. Chem.	OAD, NCI
	W. Hubbard, Ph.D.	Expert	OAD, NCI
	T. McLemore, M.D., Ph.D.	Sr. Investigator	OAD, NCI
	J. McMahon, Ph.D.	Expert	OAD, NCI
	P. Skehan, Ph.D.	Expert	OAD, NCI
	S. Stinson, Ph.D.	Biologist	OAD, NCI
	D. Vistica, Ph.D.	Pharmacologist	OAD, NCI

COOPERATING UNITS *(if any)*
Biological Testing Branch, DTP, FCRF, NCI; Natural Products Branch, DTP, NCI; Program Resources, Inc., FCRF; Johns Hopkins University, Dept. of Medicine, Pulmonary Division, and Dept. of Surgical Pathology, Baltimore, Maryland

LAB/BRANCH
Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
NCI, Frederick Cancer Research Facility, Frederick, 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.)*

New research programs were established in natural products chemistry, cellular pharmacology, ultrastructure, immunochemistry, tissue culture, and tumor cell biology. A sulforhodamine B (SRB) assay was developed for quantitating drug effects upon stratum attached cells, as was an MTT tetrazolium assay for single cell suspensions. A bicarbonate-free growth medium was developed. Evaluation of its ability to support the growth of cell lines in the screening panels is in progress. Nearly 200 human cell tumor lines have been evaluated as candidates for possible inclusion in the anti-AIDS and anti cancer drug screens. Those which grew adequately in culture, were of early passage, and were obtained from tumors with documented pathology from patients not receiving prior chemo- or radiation therapy, are undergoing extended evaluation. The histopathology, immunochemistry, ultrastructure, growth physiology, biochemistry, and chemotype of these lines are being analyzed in detail. A procedure for Stage I *in vitro* anticancer drug screening was developed. Its experimental optimization and large-scale feasibility testing are nearing completion. Pattern recognition algorithms have been developed for identifying drugs which act selectively against specific subsets of cell lines. A tentative protocol for Stage II *in vitro* anticancer screening has been developed, and is currently being optimized in a basic research mode on a subset of cell lines with a group of standard clinical drugs. A variety of tumor lines encapsulated within polymeric microspheres have been successively tested as an *in vivo* assay of drug sensitivity. A novel method was developed for propogating human lung tumor lines as orthotopic xenografts in the lungs of nude mice using intrabronchial and intrathoracic implantations. A number of cyanobacterial, tropical plant, and marine natural products with anti-AIDS activity were successfully fractionated. The chemical structures of one class of active agents has been elucidated, and several others are in the advanced stages of structure identification.

The mission of the Program Development Research Group (PDRG) is to carry out a program of basic and applied research supportive of and complementary to the new drug research and development programs of the National Cancer Institute. To this end, the PDRG investigators report progress on a number of interrelated areas.

Assay Development for Primary Screen

Three types of assays were examined in detail as possible methods for quantitating the effects upon cells of the test compounds used in the anti-AIDS and anticancer drug screens. These included 18 general biomass stains, three cellular protein stains, and two tetrazolium assays (MTT and XTT).

Two of the protein stains, Sulforhodamine B (SRB) and Bromophenol Blue (BPB), were clearly superior to other assays in resolution, signal to noise ratio, and correlation with cell number when used with substratum attached cells. However, they required multiple washings to remove low molecular weight amino acids and peptides, and were therefore not suitable for use with suspension cultures. Of the assays tested to date, only the MTT tetrazolium method was practical for use with single cell suspensions, although its linearity with cell number was limited to a narrow range of values. It was concluded that SRB was the assay of choice for substratum attached cells, while the MTT was presently the only method available for the end-point assay of poorly or non-attached cells. Alternative assays for suspension culture will be explored during the coming year.

Tetrazolium Assays and pH

Tetrazolium assays were particularly sensitive to pH fluctuation artifacts. With slight alkaline deviations, the tetrazoliums generated crystal artifacts which reduced substrate and cofactor concentrations and increased light scattering. These effects were intensified by the presence of reducing agents such as glutathione.

Thiols, particularly glutathione, were found to initiate crystal formation with tetrazolium dyes such as XTT and with electron coupling agents, such as phenazine methosulfate (PMS), which are often used to enhance tetrazolium reactions. Crystal formation was exacerbated by alkaline conditions with pH > 8, which readily occur when cell plates are removed from a 5% CO_2 incubator during drug treatment and OD reading. Studies on the effect of extracellular glucose and intracellular NADH and NADPH on tetrazolium reduction are in progress.

Bicarbonate-Free Growth Medium

Removal of cells from a CO_2 incubator for even brief periods of time causes rapid shifts in growth medium pH which can perturb cell growth, intensify drug activity, and produce artifacts with tetrazolium assays. These pH effects vary widely from one well, plate, cell line and drug to another. Efforts were therefore initiated to develop a new growth medium that did not require either exogenously added bicarbonate or a CO_2 enriched atmosphere.

A new growth medium (PDRG 1), free of exogenously added bicarbonate, is in an advanced state of development. With the cell lines tested to date, it supports growth that is comparable with and, in some cases, superior to RPMI-1740 with 5% CO_2 (the growth medium now in use) and several times better than reported for commercial bicarbonate-free medium (BFM) compositions.

Stage I Protocol for In Vitro Analysis of Anticancer Drug Activity

Specific parameters of the Stage I protocol for *in vitro* anticancer drug screening are in the final phases of large-scale feasibility testing and experimental optimization of the drug incubation period required to identify the greatest proportion of true positives, while keeping false positive identifications within acceptable limits. For substratum attached cells, the optimal protocol consisted of (1) seeding densities of 10-30 thousand cells per well in 96 well plates, (2) a 100 ul seeding volume, (3) a 1 day drug pre-incubation period, (4) addition of 100 ul of test solution in growth medium, (5) a 1-3 day drug incubation period, (6) cell fixation by a 50 ul addition of 50% TCA, (7) the SRB assay, (8) collection of time zero samples, and (9) the analysis of data by the methods of both net growth and test/control ratios. A similar protocol, using the MTT assay, has been established for suspension lines.

Pattern Recognition Algorithms for the Anticancer Drug Screen

Several computer-programmable pattern recognition algorithms are being evaluated for their ability to identify selective patterns of drug activity toward target cell lines. These methods permit three different biological drug effects to be quantitatively evaluated: the slowing of growth, cytostasis, and net cell killing. Each effect is evaluated at several different intensity levels of cellular response using both single parameter and multiparameter algorithms. Chemical concentration indices (IC50, IC90, etc.) are being used to identify cell lines differentially sensitive to low drug concentrations, while maximum response values are being used to identify cell lines which respond most intensely to a particular drug. Because the drugs most effective

against a very resistant line may seem ineffectual by these conventional criteria, percentile curves for chemosensitivity and maximum responsiveness are being constructed for each cell line. If a new drug exceeds a predetermined percentile value for a generally resistant line, it will be considered active against that line. A multiparameter test has been developed which allows the shape of a dose-response curve to be taken into account in analyzing cellular responsiveness to drugs.

Several methods of difference spectra are being tested to identify drugs similar to those with known clinical activity. Two spectra are overlayed, their cell line by cell line differences summed, and the smallness of this sum used as an index of similarity. Difference spectrum methods can be used with concentration, maximum response, and multiparameter indices of drug activity. They can be used with a wide variety of templates including alkylating agents, antifols, nucleic acids, and spindle poisons. Difference spectra methods have been tested using IC50 indices, and have proven extremely powerful at recognizing drugs with related chemistries.

Stage II In Vitro Analysis of Anticancer Drug Activity

The primary in vitro anticancer drug screen is a preliminary testing procedure designed to identify those few of a very large number of test compounds that are worthy of further evaluation. By their nature, primary screens generate significant numbers of false positive identifications which must be subsequently eliminated. Because costs escalate enormously with each successive step in the drug development process, the most cost-effective method for identifying and eliminating these false positives is in a second stage of in vitro tests.

A protocol for Stage II in vitro analysis has been developed. It asks the following questions about each apparently active drug: (1) Can the apparent selectivity pattern of the Stage I assay be confirmed by an independent method? (2) What is the biological effect of an apparently active drug - growth slowing, cytostasis, or cell killing? (3) Does drug sensitivity depend on cell density, growth rate, or nutritional status? If so, is the apparent sensitivity pattern retained under in vivo-like conditions? (4) What is the minimum period of drug exposure necessary to produce maximum biological effect on target cells? (5) Do cells recover following drug removal? If so, how rapidly? These questions are being addressed in kinetic, drug pulse, and drug recovery experiments using subconfluent monolayers, postconfluent multilayers, soft agar colonies, and plateau phase colonies with end points measured by macromolecular stains, metabolic reactions, colony forming efficiencies, image analysis of colony volumes, and colony extinction assays.

Microencapsulation of Tumor Cells

The encapsulation of tumor cells within drug and nutrient permeable polymeric microspheres has been investigated for feasibility as an in vivo assay of anti-HIV and anticancer drug activity. Microcapsules containing human T-lymphoblastoid cells were implanted in the peritoneal cavity of nude mice. The mice were then treated i.v., s.c., and orally with anti-HIV test compounds. Microcapsules were recovered at appropriate intervals and assessed for cell viability. The growth or survival of infected cells within the microcapsules was used as an index of drug antiviral activity.

Accessory technologies were developed to insure protection from the HIV aerosols generated during capsule formation, and to quantify cell viability and capsular content. Preliminary feasibility tests indicate that the modifications of microcapsule technology which we have developed provide the basis for a safe and reliable in vivo assay of anti-HIV activity.

It was concluded that (1) human lymphoid cells can be safely encapsulated using modifications of standard microencapsulation procedures, (2) cells proliferate within the capsules and are susceptible to HIV infection, (3) microcapsules implanted into nude mice can be effectively recovered and analyzed, and (4) the viability of encapsulated target cells can be quantitated using simple colorimetric methods.

Human Lung tumor Lines

Significant differences in prostanoid biosynthesis, 4-ipomeanol metabolism, cytochrome P450 activity, the regulation of P450IA1 gene expression, and glutathione-S-transferase activity were observed between paired samples of normal lung and lung tumor tissue from individual patients, as well as among different established human lung tumor lines.

A novel method was developed for propogating human lung tumor lines as orthotopic xenografts in the lungs of nude mice using intrabronchial and intrathoracic implantation methods.

Cell Line Characterization

Culture protocols are being developed for the detailed in vitro and in vivo characterization of the cell lines that are candidates for inclusion in the anticancer drug screen. The protocols include (1) the cultivation of cells as adherent monolayers and as 3-dimensional soft agar colonies, (2) the fixation and staining of culture and xenograft materials for histological and ultrastructural analysis, and (3) the analysis of cellular proteins and glycoproteins by 2-dimensional polyacrylamide gel electrophoresis (PAGE).

<u>Histopathology of Cell Lines</u>

A histopathological analysis of the cell lines which are serious candidates for inclusion in the *in vitro* anticancer drug screen is in progress. More than 30 cell lines grown as nude mouse xenografts have been imbedded for light microscopy, as have a number of cell lines grown as 3-dimensional colonies in soft agar. Slides of these samples have been prepared and are undergoing histopathological evaluation. The reamining cell lines will be prepared and evaluated in a similar manner.

<u>Ultrastructure and Immunochemical Characterization of Cell Lines</u>

A facility for ultrastructural analysis was established within the PDRG. It includes a Joel scanning-transmission electron microscope with an energy dispersive spectrometer for quantitative elemental analysis.

A preliminary ultrastructural characterization has been initiated of cell lines from the anticancer drug screen grown as 3-dimensional soft agar colonies. A comparative ultrastructural analysis has been conducted for several screen lines grown as *in vitro* monolayers, soft agar colonies, and *in vivo* xenografts.

Protocols are being developed for the immunological analysis of the tumor associated antigens of the cel lines in the anticancer drug screen. Monoclonal antibodies will be used for this purpose. Studies of the immunocytochemical localization of prostaglandin synthetase have been completed.

<u>Biochemical Characterization of Cell Lines</u>

Prostaglandins and related lipids have been implicated as mediators of tumor metastasis, host immunoregulation, and cell growth regulation. A systematic evaluation of the ability of human tumor cells to synthesize these compounds was initiated as a first step in the biochemical characterization of the human tumor cell lines being considered for inclusion in the NCI's disease-oriented *in vitro* anticancer drug screen.

Fatty acid cyclooxygenase products, synthesized from endogenous and exogenous arachidonic acid, have been determined in 28 established cell lines derived from human lung tumors and in eight established cell lines originating from three human colon, three ovarian, and two2 prostate tumors.

Employing total prostanoid biosynthesis and an index of prostaglandin H synthase (PHS) activity, our findings suggest that prostaglandin and thromboxane biosynthesis may be a unique feature of certain subclasses of non-small cell carcinomas of the lung. The level of prostanoid production and the incidence of

PHS activity > 2 picomoles/million cells were consistently higher in established cell lines derived from human non-small cell carcinomas of the lung than in lines originating from small cell carcinomas, ovarian adenocarcinomas, colorectal carcinomas, and prostate adenocarcinomas.

Analytical Chemistry of Natural Products with Anti-HIV Activity

A new natural products chemistry group has been assembled to purify and elucidate the chemical structures of natural product components samples active against the HIV virus in the NCI's primary anti-AIDS drug screen. Active extracts have been obtained from cyanobacteria, tropical plants, and marine organisms. Fractionation of cyanobacterial extracts led to the isolation and identification of a related series of compounds with moderate anti-HIV activity. Extracts from six tropical plants have also shown anti-HIV activity. A number of pure compounds have been isolated from one of these plants. Their chemical structures and anti-HIV activities are being investigated. Fractionation of the active component of a marine sponge is in progress. An improved method for the isolation and purification of Taxol has been developed.

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ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 - September 30, 1988

The Natural Products Branch is responsible for acquisition, isolation, structure determination, and testing of compounds from microbial, plant and animal sources in order to obtain new leads for further development in the NCI programs for both anticancer and anti-AIDS drugs.

The major program areas of the Natural Products Branch are: (1) acquisition of crude biological materials of plant, marine, and microbial origin for the DTP screening program; (2) contract research directed toward isolation of new agents from active extracts; (3) world-wide literature surveillance, and acquisition of natural products with demonstrated biological activity or novel structural types for evaluation; (4) procurement and preparation of large quantities of active agents for drug formulation, advanced biological testing, toxicology and clinical studies.

With the commitment of the Developmental Therapeutics Program to a new direction in *in vitro* screening, a renaissance of interest in natural products as potentially highly selective antitumor agents has begun with a major new program effort in collection of natural products from a wide variety of sources, including terrestrial plants, marine invertebrates, algae and marine microorganisms. New contracts were awarded in late FY '86 for collection of plants from diverse locations, collections of both shallow water and deep water marine organisms, and for cultivation of cyanobacteria. Contracts for marine microorganism cultivation will be awarded in late 1988.

Much of the Branch's efforts in FY '88 have been devoted towards development of the necessary procedures, operations, and data support for the repository, extraction lab, drug preparation lab and screening laboratories relevant to natural products.

Organization and Staffing

The Branch is organized into functional segments coordinated through the Chief which are: (1) acquisition of new pure compounds and supplier liaison, (2) discovery of new agents from fermentation derived extracts, (3) discovery of new agents from plant extracts, and (4) discovery of new agents from extracts of marine organisms. Many tasks require interaction between these segments, and the Branch personnel are assigned duties in whichever of the areas requires their expertise depending on changing program needs. The present full time staff consists of five professionals and two secretaries. The contracts managed by the Branch are outlined in Table 1.

Table 1.

Natural Products Branch Contracts

Contractor	Investigator	Contract RFP No.	Program Area
		CANCER	
Polysciences, Inc.	Sims	N01-CM6-7707	Large-Scale Isolation
Hauser Chemical Res.	Daughenbaugh	N01-CM8-7221	Large-Scale Isolation
Univ. of Illinois	Farnsworth	N01-CM6-7705	Literature Surveillance
Univ. of Connecticut	Collins	N01-CM5-7692	Fungal Fermentation
SeaPharm, Inc.	Pomponi	NCI-CM6-7967	Shallow Water Marine Collection
Univ. of Illinois at Chicago	Soejarto	NCI-CM6-7925	Plant Collection (Asia)
Missouri Botanical Garden	Forero	NCI-CM6-7923	Plant Collection (Africa)
New York Botanical Garden	Balick/Prance	NCI-CM6-7924	Plant Collection (South America)
Univ. of Hawaii at Manoa	Patterson	NCI-CM6-7745	Cultivation of Cyanobacteria
(In Competition)		RFP 87238	Marine Anaerobic Bacteria
(In Competition)		RFP 87239	Marine Protozoa
		AIDS	
(In Competition)		NCI-CM8-7219	Large-Scale Isolation
(In Competition)		NCI-CM8-7233	Recollection of Marine Organisms and Terrestrial Plants
(In Competition)		NCI-CM8-7226	Literature Surveillance

Fermentation

As will be noted subsequently in Table 4 numerous natural products which are in advanced development have been derived from fermentations. In the past the organisms involved have been bacteria, actinomycetes and fungi whose normal habitats are largely on land. We have developed new programs to expand beyond the microorganisms traditionally studied in the pharmaceutical industry and are taking a leadership position in utilizing novel source organisms to provide extracts for screening. Screening programs for products from fungi (University of Connecticut N01-CM-57692) and from cyanobacteria (blue-green algae) (University of Hawaii, NCI-CM-57745-16) are proceeding on schedule. Marine microorganisms are likewise being explored for the production of compounds of interest under new RFPs for marine protozoalcultivation and for marine anaerobes. An RFP for Master Agreements for the Recollection of Marine Organisms and Terrestrial Plants for testing as potential anti-AIDS agents is under competition.

A major renovation and upgrade of the Fermentation Plant at FCRF is being undertaken which will greatly enhance the capabilities for scaleup of new leads for both cancer and AIDS drugs.

Plant Program

Three major contracts for the collection of over 20,000 plant samples from the tropical rain forest regions of Africa, Madagascar, Central and South America, and South East Asia were awarded in September, 1986 for a five year period. Good progress has been made in the collection of a wide variety of taxa, and some contact has been made with shamans in certain areas who are helping in the collection of medicinal plants. Samples are being extracted and screened for activity against the human cancer cell line panels and HIV, and plants yielding selectively active extracts will be recollected in larger quantities for purposes of the isolation and development of the active agent. In the case of pure compounds exhibiting promising activity, large quantities of the drug will be isolated under contracts maintained for such scale-up isolations. Over the past year large amounts of taxol have been produced by such a contract for use in continuing Phase II clinical trials.

To date, over 6,000 samples have been received of which over 743 have been extracted to provide 1,486 extracts which are ready for testing.

Marine Program

The objective of this program is to discover, isolate and develop novel antineoplastic and anti-AIDS agents derived from marine macroorganisms, a now well established source of novel, and often biologically-active, chemical compounds. Two major contracts were initiated September 30, 1986 for the collection and documentation of 5,000 shallow-water and 5,000 deep-water organisms over a five year period. The collections include a wide variety of taxa, selected to represent the greatest possible chemical, taxonomic and environmental diversity, and priority will be given to taxa known to contain biologically-active metabolites. The Shallow Water collection contract is meeting its goal of more than 1,000 specimens per year and 709 are on hand in the FCRF Repository and will be available for screening soon. The Deep Water Marine program was terminated after one year due to escalating costs, difficulties in scheduling ship time and insufficient amounts of samples collected. Deep Water collection may become more efficient in the next few years as technology for ROVs (remotely operated vehicles) improves and these can be used in collection operations instead of the much more expensive manned submersibles. NCI may therefore wish to restart this project at a later time depending on technological advances and the results obtained on screening the 581 Deep Water samples collected to date.

Natural Products Extraction Laboratory and Repository

A natural products extraction laboratory and repository has been established at the Frederick Cancer Research Facility, and all plant and marine organism samples collected under contract are sent to this facility. Following logging in of the raw material samples by repository personnel, the samples are extracted with an organic solvent and water according to standard protocols which have been specially devised by laboratory and NCI staff to achieve

optimum extraction efficiency. These extracts, and those submitted by the contractors responsible for the fungal fermentation and cyanobacterial cultivation projects, are stored in the repository at -20°C to await testing in the human cancer cell line and HIV screens; samples of these extracts will also be held for testing in later screens as they are developed. A natural products computer support system has been developed which records the progress of each sample from receipt of the raw material, through the extraction process, to deposit of the extracts in the low-temperature repository. Reference to the various databases comprising this system enable the retrieval of detailed information concerning every organism extracted and the nature and repository storage location of each extract sample.

The extraction laboratory has been planned in a manner which will permit ready conversion to an isolation laboratory to handle the future separation, purification and structural elucidation of active agents from extracts shown to be selectively active in the anticancer and HIV screens.

Table 2.

Natural Products Acquisitions

September, 1986 - May, 1988

	Organisms Received	Organisms Extracted	Extracts Available
Deep Sea Marine Organisms	581	0	0
Shallow Water Marine Organisms	709	0	0
Cyanobacteria	340	340	680
Fungi	329	329	600
Terrestrial Plants	6,676	743	1,486
Marine Plants	190	190	380
Lichens	556	556	1,112

Worldwide Surveillance of Natural Products

This function is primarily carried out in the Office of the Chief. The objective is to acquire new natural products with biological activities which may relate to anticancer and more recently, to anti-AIDS effects, and compounds of a wide variety of new or unusual structural types which are worthy of screening for antitumor and anti-AIDS activity. The approach to identification of compounds for acquisition is multifaceted, and includes a contract for literature surveillance which identifies new natural product structures and biological activities, literature review by Branch staff, personal contacts with scientists in universities, research institutes and chemical and pharmaceutical companies, attendance at scientific meetings where new compounds are reported, and review of progress reports of NIH grantees. During calendar year 1987 a total of 292 new pure natural products were acquired for anti-cancer screening, of which 251 (86%) were donated and the remaining 41 compounds (14%) were from grantees.

Of the 292 compounds acquired, 56 (19%) were received in direct response to requests for those specific compounds, while a substantial number of unsolicited compounds received 195 (67%) have come from suppliers who were contacted by our program in regard to other compounds. The response rate to our literature requests (calculated on the basis of number of suppliers responding rather than the total number of compounds received from them) was 47% with 89% of the responders supplying the requested compounds. The balance of the responders (11%) sent regret letters, supplied other compounds or made inquiries about our program.

During a greater part of the report period, active solicitation of potential anticancer compounds selected via literature surveillance contract was curtailed considerably because of the unavailability of the _in vitro_ disease-oriented anticancer screening system which is being developed at the Institute. Hence, only 116 compounds were requested before a "Hold" status for acquisitions was instituted.

Details of the acquisition program are summarized in Table 3. Foreign suppliers were the source of 30% of the input, considerably less than previous years, which usually was about 51%. Because of the small number of compounds obtained, this is not a representative proportion. As in the previous years, Japan, many European countries, India and China were well represented, reflecting the international nature of this program.

A separate acquisition program for prospective anti-AIDS compounds was initiated in April, 1987. As there was no separate contract for the surveillance of literature of natural products with potential anti-AIDS activity during this report period, requests for compounds with possible anti-AIDS activity was made concurrently with those for anticancer compounds. A total of 166 compounds were obtained by this means and by requesting for specific compounds selected rationally. As the types or classes of compounds that may be active against this disease at this point must be determined somewhat empirically, the mechanics of literature search will be different from that for the search of prospective anticancer compounds. A contract for the surveillance of literature for natural products active against AIDS is now being negotiated.

Table 3.

Pure Compounds Acquired in Calendar Year 1987

Compounds by Type and Source

	Industry	Univ.	Res. Inst.	Other	Domestic	Foreign
Plant	27 (9%)	82 (28%)	24 (8%)	2 (1%)	87 (30%)	48 (16%)
Fermentation	29 (10%)	54 (18%)	11 (4%)	0	35 (12%)	59 (20%)
Animal	21 (7%)	31 (11%)	2 (1%)	0	52 (18%)	2 (1%)
Synthetic	3 (1%)	6 (2%)	0	0	5 (2%)	4 (1%)

Compounds by Type and Mechanism

	Grant	Contract	Lit. Surv.	Unsol.
Plant	29 (10%)	0	27 (9%)	79 (27%)
Fermentation	1 (0%)	0	17 (6%)	76 (26%)
Animal	11 (4%)	0	9 (3%)	34 (12%)
Synthetic	0	0	3 (1%)	6 (2%)

New Initiatives

1. Large-Scale Isolation of Anti-AIDS Agents from Natural Sources

   The isolation of pure compounds exhibiting promising anti-AIDS activity from plants and marine organisms will necessitate the further isolation of large quantities of the active agents for the purposes of preclinical and clinical development. Such scale-up isolations will be performed under a Master Agreement contract which is currently in competition.

2. Workshop on Marine Microorganisms

   The NCI together with the Office of Sea Grant of NOAA and the California Sea Grant Commission conducted a conference on the topic of medicinal applications of marine microorganisms which explored the possibilities in this area and discussed the technical problems and requirements for culture isolation, fermentation, and scale-up. This was attended by many experts in this small field and was extremely useful in determining NCI plans for this area.

3. Sample Acquisition for the HIV (Anti-AIDS) Screening Program

   The addition of an HIV drug discovery and development program to the Division has placed additional responsibility on all staff members since crude extracts from the plant, marine and microbial areas as well as pure compounds acquired through the literature surveillance program will all be submitted for evaluation. In addition to selection and prioritization of materials for screening, the Branch also is responsible for acquiring additional quantities of interesting leads and bulk isolation of development candidates.

Accomplishments

A new major program in collection of natural products for screening, extraction and storage of extracts, and isolation of active constituents from these extracts has been developed, and contracts have been awarded. This major initiative of the Division of Cancer Treatment will supplement the acquisition of new natural products through the literature surveillance project which has continued to play an indispensable role in the drug discovery program. The

Natural Products Branch has worked closely with DTP contractors and FCRF staff in development of a major new data system to handle all records on crude natural products, the Natural Products Repository System (NPRSS). This system contains detailed information on the taxonomy and habitat of the organisms collected, locations and amounts of samples at all stages from raw material to aliquotted extracts, methods of extraction used, shipping records and all other data needed to trace every sample's history and to enable recollection of the same organism for additional study as screening leads arise.

Natural products have continued to be an important source of compounds in preclinical development and early clinical studies as outlined in Table 4.

Table 4.

Natural Products in Advanced Development

NSC #	Drug	Origin	Status
163501	Acivicin	Fermentation	Phase II Clinical Trial
218321	Pentostatin	Fermentation	Phase II Clinical Trial
526417	Echinomycin	Fermentation	Phase II Clinical Trial
141633	Homoharringtonine	Plant	Phase II Clinical Trial
125973	Taxol	Plant	Phase II Clinical Trial
269148	Menogaril	Fermentation	Phase II Clinical Trial
325319	Didemnin B	Marine Animal	Phase II Clinical Trial
328426	Phyllanthoside	Plant	Toxicology
356894	Deoxyspergualin	Fermentation	Phase II Clinical Trial
303812	Aphidicolin Glycinate	Fermentation	Phase II Clinical Trial (EORTC)
339638	CI-920	Fermentation	INDA Filing
332598	Rhizoxin	Fermentation	Toxicology (EORTC)
364372	Elactocin	Fermentation	Toxicology (CRC)
369327	Elsamicin	Fermentation	Phase I Trial (EORTC)
333856	Tetrocarcin A	Fermentation	Activity Studies (EORTC)
349156	Pancratistatin	Plant	Formulation (CRC)
608418D	Discreet	Fermentation	Formulation
339555	Bryostatin I	Marine Animal	Bulk Production

Major presentations on the new screening program have been given at chemistry and pharmacognosy conferences, and have resulted in considerable interest from outside investigators. The conferences included a symposium on natural antitumor agents sponsored by the Japanese Antibiotic Research Association, and meetings of the Australian Chemical Society, the American Society of Pharmacognosy and the Society of Economic Botany.

A collaboration with the Cancer Research Campaign (CRC) in the United Kingdom and the European Organization for Research in Treatment of Cancer (EORTC) has resulted in several new drugs entering study in Europe (Table 4).

Several new collaborations have been established with outside investigators for screening and discovery of new agents including Dr. Paul Cox of Brigham Young University on Samoan medicinal plants, and Dr. Won Sick Woo of the Seoul National University on Korean medicinal plants. Several of these medicinal plant extracts have shown preliminary activity against HIV and the chemistry of these leads will be a top priority during the coming years.

Taxol, isolated from the bark of _Taxus_ _brevifolia_ (Pacific or Western Yew) has shown some promise in clinical trials against melanoma, and is showing a 30% response rate in an ongoing Phase II trial against ovarian carcinoma. A 60,000 lb collection of the dried bark of _T._ _brevifolia_ is due for completion in 1988, and further collections are envisaged in 1989. Excellent collaboration is being offered by the Forestry Service of the U.S.D.A. in issuing permits to collectors and exercising control over the harvesting of the bark. _T._ _brevifolia_ is a very slow-growing tree, and only one or two more large collections will be possible without causing environmental problems. The participation of companies in the forestry industry is being solicited in investigating the mass propagation of seedlings as a continuing source of the drug.

Bryostatin I has been selected for advanced development and a major collection of 11,000 gallons of the source organism, _Bugula_ _neritina_ (a marine animal in the Bryozoa) is underway as is methods development for large scale extraction and isolation. Bryostatins are members of a totally new chemical class, the bryopyrans, and have extremely potent biological activities as activators of protein kinase C. They have shown extremely good selectivity in the disease oriented screen and in addition stimulate growth of granulocyte-macrophage colony forming units and show potentiation of IL-2 cytotoxicity. There is very high interest in the Division of Cancer Treatment in development of the bryostatins.

Staff Publications

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2. Bray DH, Boardman P, O'Neill MJ, Chan KL, Phillipson JD, Warhurst DC, and Suffness M. Plants as a source of antimalarial drugs, Phytotherapy Res 1987;1:22-24.

3. Boyd MR, Shoemaker RH, Cragg G and Suffness M. New avenues of investigation of marine biologicals in the anticancer drug discovery program of the National Cancer Institute. In Jefford, CW, Rinehart KL, and Shield LS (eds.), Pharmaceuticals and the Sea, Technomic Publishing Co, Lancaster, PA, 1988;27-44.

4. Brossi A, Yeh HJC, Chrzanowska M, Wolff J, Hamel E, Lin CM, Quinn F, Suffness M and Silverton J. Colchicine and its analogues: recent findings, Medicinal Res. Rev. 1988;8:77-94.

5. Cragg G and Suffness M. Metabolism of plant-derived anticancer agents, Pharmacol Ther. 1988;37:425-461.

6. Spjut RW, Cassady JM, McCloud T, Suffness M, Norris DH, Cragg G, and Edson, CF. Variation in cytotoxicity and antitumor activity among samples of the moss _Claopodium_ _crispifolium_ (Thuidaceae), Economic Botany 1988;42:62-72.

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The fundamental objective of the Drug Synthesis and Chemistry Branch (DS&CB) is the discovery and development of novel synthetic anticancer leads through the acquisition, synthesis structure-activity optimization and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents in the Developmental Therapeutics Program (DTP). Recently, DS&CB has been assigned additional responsibilities for the discovery and development of novel synthetic anti-AIDS preclinical leads. The DS&CB achieves its central mission by engaging in a variety of Program activities, namely, acquisition of a large number of synthetic compounds of diverse biological and structural types through the development and maintenance of an extensive net-work of scientific liaison on a world wide basis, structure-activity optimization through the synthesis of congeners and prodrugs, radiolabelled syntheses, task order resyntheses, bulk synthesis for toxicology and Phase I Studies, storage, inventory and distribution and computer-assisted structure-activity analysis. The synthesis contracts for toxicology and Phase I Studies, both cancer and AIDS, have been transferred to the Pharmaceutical Resources Branch to better facilitate overall DTP coordination. Collaborative programs are under-way with the intramural laboratories; the Laboratory of Biological Chemistry and the Laboratory of Molecular Pharmacology. In addition, DS&CB supports the activities of the Information Technology Branch (ITB), the Radiation Research Program (RRP), the European Organization for Research on Treatment of Cancer (EORTC) Program and the U.S.-Japan Cooperative Cancer Research Program.

The contracts managed by DS&CB are outlined in Table 1. In addition, several RFPs have been prepared and are being competitively reviewed for the new anti-AIDS program. Presently, the DS&CB is staffed with six professionals, and two clerical personnel; one-staff member is on detail to ITB.

The DS&CB played a central role in organizing the Acquisition Input Committee (AIC) of DTP which is now functioning effectively. The major challenge during this year has been to anticipate the needs of our evolving "disease-oriented" anti-cancer drug discovery program and restructure the program activities of DS&CB and the staff to respond in a creative and timely fashion to the new opportunities. An additional challenge has been to anticipate, plan, and respond to the new opportunities created by the anti-AIDS drug discovery and development effort.

The significant accomplishments of DS&CB include the following:

1. Synthetic compounds of diverse biological and structural types are being acquired for biological evaluation as dictated by the capacities of both the anti-cancer and anti-AIDS screens. New DN-2A candidates are emerging from this effort.

2. We are developing a computer-based clustering model to group and prioritize compounds for screening from our large repository of unique compounds.

3. Within a very short period of the operation of the new AIDS screen, NSC 614846 (Carbovir) was identified as an interesting anti-AIDS lead with good potential.

4. A novel carbinolamine derivative, NSC 602668, developed in one of the drug design contracts, with excellent anti-tumor activity against a broad spectrum of tumors, is being rapidly developed further.

5. The large-scale synthesis of several compounds have been accomplished, for example, DDA (NSC 98700), DDC (NSC 612049) and Penclomedine (NSC 338720).

6. The synthesis of Several radiolabelled for preclinical and clinical studies were completed; for example Bayer compound (NSC 320846), AZT, DDI (NSC 612049) and DDC (NSC 606170).

7. The master resynthesis contracts have provided a variety of compounds of program interest that are not available in sufficient quantity from the original suppliers (approximately 60 compounds) in a cost-effective way.

8. The storage and distribution contractor, has organized, repackaged and reshelved more than 112,000 compounds. The contractor has shipped approximately 8000 compounds to foreign and domestic investigators.

9. Several contracts, for resynthesis, radiolabelled synthesis, and congener and pro-drug synthesis in support of the AIDS program have been established.

10. The syntheses of Castanospermine analogs and the Cyanobacteria anti-AIDS lead have been initiated.

11. Collaborative programs with the intramural groups resulted in the synthesis of a variety of compounds, e.g., several fatty acid derivatives of Coenzyme-A, indanone mustards, B-methylene TAD and cyanotyrosines.

TABLE I

CONTRACTS - FY 88

Contractor	Investigator		Contract No.
Alabama, University of	Baker		N01-CM-87267
Aldrich Chemical Co., Inc.	Kulkarni		N01-CM-67771
Ash Stevens, Inc.	Khan		N01-CM-67872
ERCI Facilities Service Corp.	Groover		N01-CM-73721
Georgia Technology Res. Center	Zalkow		N01-CM-87269
Japanese Foundation	Tsukagoshi		N01-CM-36011
Moravek Biochemicals, Inc.	Moravek	(SBIR)	N44-CM-77830
New York, State University of	Anderson		N01-CM-67698
New York, State University of	Anderson		N01-CM-87216
Purdue Research Foundation	Cushman		N01-CM-67699
Purdue Research Foundation	Cushman		N01-CM-87268
Raylo Chemicals, Ltd.	Langford		N01-CM-67866
Research Triangle Institute	Kepler		N01-CM-67703
Research Triangle Institute	Kepler		N01-CM-87227
SRI International	Tracy & Brown		N01-CM-47611
Starks Associates	D. Starks		N01-CM-67798
Starks C.P.	Perchonock		N01-CM-47608
Z, Inc.	Sobers		N01-CM-73720

Master Agreements (Task Order) Contracts:

Contractor	Investigator	Contract No.
Alabama, University of	Baker	N01-CM-67971
Darmouth College	Curphey	N01-CM-67976
H.G. Pars Pharmaceutical Labs.	Pars	N01-CM-67972
New Mexico State University	Guziec	N01-CM-67974
Polysciences, Inc.	Parasaran	N01-CM-67977
Raylo Chemicals, Ltd.	Lee	N01-CM-67975
Research Triangle Institute	Seltzman	N01-CM-67970
South Florida, University of	Owen	N01-CM-67973
Sothern Research Institute	Temple	N01-CM-67968
SRI International	Tanga	N01-CM-67969
Starks Associates	Hsiao	N01-CM-67978

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PATENTS:

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ANNUAL REPORT OF THE BIOLOGICAL TESTING BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Biological Testing Branch (BTB) has responsibilities which include the development and implementation of a disease-oriented in vitro screening program for a large number of candidate cancer chemotherapy compounds and for follow-up in vivo testing of selected agents.

The BTB has responsibility for developing an AIDS testing program which includes the establishment and implementation of an anti-HIV screen (in vitro) with follow-up in vivo testing capabilities.

The BTB manages a large resource for the production, quality control, and distribution of genetically and biologically defined rodents. These disease free experimental animals are distributed to other NCI Divisions, intramural investigators at Bethesda and FCRF, other investigators within the NIH, other governmental agencies, and NIH grantee investigators on a cost reimbursement system.

The BTB maintains a large repository of experimental animal and human tumor lines for usage by DCT and other NCI programs. Tumors are also distributed to qualified cancer research investigators on a cost reimbursement basis.

The objectives of the BTB are to:

I. Cancer Area

1. Expand in vitro testing capabilities from the current capacity of approximately 50 human tumor lines to encompass approximately 100 well characterized and defined lines of diversified origin representing the major types of cancer.

2. To expand in vitro screening capacity from the current level of approximately 2,000 compounds to our goal of 10,000 compounds on an annualized basis.

3. Develop models and enhance in vivo testing capacity to the extent that selected compounds from the in vitro screen can be tested for confirmation purposes. The time frame for accomplishing adequate in vivo testing capacity is dependent upon the renovation of space at FCRF (APA buildings).

II. AIDS Area

1. To implement in vitro screening capacity to an interim level of 10,000 compounds and a final level exceeding 20,000 compounds on an annualized basis. The time frame for reaching the 20,000

compound testing capacity is dependent upon the completion of Building 431 at FCRF and the hiring of additional technical staff by the contractor.

2. To develop models and testing capacity to the extent that initial _in vivo_ testing of candidate compounds from the _in vitro_ screen can be accomplished. The time frame for reaching appropriate _in vivo_ testing capacity is dependent upon the renovation of space at FCRF (APA buildings) and/or the utilization of extramural contracts.

III. Animal Production and Tumor Distribution

1. To continue to produce laboratory animals of highest quality (from both a health and genetic viewpoint) and necessary volume to meet the needs of the various programs using these services and to make the necessary adjustments to make this area as cost effective as possible.

2. To encourage suppliers of human tumor cell lines and those applicable for AIDS testing to limit restrictions to the extent that these cell lines can be expanded and supplied to qualified investigators in order to make overall research accomplishments in these areas more uniform. To make cell line expansion and distribution as cost effective as possible.

Accomplishments:

I. Cancer Area

1. _In vitro_ screen

During the past year, feasibility studies were accomplished to assure the capability of testing 10,000 compounds annually in the _in vitro_ screen against a large number of human tumor cell lines utilizing a selected protocol. Protocols are in the final stages of testing and selection will be made in the early part of the current year.

2. Secondary testing

A. _In vitro_

Confirmatory protocols have been established for initial actives from the _in vitro_ primary screen. Protocol selection will be based on tumor type and desired information.

B. _In vivo_

Model development studies have continued, but have been limited due to space and staffing problems. In the absence of quantitative data for potential evaluation models, it has been decided that the subcutaneous model which provides significant tumor inhibition/cell kill information, and which has been studied

extensively in murine models will be utilized. Comparative *in vivo* model development studies will continue.

II. AIDS Area

1. *In vitro* screen

The *in vitro* screen has been implemented to the extent that testing has exceeded the 10,000 compound level on an annualized basis. The level of effort should be doubled for *in vitro* AIDS screening during the current fiscal year.

2. *In vivo* screen

Initial studies have been conducted with a microencapsulation model using the AIDS virus. Additional studies will be performed and implemented as appropriate with feline and/or bovine lentivirus models and other retroviruses.

III. Animal Production and Tumor Distribution

1. Quality standards for animal production have been maintained. Animals have been supplied to qualified investigators which are free of pathogenic organisms and genetically sound. The payback system has continued to work extremely well in making the animal production system cost effective. Adjustments have been made in animal production to reflect changes in DTP objectives including more reliance on athymic mice and less overall volume of usage.

2. The tumor bank has expanded its capacity to accomodate a number of the cell lines utilized in the disease-oriented screening program. Steps have been taken to enhance the acquisition of cell lines from both the cancer and AIDS testing programs and to make these lines available for distribution. The payback system for cell line distribution is working successfully.

BIOLOGICAL TESTING BRANCH

FY 1988

PRIMARY GENETIC CENTERS (3)	$3,325,000
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
RODENT PRODUCTION CENTERS (1)	186,638
Large-scale production of nuce mice under barrier controlled environment.	
DIAGNOSTIC & HISTOCOMPATIBILITY PROJECTS (7)	632,328
To monitor animal health and genetic integrity.	
DEVELOPMENT OF STANDARDS & GUIDELINES (1)	38,500
For animal care and breeding.	
MAINTENANCE OF FROZEN TUMOR BANK (1)	180,000
IN-VIVO SCREENING (2)	1,200,990
Screening of potential anti-cancer drugs.	
SCREENING QUALITY CONTROL (1)	432,727
FREDERICK CANCER RESEARCH FACILITY (2)	
Animal Production	$2,550,000
In Vitro Cell Line Screen	4,000,000
In Vivo Model Development	200,000
Tumor Procurement and Preparation	325,000
BTB Support	65,000
Operational Support for APA-FCRF	100,000
Biological Laboratory Training Program	300,000
	$7,540,000

TOTAL	$13,536,183
Less Reimbursements	2,010,000
NET COST TO DTP	$11,526,183

DTP-AIDS PROGRAM AT FCRF

AIDS Cell Line Support	$ 45,000
AIDS *In Vitro* Screening	1,450,000
AIDS *In Vivo* Screening	1,150,000
AIDS Samples Prep Laboratory	210,000
TOTAL DTP-AIDS - FCRF	$2,855,000

ANNUAL REPORT OF THE PHARMACOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Pharmacology Branch continues its efforts in the management and technical direction of contracts concerned with drug development of anticancer agents and anti-AIDS drugs. Contracts concerning preclinical studies are involved with two areas of drug development: 1) Detailed therapeutic evaluation of candidate drugs, 2) Methods development for drug measurements and pharmacokinetics. The staff of this Branch and selected contractors or independent investigators presented to the Decision Network Committee pharmacological data for several drugs: Cyclopentenyl cytosine (NSC 375575); Pyrazoloacridine (NSC 366140); 8-Chloro-cAMP (NSC 284751) and Carmethizole hydrochloride (NSC 602668).

The staff also cooperated with CTEP in preparing INDA's for the FDA on several drugs and clinical brochures by providing summaries concerning preclinical therapeutic information, methods of measurement and pharmacokinetic information for Hepsulfam (NSC 329680); Chloroquinoxaline sulfonamide (NSC 339004); Pyrazine diazohydroxide (NSC 361456); BSO (NSC 326231) and Oxantrazole (NSC 349174). Collaborative efforts with CTEP personnel also resulted in the preparation of manuscripts for five drugs: Flavone acetic acid (NSC 347512); NMF (NSC 003051); and Pyrazole (NSC 045410); Merbarone (NSC 336628); and Arabinosyl-5-azacytosine (NSC 281272).

A considerable effort of this Branch is involved in the design and evaluation of special studies conducted to explore the therapeutic efficacy of agents of interest to DTP. Such studies involved evaluation of treatment regimens including constant infusions, routes of administration, formulated products, activity against advanced stage and metastatic tumors, and evaluation of drug resistant profiles. Included in the compounds tested in one or more of these studies were Batracylin (NSC 320846); CPEC-C (NSC 375575); Carmethizole hydrochloride (NSC 602668); 8-Chloro-cAMP (NSC 284751); Chloroquinoxaline sulfonamide (NSC 339004); Fetindomide (NSC 373965); Penclomedine (NSC 338720); Pyrazine diazohydroxide (NSC 361456) and Pyrazoloacridine (NSC 366140). Other studies were conducted to address specific concerns arising during the drug selection and development processes. For example, in efforts to improve the formulation of insoluble agents, soluble prodrugs of Taxol (NSC 125973) and Rapamycin (NSC 226080) and a new microdispersed formulation of Merbarone (NSC 336628) were tested.

Another major effort of the Branch is to see that drugs, of sufficient interest to undergo preclinical toxicology testing and phase I clinical trials, have methods for analysis developed and pharmacokinetic measurements conducted. In addition, more detailed mechanistic and metabolism studies are conducted on particularly interesting agents to supplement ongoing preclinical or clinical studies. Agents studied this year include: Flavone acetic acid (metabolism and binding studies), 8-Cl-cyclicAMP, Histidinol, Pyrazoloacridine, Hepsulfam,

Penclomedine (additional metabolism studies), Carmethizole hydrochloride, Batracylin, CPE-C and Vince's compound (discreet).

Some effort of the Branch was devoted to designing studies to answer questions raised during the early clinical trials of cytotoxic agents and to modulate the activity of effective anticancer agents. With regard to the former, a concern was expressed that alkalinization of the urine of patients receiving Flavone acetic acid (NSC 347512) would reduce the antitumor activity of the compound. In mice, it was found that alkalinization of the urine by administration of bicarbonate caused parallel shifts in the dose response curves for both lethality and therapeutic efficacy. Both effects were observed at higher doses in the mice receiving bicarbonate. Pharmacokinetic studies run in parallel indicated more rapid clearance occurred following alkalinization. To evaluate further the relative importance of a "therapeutic window" and total drug exposure on the activity of Flavone acetic acid, s.c. bolus and 24 and 48 h infusions were compared in a murine solid tumor model. While two courses of bolus treatments caused complete tumor regressions, 24 and 48 h infusions caused no partial or complete responses at non-lethal doses. The 48 h infusion total dose was twice the total dose tolerated with the bolus regimen. With regard to efforts to improve the therapeutic index of antitumor agents, a continuation of studies with Histidinol using infusion schedules instead of bolus treatments still failed to produce significant results.

The Branch has been actively involved in the initiation of projects to evaluate in mice agents identified by the _in vitro_ anti-human immunodeficiency virus (HIV) screen. A contract with the objective of optimizing _in vivo_ antiviral activity of new agents was awarded in May. Tasks include evaluation of the influence of drug schedule and route of administration on antiviral activity and determination of antiviral activity of formulated products. In addition, final stages of the contract award process are in progress for a project to screen a series of antifols against dihydrofolate reductase and analogs of p-aminobenzoic acid against dihydropteroate synthetase. Both enzymes will be isolated from _Pneumocystis carinii_ and _Toxoplasma gondii_, two causative agents of opportunistic infections in AIDS patients. A series of contracts are currently being negotiated to develop methods of analysis and to conduct pharmacokinetic studies on agents of interest for AIDS.

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ANNUAL REPORT OF THE INFORMATION TECHNOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Information Technology Branch (ITB) provides data processing support for the Developmental Therapeutics Program. Major chemical, biological, and administrative databases are maintained regarding materials screened for anticancer activity during the past 30 years. In addition, ITB provides support for new initiatives and on-going activities, including DTP's disease-oriented, in vitro-based anticancer drug screen and, beginning in calendar year 1987, anti-HIV drug screening. Support is provided for all phases of the Program from sample acquisition through inventory, testing and data reporting.

ITB provides support principally through a portfolio of extramural contracts. Management of the various contracts is provided by ITB staff. The staff of ITB is a valuable resource with diverse computer programming capabilities. For example, several dBase programs which interface with the Drug Information System (DIS) were written this year by ITB staff. These programs relieve the user of the frustration of dealing with the slowness and poor reliability of the DIS operating on the DEC-10. Also, a microcomputer-based alternative to the troublesome AVAIL and ASGN programs which were intended to support scheduling of work within the in vitro drug screening laboratories was developed by ITB staff. A variety of other specialized programs written in PL/1, Fortran, C, SAS, or Wylbur Command Procedures have been created by staff and are in wide use by DTP scientists.

A special project of the ITB staff is the development of a viable robotics program. The first successful application of robotics in DTP involves a robot which weighs bottles containing drug samples. This simple application serves a useful purpose, but equally important is that it demonstrates that robots can be adapted to perform useful tasks within DTP. The next application of robotics is anticipated to be at NCI-FCRF. Technicians there must accurately add solutions of test compounds to 96 well microtiter plates. The anticipated advantages of having a robot perform these drug additions are speed, accuracy, and reliability in the dispensing operation. It is not yet known when the robot will be ready to perform the drug addition step. However, given an adequate priority for the development of this capability, it may be possible that trials could begin within the year for laboratory robotics. It is anticipated that a variety of additional applications will be identified.

The Biological Data Processing System (BDPS) provides support for acquisition and processing of anticancer and anti-HIV drug screening data (VSE Corporation is the incumbent contractor). This system includes a laboratory microcomputer system interfaced to microtitre plate readers and to mainframe computers at NIH's Division of Computer Research and Technology (DCRT). Using this system the feasibility of large-scale, in vitro anticancer drug screening has been demonstrated. More than 3,000 test materials have been screened for anti-HIV activity. This system also provides for archival storage of biological testing data generated in previous in vivo screening models. In principle, the BDPS begins by interacting with the DIS operating on a mainframe computer, a DEC-10, to capture information on chemical samples sent for evaluation in either the anticancer drug screening program or the anti-HIV screening program or both. This information is passed to another mainframe computer, an IBM 3090 system, and then entered into an assembly of programs, AVAIL and ASGN, intended to assist with the informational requirements of sample formulation and to effectively schedule and list those samples awaiting testing. Part of this process involves passing parts of this information from the mainframe computer location on the NIH campus to microcomputers located at the NCI-Frederick Cancer Research Facility. This information is used and modified by the sample preparation group at NCI-FCRF and the incremented information then is further used by the in vitro anticancer and anti-HIV drug screening laboratories at NCI-FCRF. A new stream of information is generated by these laboratories as they test the various chemical samples. This stream is captured by another subsystem of the BDPS called the In Vitro Screening System (IVSS), calculations are performed, and the calculated values are transmitted back to Bethesda where it is placed into multiple ASCII files on an IBM mainframe. The latter files are accessed by a variety of specialized programs which process the data for display and analysis.

Virtually all of the subsystems of the BDPS have come under critical scrutiny in the last few months because they have repeatedly malfunctioned. The initial data capture system for getting shipping information from the DIS was found to consist of incompletely automated programs requiring manual intervention which were run too infrequently to be of optimal usefulness for their intended functions. This has been changed and the fully automated system is run daily providing the information flow necessary to make improvement of the function of the seriously flawed AVAIL and ASGN programs possible. However, improvement of the AVAIL and ASGN programs has not been so simple to accomplish. An interim solution was provided by a dBase III program designed and coded by a ITB staff programmer. A more comprehensive long term solution is being attempted by VSE programmers. The IVSS in its current form is probably incapable of meeting the increased demands of large scale screening anticipated as the new in vitro laboratories become operational. Consequently, VSE conducted a comprehensive analysis of the functional requirements for screening laboratory support. The tentative results of this

analysis are still being evaluated, but it is clear that an acceptable design for a new IVSS is not yet in hand. Finally, the file structure of the *in vitro* database is being re-examined. Studies to evaluate using the IBM relational database system DB2 for managing the *in vitro* data have been initiated.

Fein-Marquart Associates developed the DIS and currently is responsible for modifying and maintaining it. The DIS is essentially a custom-designed chemical structure database which also is used as a general purpose database to store material inventory information, shipping information, and currently stores screening information for a subset of the compounds screened by DTP. Its development began in 1982 and has continued over a six year period. The performance of this system has never been satisfactory, particularly with respect to the very poor response times required for many queries and update procedures attempted with it. Over the last several years, the troublesome poor performance characteristics have been made intolerable by the added burden of the poor reliability of the aging host DEC-10 system on which it was designed to run. Consequently, a decision was made to rehost the DIS on a more powerful modern computer. The DEC-10 then proved to be not only an unsuitable host for the DIS from the very beginning because of its undesirable performance and reliability characteristics, but it also proved in the end to be an unfortunate choice for a host because there was a problem associated with replacing it. The DEC-10 is a 36-bit computer no longer supported by the Digital Equipment Corporation. No modern alternative computers are available which will accept the 36-bit code of the DIS. Therefore, in order to rehost the DIS on a computer other than the DEC-10 the original programs of the DIS have to be rewritten in 32-bit form. Fein-Marquart Associates has been contracted to do the 36-bit to 32-bit conversion. The "core" of the DIS has been rewritten and ported to a VAX 8700 for the purpose of benchmark trials. These trials demonstrated that at least a fourfold increase in speed is possible through a combination of program redesign and the use of the more modern computer. The demonstration provided a rational basis to proceed with the procurement of a powerful, dedicated VAX on which to rehost the DIS.

The advent of large-scale natural product collection and extraction activities led the ITB to design a DIS-like computer support system. Fein-Marquart was selected to implement this system. The Natural Products Repository Support System (NPRSS) is currently being written. The 36-bit code currently being written for the DEC-10 version of the NPRSS will also have to be rewritten in 32-bit code for it to run on the new host computer.

ORI Inc. is the incumbent for a Task Order Managed Computer Support contract. The contract provides a readily available resource for high-priority projects and invaluable technical advice and support in a wide variety of important information

management problem areas. This group had a key role in developing the specifications for the new host computer system for the DIS. They have also provided valuable advice regarding the DTP networking requirements.

ORI Inc. continues to provide critical operational and technical support for the production-scale laser printer system. Major upgrades to this system were installed this year. The upgrades significantly increased the functional capacity of the laser printer system. For example, the first versions of the cancer program supplier report graphics program required more than ten minutes to produce each report. Upgrades to the laser system decreased the time requirement per report to a few minutes. Very recently, by conversion of the supplier report programs from graphics mode to text mode, the time required to produce each report was reduced to less than 1 second. The ability of the laser printer system to produce the cancer program supplier reports at the much faster rate will significantly reduce the turnaround time for DTP staff printing jobs of all kinds due to the freeing up of the laser printer resource especially at peak periods such as when the supplier reports are being printed.

Another example of ORI Inc. tasks this year will be mentioned because it illustrates well the value of this type of programming support. On-line access to either AIDs or Cancer screening data is provided to DTP staff via a series of SAS, Fortran, and Wylbur Command Procedures programs developed by ITB management and staff. As the size of the respective AIDs and cancer databases continued to grow at a rapid rate, the cost of a single pass through these large databases by either the SAS (for AIDs) or Fortran (for cancer) programs also continued to grow. At one point, the cost for a single run of the Fortran cancer database accessing program reached $35. The ORI Principal Investigator rewrote the Fortran program in Pl/1 and utilized efficiency-enhancing procedures. The cost was reduced to $ 0.85 per database access. A similar rewrite of the SAS AIDs database access program resulted in similar efficiency enhancements and cost reductions. This contractor is currently developing an integrated, microcomputer-based display system which is expected to greatly improve the data access, display, and analysis capabilities of the DTP.

Phase II Small Business Innovative Research Contracts:

Civilized Software -

This project is developing a version of the mathematical modelling program, MLAB, which will run on a personal computer of the IBM AT class. MLAB is a popular and powerful system currently resident on the DCRT DEC-10. The DEC-10 version is

written in SAIL which limits MLAB's portability to other computer systems. The PC version being developed by this project is being written in C, a computer language well-known to be highly portable to a variety of computer systems.

Creare -

Like the Civilized Software Phase II project, this project will develop a PC version of MLAB. In this case, however, the target computers are those with the Intel 80386 microprocessor and, specifically, those using the UNIX operating system. This combination of hardware and software endow this system with certain capabilities which are expected to translate to speed, power, and flexibility for the user. The C language will provide a high degree of portability to other systems. Both this language and the operating system UNIX auger toward portability among microcomputer systems and to large computers such as a VAX with only minor adjustments.

Fein-Marquart -

This is a project to convert the 36-bit version of the DIS to a 32-bit version which can run on a VAX. A portion of the converted code, the "core" part, has been benchmarked to compare the performance of the converted code running on a VAX 7800 to the unconverted code running on the old DEC-10. So far, performance increases of four-fold have been realized. These performance increases, so important to the concept of porting the DIS to a more powerful computer, were partially due to changes in the way the DIS accesses disk memory and partially due to the greater speed of the VAX.

Problem Areas and Actions to be Taken:

As mentioned above, several major problem areas have been identified during the past year. As the anticancer drug screening program has been scaled up, major deficiencies have been identified with the laboratory support system. To a large extent these problems are shared by the anti-HIV drug screen. The program used to coordinate scheduling of testing (the "AVAIL and ASGN" program) has repeatedly failed to function as designed. The _in vitro_ database has been found to contain errors and to be very difficult to correct. Support for processing of chemical information, provided mainly via the DIS, has been hampered by repeated failures of the DEC-10 computer facility at DCRT. This has been particularly troublesome for processing of natural product samples at NCI-FCRF which depends strongly on software running on the DEC-10.

In response to the problem areas described above, several actions are planned for the next year. A key innovation will be the establishment of an expert consultant group (ECG). The purpose of the ECG will be to help devise state-of-the-art computer science solutions to information technology problems identified

by the branch management. The selection of the ECG members is a high priority objective for the next year. As an initial step in this selection process, a comprehensive review of existing DTP computer support requirements and contracts will be conducted. It is anticipated that this review will be performed by invited non-NCI scientific information technology specialists. In addition to these fundamental management changes, a variety of specific technical innovations are already envisioned. These are listed below:

- To alleviate problems associated with the DEC-10 system at DCRT as well as telecommunications problems between DCRT and NCI-FCRF, plans are underway to obtain a dedicated computer for support of DTP activities. This computer (probably a VAX 8820) will be located at the NCI-FCRF and connected to the screening laboratories via Ethernet.

- In order to achieve database integrity and to provide for ready corrections and manipulation of data, the in vitro data will be transferred to a commercial database management system. The IBM-based DB2 will be used initially, but others will be evaluated for use on the VAX 8820 system.

- A new generation of laboratory support software will be developed. This software will be optimized for the current laboratory environment and will utilize microcomputers employing the Intel 80386 processor operated at 20 MHz.

In summary, a number of problems have been identified in the computer support systems of DTP. Several positive steps have been tried, taken, initiated, or planned in the last several months. However, a great deal remains to be done to resolve the current crisis in DTP automated information systems.

Publication:

Milne GWA. A data base system that relies heavily on graphics. In: Warr W, ed. ACS Symposium #341. Washington: American Chemical Society, 1987;102-109.

ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Pharmaceutical Resources Branch (PRB) is structured to provide comprehensive pharmaceutical services to the various Programs of the Division of Cancer Treatment and recently, to provide similar services to the AIDS Clinical Programs of the National Institute of Allergy and Infectious Disease (NIAID). The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. During this report period, the Branch supervised a total of 23 contracts with a combined annual budget of approximately seven million dollars.

The major contract areas include: chemical preparations and pilot plant production, analytical services, pharmaceutical research and development, and pharmaceutical manufacturing. Additionally, the Branch is responsible for shelf life surveillance, storage and distribution, and computerized inventory maintenance of all drug products used in the Clinical Programs of the Division of Cancer Treatment (DCT).

Staff

The Pharmaceutical Resources Branch is presently staffed with eight senior professionals, one technical and two secretarial personnel. The classification of the senior professionals is as follows: five PHS Commissioned Corps Pharmacists, one Ph.D. analytical chemist, and two Ph.D. medicinal chemists.

The Branch consists of four critical service areas:

Chemical Resources

The primary functions of Chemical Resources are to provide for resynthesis, large-scale production and procurement of chemical substances. These services are accomplished by the management and supervision of a contract program for resynthesis and pilot plant production of various quantities of bulk substances primarily intended for pharmaceutical manufacture of investigational products for clinical evaluation. Chemical Resources supervises eight chemical prep lab contracts consisting of a combined annual effort of approximately 34 man years.

An important aspect of Chemical Resources is the interaction with chemical suppliers of bulk drug substances. The PRB chemists must contact the suppliers and ascertain that materials they are to provide are prepared under FDA required Good Manufacturing Practices (GMP) and are of the highest quality possible. An increasing quantity of chemical bulk substances are being provided by suppliers for PRB contract formulation.

Analytical

The analytical service provides for: (1) analytical characterization of new investigational agents and (2) analytical assessment of chemical and formulated products. The analytical service supervises contractors engaged in the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions, and identification of impurities and/or degradation products. The analytical chemist prepares bulk chemical specifications used for acceptance criteria of additional supplies either from commercial sources or chemical preparation contractors. The specifications and validated analytical methodology are prepared in a format suitable for submission to the Food and Drug Administration as part of the NCI's Investigational New Drug Application. The analyst is also responsible for chemical evaluation of new investigational dosage forms.

Presently, the analytical chemist supervises four analytical contracts representing a combined 18 staff year effort. These contractors have the expertise to chemically characterize a very structurally diverse group of chemicals. These contractors are also responsible for the development and application of stability-indicating methods for all new drug substances.

Analytical data developed on new investigational compounds is assembled and published in a book entitled "NCI Investigational Drugs - Chemical Information". This text contains stability-indicating methods, spectral data, approximate solubility and stability data, and other appropriate information on a large number of agents. The publication is distributed on request and without charge to investigators throughout the country.

Pharmaceutical Development

The dosage form development component is responsible for conversion of bulk chemicals into pharmaceutical products suitable for clinical use in chemotherapy and AIDS programs. About one-half of the drugs required for intravenous delivery do not exhibit adequate solubility or stability and some form of pharmaceutical intervention is required. Standard approaches (salts, solvents and surfactants) are initially evaluated. Emphasis is also given to evaluation of newer techniques to improve solubility or stability (emulsions, prodrugs and complexation). The developed dosage form is evaluated for chemical content, antitumor activity in the rodent screen, and feasibility for manufacture on production scale.

All of the production development effort is conducted under contract with the Pharmaceutical Development staff servicing as project monitors.

The Product Development service is responsible for the supervision and management of two pharmaceutical R & D contracts with a combined annual effort of eight man years, one combined R & D (one and one-half man years), and a pharmaceutical contract.

Clinical Products Service

The Clinical Products Service manages six pharmaceutical contracts with capabilities to produce a broad variety of pharmaceutical products. The service manages a storage and distribution contract with computer capabilities for accurate accountability of the disposition of all investigational products, and also manages a shelf life contract involving an annual three and one-half man year effort.

In addition, the service manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this report period, drug purchase expenditures were in excess of 2.5 million dollars. A similar effort was begun during the year to record the expenditures for drugs for use in the AIDS program. This involved establishing a dual record system to accurately account for the expenditures for AIDS drugs.

A significant amount of staff time is expended in preparing purchase specifications, award justifications, and performing financial recordkeeping functions. Several different NIH mechanisms to obtain contracts are utilized to obtain drugs, such as blanket purchase agreements, indefinite delivery contracts, direct purchase contracts, etc.

The contractors managed by the Clinical Products Service produced over 400,000 injectable units, and slightly less than 200,000 oral dosage forms for clinical distribution.

Investigational product literature in the form of Investigational Drug - Pharmaceutical Data Sheets is prepared by the staff. These information sheets are also supplied in bound book form (NIH Publication No. 86-2141) which is updated periodically. During this reporting period, over 4,300 issues were distributed.

Goals and Accomplishments

During this reporting period an emergency drug distribution system was put in place for off-hour AIDS drug requests. The system provides weekend delivery of emergency drug supplies to AIDS patients. Several emergency orders have been received and the drug supplies have been packaged and delivered in a timely manner.

During this reporting period the Branch experienced a significant increase in the receipt and distribution of a variety of biological products for both the AIDS and Cancer Programs. These products usually require dry ice or wet ice packaging and special mailing procedures. These procedures are labor intensive and expensive and have resulted in administrative and financial contract modifications.

A noteworthy accomplishment during this reporting period involved the design and implementation of a special dose-pak for a five-arm blinded trial with the AIDS drug AZT and placebo. The special packaging was designed by PRB staff and involved more than 160,000 units requiring PRB on site monitoring. The project was accomplished in a timely manner without incidence of errors or loss of drug.

Also, during this reporting period a computerized Pharmaceutical Data System was implemented. This system is designed to track analytical, chemical, pharmaceutical, and distribution actions of DCT investigational agents. The system is expected to improve drug product files and make more efficient use of staff time.

The Chemical prep lab contractors in the Branch successfully accomplished their objectives in providing high quality bulk chemicals and pharmaceutical products to the various programs in the Division of Cancer Treatment. During this reporting period, the prep lab contractors prepared over 25 compounds totaling more than 180 kilograms. Examples of bulk pharmaceutical substances delivered include: dideoxyadenosine (NSC-98700), merbarone (NSC-336628), HMBA (NSC-95580), pyrazine diazohydroxide (NSC-361456), N-methylformamide (NSC-3051), oxantrazole (NSC-349174), DDI (NSC-6120490, deazaneplanocin (NSC-606385), and dideoxycytosine (NSC-606170).

The analytical contractors have submitted reports describing the analysis of over 100 lots of bulk chemicals and formulated products. Examples of bulk drugs and formulated products assayed included: dideoxyadenosine (NSC-98700), dideoxyinosine (NSC-612049), hepsulfam (NSC-329680), pyrazine diazohydroxide (NSC-361456) and clomesone (NSC-338947). Many plant material extracts were assayed for taxol content. These data will aid in the future collection and procurement of plant materials from which taxol will be isolated for use in clinical trials.

Pharmaceutical R&D contractors successfully completed dosage development on eight new agents including: cyclopentenyl cytosine (NSC-375575), nitrosopyrazinamine (NSC-361456), helsulfam (NSC-329680) and special oral granule formulations of the AIDS drug dideoxyinosine (NSC-612049). Compounds exhibiting significant solubility and stability problems continue to be encountered. New delivery systems to be evaluated include parenteral emulsion systems, lipsomal encapsulation of cytotoxic agents and micro particle suspensions for intravenous use.

The next twelve-month period should involve an increase in contract production of investigational agents to maintain adequate clinical supplies for new treatment protocols and new agents under development. Also, the increased use of biological products such as IL II, in combination with chemotherapeutic cytotoxic agents, will involve considerably more interaction with suppliers.

During the next reporting period the PRB will concentrate on evaluating new parenteral drug delivery systems such as with liposomes, emulsions and micro particles for intravenous use.

We anticipate two to four new agents from the AIDS HIV screen to be approved for development during the next reporting period. In addition, we anticipate another two to three candidate agents provided from non government sources that may require product development for AIDS trials. A contract program is in place and all new agents will receive a high priority for development.

Publications by Staff

1. Ahluwalia G, Cooney DA, Mitsuya H, Fridland A, Flora KP, Hao Z, Dalal M, Broder S, Johns DG. Initial Studies on the Cellular Pharmacology of 2', 3'-Dideoxyinosine, an Inhibitor of HIV Infectivity, Biochem Pharmacol 1987;36:3797-3800.

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3. Glover A, Chun HG, Kleinman LM, Cooney DA, Plowman J, Grieshaber CK, Malspeis L, Leyland-Jones B. Merbarone: an antitumor agent entering clinical trials, Invest New Drugs 1987;5:37-143.

4. Grem JL, Shoemaker DD, Hoth DF, King SA, Plowman J, Zaharko D, Grieshaber, CK, Steadman DH Jr, Cradock JC, Jones BLJ. Arabinosyl-5-azacytosine: A novel nucleoside entering clinical trials, Invest New Drugs 1987;5:315-328.

5. Silverton JV, Quinn FR, Haugwitz RD, Todaro LJ. Structures of Two Dideoxy-nucleosides: 2,3'-Dideoxyadenosine and 2',3'-Dideoxycytidine, Acta Crystallogr 1988;C44:321-324.

6. Trissel LA, Flora KP. Stability Studies - Five Years Later. [Invited Commentary] Amer J Hosp Pharm In Press.

7. Vishnuvajjala BR. Method of preparing 1,2-Diaminocyclohexane tetra-chloroplatinum (IV) isomers, U.S. Patent Pending (Applied October 1987).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07183-02 PRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The influence of molecular structure on chemical and biological properties

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

PI : Frank R. Quinn Chemist PRB, NCI

COOPERATING UNITS (if any)

Norman E. Sharpless, LCP, NIDDK
James V. Silverton, LC, NHLBI
Rudiger D. Haugwitz, DSCB, NCI

LAB/BRANCH

Pharmaceutical Resources Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

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.)

X-ray crystallographic, energy minimization and quantum mechnical calculations been employed on compounds of biological interest to give insights into and explanation of their modes of behavior.

Various compounds showing promise against the AIDS virus are being systematically investigated to obtain structural and electronic properties which may help elucidate the mechanism of their action and thus lead to improved analogs. The x-ray structures of 2′,3′-dideoxyadenosine (DDA) and 2′,3′-dideoxycytidine (DDC) have been determined and published. The x-ray structures of 2′,3′-dideoxyinosine (DDI) and 2′,3′-dideoxyguanosine are being determined. Strain energies and quantum calculations have been carried out on these compounds. Calculations have been completed on the eight possible empimers of 3′-azido-2′,3′-dideoxythymidine (AZT), as well as the corresponding bases: cytidine, adenosine, inosine, 2′-deoxycytidine and 3′-amino-3′-deoxyadenosine for comparison. Strain energies and quantum calculations on colchicine, which binds to tubulin, and isocolchicine, which does not, have been completed. Isocolchicine is more strained by about 8 kcals./mol. The factor which differentiates the binding ability of the two isomers seems to be different inter-oxygen distances which affect hydrogen bonding ability in isocolchicine.

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

ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The objectives of the Developmental Therapeutics Program center on the discovery and preclinical development of agents with anticancer, and presently, with anti-HIV potential. Studies focusing on the hazards of new investigational agents to healthy organs in intact experimental animals are the final steps in the preclinical stages of new drug development. These investigations comprise the primary responsibility of the Toxicology Branch. The functions of the Branch can be separated into five distinct but interrelated areas:

1. Determine maximally tolerated doses in rodents and dogs;

2. Determine end-organ toxicities and additionally establish the dose-responsiveness and schedule-dependency of toxicity;

3. Determine plasma drug concentrations and correlate levels to safety, toxicity and _in vitro_ efficacy;

4. Establish a safe starting dose for the Phase I clinical trials in humans.

5. Determine the reversibility of toxicity during a post-dosing recovery period;

During Fiscal Year 1988, the Toxicology Branch initiated protocols for preliminary studies on compounds which are just beginning preclinical development. These studies include _in vitro_ assessment of toxicity in bone marrow cells, _in vitro_ metabolism of potential anti-HIV compounds in cultured lymphocytes/monocytes and _in vivo_ determination of pharmacokinetics, oral bioavailability, maximally tolerated doses and an estimation of the dose-limiting toxicity. These studies enable the program to evaluate animal toxicity and potential human toxicity of compounds in early development as well as to evaluate the pharmacokinetic and metabolic characteristics prior to committing the program to full scale drug development. Additionally, the pharmacokinetic information can be used to reliably extrapolate toxic effects across species and permits a more rational evaluation of the role of schedule dependence in the efficacy of new agents as well as in the development of toxicity.

The information generated from the toxicology and pharmacology studies comprise the major portion of the evaluable preclinical information required by the Food and Drug Administration for an Investigational New Drug Application. The Division of Cancer Treatment maintains a master file with the FDA which contains toxicity study protocols for potential anticancer drugs. These protocols set forth jointly agreed to procedures for animal toxicity studies of antineoplastic drugs. Data from studies conducted under the protocols are accepted for regulatory purposes in INDA approval. The Toxicology Branch has amended the protocol files to accurately reflect newer methods and techniques. Presently 28 protocols are on file with the FDA. Individualized protocols (drug specific) are routinely developed from these basic designs to account for agent specific chemical and/or physical properties.

Final reports on the following agents have been received, or are anticipated to be received by the Branch in FY'88:

L-Buthionine sulfoximine	NSC-326231
Batracylin	NSC-320846
Pyrazine Diazohydroxide	NSC-361456
Ipomeanol (Special Study)	NSC-349438
2',3'-Dideoxyadenosine*	NSC-098700
2',3'-Dideoxyinosine*	NSC-612049

Toxicology studies have been, or are anticipated to be initiated on the following compounds in FY'88:

Pyrazoloacridine	NSC-366140
Carmethizole	NSC-602668D
Cyclopentenyl cytosine	NSC-375575
Penclomidine	NSC-338720
Carbovir*	NSC-614846

*anti-HIV compounds

Publications:

Manuscripts:

Glover A, Chun HG, Kleinman LM, Cooney DL, Plowman J, Grieshaber CK, Malspeis L, Leyland-Jones B. Merbarone: an antitumor agent entering clinical trials. Invest New Drugs 1098;5:137-43.

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ANNUAL REPORT OF THE GRANTS AND CONTRACTS OPERATIONS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

This Branch provides an administrative and managerial focal point for DTP extramural activities. These activities encompass grants, contracts and cooperative agreements such as those involving the National Cooperative Drug Discovery Groups (NCDDGs). The activities for this year are summarized below by funding category.

Grants

A breakdown of the grants' portfolio for the Biochemistry and Pharmacology Program is shown in Table 1.

Contracts

Requests for Proposals (RFPs) were issued and/or awarded during the year and these are listed in Table 2 together with the title of the solicitations and associated dollars.

Cooperative Agreements

The NCDDG Program was initiated in 1983 to exploit exciting developments in biomedical research into new and more effective treatments for cancer. Multidisciplinary and multi-institutional teams of the nation's most talented scientists from academic, non-profit research and commercial organizations are brought together to conceive and develop new drugs and treatment strategies. Currently there are seven funded Groups as shown in Table 3. During the past year another competition was held to expand the program. Thirty-three applications were reviewed in response to Requests for Applications (RFAs) which encouraged three conceptually different research approaches to the discovery of new therapies: general mechanism of action, a disease-oriented strategy, and a new effort to stimulate the development of novel models which will more accurately predict the efficacy of new treatments. Awards are planned by the end of September for the most meritorious applications. A plan for the establishment of National Cooperative Natural Products Drug Discovery Groups received concept approval from the Board of Scientific Counselors of the Division of Cancer Treatment. An RFA is currently being prepared.

Workshops

The Branch collaborated with the National Institute of Allergy and Infectious Diseases (NIAID) in sponsoring a workshop on "Anti-Sense Oligonucleotides as Therapeutics Agents" on September 13-15, 1987 in Annapolis, MD. The gathering provided an ideal forum for international experts to inform one another of recent developments in this relatively new research area and to speculate on future directions in the development of new anti-viral and anticancer therapies using this technology. On October 5-7, 1987, the Branch organized a workshop in Downingtown, PA to acquaint DTP contractors with the new *in vitro* human

tumor cell line approach to anticancer drug screening, to update them on recent changes in contract regulations, and for information exchange between NCI Project Officers and contract Principal Investigators. Lastly, the Branch cooperated with the Biological Response Modifiers Program in sponsoring a one-day workshop entitled "Specific Cancer Cell Targeting Using Molecular Genetic Technology." This meeting was held June 1 in Bethesda and will likely stimulate the issuance of RFAs to promote this new area of chemotherapy.

Publications

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2. Rahman A, Roh JK, Wolpert-DeFilippes MK, Goldin A, Venditti J, Woolley PV. Therapeutic and pharmacologic studies of Tetraplatin, Tetrochloro (d,l-trans)1,2-diaminocyclohexane Pt (IV): A new platinum analog. Cancer Res 1988;48:1745-52.

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TABLE 1

BIOCHEMISTRY AND PHARMACOLOGY GRANTS

PROGRAM AWARDS BY SUB-CATEGORY

FY 1987 (ESTIMATED)

A	Synthesis and Chemistry	83	$11,339,000
B	Natural Products	46	6,044,000
C	Screening and Experimental Therapeutics	45	5,125,000
D	Comparative Pharmacology	37	3,033,000
E	Other Preclinical Aspects	5	654,000
F	Mechanism of Action	106	14,837,000
	Program Projects	9	6,092,000
	Total	321	$47,124,000

Includes Traditional (R01), New Investigator (R23/R29), Small Business Innovation Research (R43/R44), Academic Research Enhancement (R15), Merit (R37) and Outstanding Investigator (R35) Awards. Does not include Conference (R13) or Equipment (R15) Awards.

TABLE 2

Total Contract Value (Estimated/Awarded)

Antitumor Projects

Project	Value
Performance of Protocol Toxicology Studies	$ 8,076,444
Performance of Protocol Toxicology Studies by Small Business	3,697,789
Pathology & Veterinary Support Services for Protocol Studies	677,507
Maintenance of a Rodent Production Center	670,340
Iso-Antigenic Typing of Mouse Strains	779,672
Biochemical Genetic Monitoring of Rodents	351,050
Maintenance of the NCI Drug Information System	2,189,708
Preclinical Pharmacology Investigations of Antitumor Agents	2,254,038
Quality Control & Model Development in Rodents	4,973,067
Master Agreements for Large Scale Isolation of Antitumor Agents from Natural Sources	2,762,815
Preparation of Radiolabeled Materials	1,933,972
Literature Surveillance & Selections of Promising Natural Products	428,740
Procurement of Human Breast Cancer Cell Lines	945,750
Procurement of Prostate Cancer Cell Lines	945,750
Master Agreements for Chemical Synthesis	1,418,625
Primary Rodent Production Centers	11,979,500
Development of Dosage Forms and Delivery Systems for New Antitumor Agents	1,134,900
Development and Production of Pharmaceutical Dosage Forms	1,576,250
Partial Support of Institute of Laboratory Animal Resources	237,500
Development of Dosage Forms & Delivery Systems for New Antitumor Agents	1,134,900
Operations and Maintenance of DTP Biological Data Processing System	5,304,606
Total	$ 53,472,923

Anti-AIDS Projects

Project	Value
Synthesis of Congeners & Prodrugs of Anti-AIDS Compounds	$ 2,147,506
Master Agreements for the Large Scale Isolation of Anti-AIDS Agents from Natural Sources	2,762,815
DTP AIDS Screening Data Base Support	1,090,942
AIDS DTP Computer DIS Installation	410,000
Preclinical Pharmacology Studies of Anti-AIDS Agents	2,522,000
Large Scale Preparation of Anti-AIDS Drugs for Phase II and III Clinical Trials	1,224,305
Literature Surveillance for Natural Products with Potential Anti-AIDS Activity	828,845
Preparation of Radiolabeled Anti-AIDS Compounds	1,379,084
Analysis of Chemicals and Pharmaceutical Formulations for Anti-AIDS Agents	2,102,316
Chemical Synthesis of Anti-AIDS Compounds	2,206,750
Chemical Synthesis by Small Business of Anti-AIDS Compounds	735,568

Anti-AIDS Projects (continued)

Project	Amount
Detailed Drug Evaluation of Anti-AIDS Agents	$ 1,326,403
Master Agreements for Recollection of Marine Organisms and Terrestrial Plants for Anti-AIDS	1,657,689
Special Studies in Toxicology and Pharmacology of Anti-AIDS Agents	514,011
Primary Screening of HTLV III/LAV (Human AIDS Virus)	3,039,095
Antifolate Screen for Drugs Against Opportunistic Infections in Patients with AIDS	1,381,407
Storage & Distribution of Clinical Drugs for AIDS	3,315,379
Development & Implementation of Mechanistically-oriented Anti-HIV Drug Screen	18,705,257
Development & Manufacture of Oral Dosage Forms of Anti-AIDS Agents	3,315,379
Preclinical Toxicology & Pharmacology of Drugs Developed for AIDS & Related Illnesses	5,640,327
Dosage Form Development of New Agents for the Treatment of AIDS	394,062
Large Scale Preparation of Anti-AIDS Drugs for Preclinical Toxicology and Phase I Clinical Studies	2,364,375
Large Scale Preparation of Anti-AIDS Drugs for Phase II and III Clinical Trials	4,965,187
Total	$ 64,028,702

Antitumor and Anti-AIDS Projects

Project	Amount
Selective Acquisition of Compounds for Anticancer & Anti-AIDS Screening	$ 4,144,224
Cultivation of Marine Anaerobic Bacteria	384,776
Cultivation of Marine Protozoa	384,776
Services in Support of the Developmental Therapeutics Program	1,779,508
Total	$ 6,693,284

SBIR Contracts

Project	Amount
Multi-drug Resistance Patterns in Human Tumor Cells	777,905
Development of the Portable and Extended MLAB Modelling Laboratory	482,785
Total	$ 1,260,690
GRAND TOTAL	$125,455,599

TABLE 3

NATIONAL COOPERATIVE DRUG DISCOVERY GROUP PROGRAM

TOTAL COSTS FOR FY 1987

Grant Number	Investigator/Institute/Title	Costs
1 U01 CA 45967-01	Brattain, Michael G. Baylor College of Medicine Growth Regulation of Human Colonic Neoplasms	$ 316,554
1 U01 CA 45962-01	Corbett, Thomas H. Wayne State University Drug Discovery - Anticancer Agents for Colorectal Cancer	$ 588,367
1 U01 CA 46088-01	Johnston, Michael R. University of Colorado Health Sciences Center Targeted Therapy for Lung Cancer	$ 503,869
5 U01 CA 37655-04	Levin, Victor A. Northern California Cancer Center Approaches to the Inhibition of Oncogene Expression	$ 332,751
5 U01 CA 37641-03	Mendelsohn, John Memorial Sloan Kettering Cancer Center Anti-Receptor Monoclonal Antibodies in Cancer Treatment	$ 666,443
5 U01 CA 37606-04	Porter, Carl W. Roswell Park Memorial Institute Inhibitors of Polyamine Biosynthesis and/or Function	$ 624,270
5 U01 CA 40884-03	Ross, Warren E. University of Florida Topoisomerases as New Therapeutic Targets	$ 568,955
	Total	$3,601,209

ANNUAL REPORT OF THE LABORATORY OF BIOLOGICAL CHEMISTRY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Laboratory of Biological Chemistry was established in 1985 to identify as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. Agents are designed to interact with these targets and are evaluated for biochemical and antitumor effectiveness. An important aspect of this mission is to develop appropriate _in vivo_ systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler _in vitro_ model systems. Accordingly, the Laboratory is involved in identifying endogenous factors present _in vivo_ that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. Approximately 75% of the Laboratory's resources is applied to non-traditional targets for antitumor drug design and study. These non-traditional targets include early key biochemical events signaling cell proliferation or differentiation. The other 25% of Laboratory resources is applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated projects to develop new chemotherapeutic agents to block the action of growth factors. Non-traditional targets selected for drug design and study include: second messengers inositol triphosphate and diacylglycerol; myristoylation of cellular oncogene products; protein kinase C; and selected G-proteins.

Recent findings indicate that inositol-phosphates formed from phosphatidylinositides may be the second-messengers which mediate the action of many growth factors. In addition, the products of two distinct oncogenes (src and ros) are associated with increased levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. The synthesis of phosphatidylinositol polyphosphates and their subsequent hydrolysis therefore provide attractive well-defined targets for the design of inhibitors for use in chemotherapy. A project was initiated that has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell replication. Second, to design, synthesize, and evaluate drugs to inhibit this process; specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme which produces the inositol phosphates. Test systems have been

developed to identify drugs that inhibit phosphatidylinositol turnover or synthesis. During this period, the metabolism of inositol phosphates by homogenates of GH3 rat pituitary cells was characterized in detail and establish that independent routes of dephosphorylation are followed for $I(1,3,4,5)P_4$ and $I(1,4,5)P_3$. Studies of the activation of phospholipase C demonstrated that this activation is attenuated by pretreatment with agents that activate protein kinase C such as phorbol esters and diacylglycerols. This effect seems to be mediated by protein kinase C because down regulation of this enzyme by prolonged exposure to phorbol esters eliminates the ability of the phorbol esters to attenuate phospholipase C activation. A role for a G-protein in the activation of phospholipase C was implicated by the demonstration of increased accumulation of inositol phosphates in the presence of AlF. A series of analogs of _myo_-inositol were synthesized and evaluated. The 5-deoxy-5-fluoro-inositol analogue is incorporated into cellular phospholipid and phosphorylated to a compound similar to PIP but not further phosphorylated to the corresponding PIP2 derivative. The product formed by PI synthetase from the 5-deoxy-5-fluoro -myo-inositol was partially characterized.

It may be possible to alter the activity of an oncogene product by interfering with its localization in the plasma membrane. The early events signaling cell proliferation occur in the plasma membrane of the cell, the location of most of the known cellular oncogene products. Myristoylation has been shown to be critical for the membrane localization and cellular transforming activity of p60src and has been implicated for other transforming proteins. Three mechanisms for blocking myristoylation dependent transformation are under investigation: (1) direct chemical inhibition of transforming protein myristoylation; (2) inhibition of translocation of the myristoylated transforming protein to the plasma membrane; and (3) displacement of the myristoylated transforming protein away from the membrane, into the cytoplasm, and thus away from its substrate. Methods developed in the p60src system will be applied to malignant cells where membrane-associated oncogene products are suspected. N-Myristoyl transferase was partially purified from bovine brain. Several potential inhibitors of the enzyme were synthesized and are being tested for their _in vitro_ effects on the purified enzyme. N-Myristoyl and nonmyristoyl peptides homologous to the N-terminus of $p60^{src}$ were prepared and were used to identify high affinity protein acceptors in red cell membrane vesicles. The results suggest the existence of _src_-specific acceptors in the plasma membrane which might be involved in the normal cellular regulation and transforming activity of _c-src_ and _v-src_, respectively. A new method for identifying N-myristoyl-proteins was developed. The N-myristoylglycine from myristoylated proteins is released by mild acid hydrolysis, derivitized to p-nitrobenzylazlactone, and identified at nmole levels by reversed phase HPLC. This procedure was used to identify the alpha subunit of the GTP-binding protein, G_o, as a myristoylated protein.

Investigations were continued in three inter-related projects that center around the role of protein phosphorylation in various regulatory systems. The first project deals with the role of the proto-oncogene tyrosine protein kinase, c-_fes_ in myeloid cell differentiation. The c-_fes_ kinase was purified and characterized from HL-60 cells expressing the granulocyte phenotype as a result of treatment with DMSO. The genomic DNA encoding c-_fes_ was transfected into HL-60, KG-1a (GM-CSF-independent) and K562 (resistant to

differentiation) cells to study the role of the c-fes protein in the ability of these cells to differentiate along the myeloid or monocytic pathway. These investigation should give us some clue as to the obligatory role of c-fes in this process and the cellular mechanisms (transcriptional or translational) involved in its expression. The second project deals with the role of calcium- and phospholipid-dependent protein kinase (protein kinase C) in multidrug (mdr) resistant cells. We found that not only is there an overabundance of protein kinase C in mdr cells but that the isoform pattern for this family of kinases (seven at last count) also differs from their sensitive counterparts. Therefore, this study is directed at the role of protein kinase C and its processing to the catalytic fragment (termed M-kinase) in mdr cells. The other facet of this project is to determine the endogenous protein substrates for these protein kinases. Candidates to be studied are the cytoskeletal protein, vinculin, and the cell membrane resistance-associated family of proteins termed P-glycoproteins. The ultimate goal of this project is to determine how these proteins are regulated by phosphorylation via protein kinase C and how this affects the expression of the mdr phenotype. The third project addresses the role of protein phosphorylation in the regulation of replication of the human immunodeficiency virus (HIV) which causes AIDS. Two genes of the provirus genome code for 23 and 27 kDa products of unknown function. These genes are termed sor and 3'-orf, respectively. It is known that the HIV does not replicate when the sor gene is deleted, but replicates at a faster than normal rate when the 3'-orf gene is deleted. Thus, the products of these two genes impose negative and positive regulatory functions, respectively. We found that lysates of HIV contain a unique Mn^{2+}-dependent protein kinase activity that is not present in lysates of the ribosome fraction from uninfected cells. We are presently trying to determine the open reading frame in HIV encoding this protein kinase using both viral lysates and systems employing the cloned sor and 3'-orf genes.

Studies were extended on ARF, a recently characterized G-protein. In the past year the bovine ARF gene was used to clone two homologous genes, ARF1 and ARF2, from yeast. Current studies will determine the phenotypic consequences of single and double disruptions of the ARF genes. Three phenotypes resulting from disruption of ARF1 have been defined: slow growth at 30°C, cold sensitivity, and supersensitivity to fluoride ion. We have isolated a number of pseudorevertants most of which have wild type phenotype. Several complementation groups of recessive revertants have been found and are being analyzed. It is likely that at least some of the resulting genes will be in the ARF pathway and should help to identify functionally related gene products. Indirect immunofluorescent staining of yeast cells with affinity purified ARF antibodies suggest that ARF may be localized or concentrated in microtubules. These results are preliminary but point to a new site of action of regulatory G-proteins. In addition to the genetic studies in yeast we have collaborated with Dr. Hsiang-fu Kung on the cloning and sequencing of the human ARF gene. The coding region of human ARF is identical to bovine ARF, thus allowing the use of affinity purified peptide antibodies originally obtained from bovine sequence, on human cells. The human ARF cDNA and antibodies will be used to screen a number of human tumors to look for aberations in the expression of ARF. The development of plasmids containing ARF genes for expression in bacterial, yeast and baculovirus is continuing. The bacterially expressed bovine (or human) ARF is being purified and characterized biochemically. This material will be used in

studies of the effects of microinjection of ARF or ARF antibodies in Xenopus oocytes; which has proven to be a very fruitful system for studies of G-protein functions. Studies were continued on the role of fatty acylation of GTP-binding regulatory proteins as it relates to function or cellular localization. These studies will be aided greatly by the yeast work and possible localization of ARF at or in microtubules. Of more immediate interest is the finding that ARF associates with plasma membranes in a GTP-dependent manner. When GTP is bound there is a much lower or no affinity of ARF for the membrane. We will be focusing on how this result (and the implication of a plasma membrane site of action) relates to the apparent localization of ARF in microtubules in yeast.

Although many biological effects of retinoic acid have been described the mechanism for these actions is unknown. We have now discovered that in the human acute myeloblastoid cells line, HL60, a covalent bond is formed between retinoic acid and protein. Based on sensitivity to hydrolysis with hydroxylamine, about 70% of the retinoic acid moiety is linked to protein _via_ either an oxygen-ester or a thio-ester bond. Fractionation of cells labeled with radioactive retinoic acid indicate that greater than 80% of the retinoylated protein is associated with the membrane fraction.

A method has been developed for the fixation and permeabilization of HL60 cells so that intracellular antigens can be detected with the use of a fluorescence activated cell sorter (FACS). This method has been applied in a study of the changes in the level of c-_myc_ oncogene protein during differentiation of HL60. Our results indicate that c-_myc_ protein decreased during differentiation at a much slower rate than would be expected from the decreases in c-_myc_ mRNA levels under the same conditions. These results are consistent with changes in the stability of c-_myc_ protein under differentiation induction conditions.

Multidrug-resistance is a well documented phenomenon that limits the chemotherapeutic effectiveness of many traditional antitumor agents. An understanding of the physiologic function of proteins associated with multidrug-resistance could lead to the design of more effective chemotherapeutic strategies with existing agents or the associated proteins may be considered as targets for drug design, in which case a new class of antitumor agents might arise. A radioactive photoactive analog of vinblastine was used to identify a specific vinblastine binding P-gp in multidrug-resistant cell lines. P-gp vinblastine photolabeling was blocked by a number of indole alkaloids previously shown to increase anticancer drug cytotoxicity and increase drug retained by cancer cells. It was also found that there is a correlation between the level of vinblastine photolabeling of P-gp and the cellular collateral sensitivity of CEM/VLB cells to verapamil up to about 40-fold vinblastine resistance. At higher levels of resistance this relationship was not maintained suggesting that collateral sensitivity of these cells to verapamil may be mediated in part by mechanisms other than P-gp. P-gp has also been identified in HL60/Vinc but not HL60/ADR cell lines by photolabeling with vinblastine or the calcium channel blocker, azidopine. These results suggest that mechanisms of drug resistance in HL60/Adr sublines have features which are distinct from cells containing P-gp and may represent a new model for investigating experimental and clinical drug resistance.

The mixed disulfide of methyl mercaptan and L-homocysteine, S-(methylthio)-L-

homocysteine (L-SMETH), inhibits the growth of L-1210 leukemia cells in culture at micromolar concentrations. The inhibition is markedly promoted by added cupric ion, but not by ions of other metals, is steriospecific, and is competitive with glutamine. For example, at 10 M each of L-SMETH and Cu^{++}, complete growth inhibition was observed if cells were grown in 1 mM glutamine, 50% inhibition at 2 mM glutamine and none at 4 mM glutamine. The inhibition is also completely relieved by cytidine in a non-competitive manner, but not by guanosine or uridine, indicating that the principal damage to the cellular economy resides in the amination of uridine to cytidine. This was confirmed by HPLC analysis of cell extracts, which showed a marked decrease in CTP with increases in the levels of UTP, GTP, and ATP.

A project involving traditional targets for drug design and study is a continuing project to determine the relative dependency of host and tumorous tissues on *de novo* vs salvage pathways for the synthesis of pyrimidine and purine nucleotides *in vivo*. This can be accomplished because of two recent advances in our Laboratory: (a) development of GC/MS methodology and a novel method of data analysis to quantitate pyrimidine *de novo* pathway activity and (b) discovery of an inhibitor of uridine kinase that is an effective inhibitor of pyrimidine salvage *in vivo*. These two tools will be used to assess the *de novo* and salvage pathways as targets for future antitumor drug development, as well as the therapeutic value of concurrent inhibition of both pathways. Early comparative data indicate that uridine salvage makes a substantial contribution to the pyrimidine nucleotide pools of mouse liver, intestine, and kidney in the intact animal. Activation of uridine salvage is an early event in the mitogenic response, occurring within minutes of serum stimulation of quiescent fibroblasts. Data from our early experiments into the mechanism response for this activation indicate that it is linked to the synthesis of components of the extracellular matrix (specifically, hyaluronate). Current studies will evaluate the effects of inhibitors of hyaluronate synthesis on the activation of uridine salvage and on the invasive and metastatic properties of cancer cells.

The preceding summary outlines the objectives of the Laboratory of Biological Chemistry and describes some of the research carried out within the Laboratory during the past year. The individual Project Reports, which follow, describe this research in greater detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06163-04 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Pharmacologic Aspects of Nucleotide Metabolism

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

PI:	R. Cysyk	Pharmacologist	LBC,NCI
Others:	L. Anderson	Chemist	
	C. Chisena	Biologist	
	J. Kasofsky	IRTA Fellow	LBC,NCI
	N. Malinowski	Chemist	LBC,NCI
	J. Moyer	Sr. Staff Fellow	LBC,NCI
	E. Napier	Chemist	LBC,NCI
	J. Strong	Pharmacologist	LBC,NCI
	D. Zaharevitz	Sr. Staff Fellow	LBC,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The overall goal of this Project is to determine the relative importance of the *de novo* and salvage pathways for the synthesis of pyrimidine nucleotides in normal and malignant tissues *in vivo*, i.e., in the intact animal. This can be accomplished because of two recent advances in our Laboratory: (a) development of GC/MS methodology and a novel method of data analysis to quantitate pyrimidine *de novo* pathway activity and (b) discovery of an inhibitor of uridine kinase that is an effective inhibitor of pyrimidine salvage *in vivo*. These two tools will be used to assess the *de novo* and salvage pathways as targets for future antitumor drug development, as well as the therapeutic value of concurrent inhibition of both pathways. Early comparative data indicate that uridine salvage makes a substantial contribution to the pyrimidine nucleotide pools of mouse liver, intestine, and kidney in the intact animal.

Activation of uridine salvage is an early event in the mitogenic response, occurring within minutes of serum stimulation of quiescent fibroblasts. Data from our early experiments into the mechanism responsible for this activation indicate that it is linked to the synthesis of components of the extracellular matrix (specifically, hyaluronate). Current studies will evaluate the effects of inhibitors of hyaluronate synthesis on the activation of uridine salvage and on the invasive and metastatic properties of cancer cells.

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

Objective:

The overall objective of this project is to determine the relative dependency of host and tumorous tissues on de novo vs salvage pathways for the synthesis of pyrimidine and purine nucleotides in vivo. The de novo biosynthetic pathways supply pyrimidines and purines for nucleic acid synthesis and are therefore considered to be important pathways for cell proliferation. Therefore, there has been a considerable effort throughout the past several decades to develop specific inhibitors of enzymes of these pathways. Although a number of potent inhibitors (e.g. PALA, pyrazofurin, 6-azauridine) exhibit excellent in vitro activity against isolated enzymes and cultured cells and in vivo activity against certain murine tumors, only marginal clinical success has been achieved with these agents. It would appear that factors other than potency of inhibition are important because very potent enzyme inhibitors (PALA, pyrazofurin) are only marginally effective. Accordingly, lack of clinical success with the pathway inhibitors developed to date is an indication that the importance of the de novo pathway to cell survival in vivo has been overestimated. If so, then the development of additional inhibitors of the de novo pathway would be a futile effort unless there is a coordinate development of agents that either interfere with the salvage pathway or with the synthesis and export of preformed nucleosides by donor organ(s).

This Project is divided into the following specific aims: to determine the physiologic importance of circulating pyrimidines and purines and their role in modulating the antitumor activity of antipyrimidine and antipurine chemotherapeutic agents; to study the liver as a modulator of circulating nucleosides and as a possible target for chemical manipulation; to develop agents to interfere with nucleoside salvage to be used in combination with inhibitors of de novo synthesis; to develop methodology for monitoring and quantitating the flux through the de novo pathways of host and tumorous tissues in vivo.

Major Findings:

Development of Inhibitors of Nucleoside Salvage. Results discussed in previous annual reports of this project indicate that inhibition of nucleoside salvage could enhance the effectiveness of inhibitors of de novo pyrimidine nucleotide synthesis. Our previous results indicate that cyclopentenyluracil (an inhibitor of uridine kinase) is at present the most effective agent for blocking salvage in the intact animal. A large scale synthesis of cyclopentenyluracil yielded enough material to begin in vivo therapy studies. A study of the toxicity of cyclopentenyluracil in male BDF_1 mice showed no toxicity and treated mice gained weight at the same rate as the control mice. This dose is similar to those shown previously to provide significant inhibition of uridine salvage. The ability of multiple dose regimens to block uridine salvage in mice was evaluated. In the period 8-9 h after the second of two doses at a 12 h interval, salvage of uridine was inhibited by 64-79% in all tissues examined as well as P388 ascites tumors. At 11.5-12 h after the last of 5 doses at 12 h intervals the inhibition was 31-58%. These experiments have established that an effective inhibition of uridine salvage in vivo can be produced at non-toxic doses of cyclopentenyluracil. Future studies will characterize the extent and duration of inhibition in vivo, the effect of this inhibition on nucleotide pools in tumors and normal tissues, and the consequent

changes in the rate of nucleotide synthesis de novo. We will evaluate the anti-tumor effects of a combination of cyclopentenyluracil and PALA (an inhibitor of pyrimidine synthesis de novo) on model murine tumors only slightly inhibited by PALA alone. This study will reveal the extent to which the therapeutic index of inhibitors of pyrimidine nucleotide synthesis can be improved by simultaneous inhibition of nucleoside salvage.

Quantitation of pyrimidine synthesis de novo in the intact animal. We developed a method which utilizes ^{15}N-labeled precursors in conjunction with GC/MS and a novel method of data analysis to quantitate the actual amount of product formed by the pyrimidine de novo pathway in isolated rat hepatocytes. An important advantage of this method is that it can account for the amount of product formed by the pyrimidine de novo pathway that is not labeled. Also, values for the enrichment of pathway precursors are not required for the calculation and, in fact, this method allows the enrichment of the precursor pool to be determined; an important advance for the study of cellular compartmentation of metabolic pathways. We are currently applying this method in the intact animal. We are at the stage now of determining a suitable ^{15}N-precursor and the proper dosing schedule to achieve a sufficient amount of dilabeled molecules in mouse tissues to permit the calculation. We have evaluated ^{15}N-aspartate, ^{15}N-glutamine, and ^{15}N-alanine in addition to $[^{15}N]H_4Cl$ which we used in the in vitro rat hepatocyte experiments. The most promising candidate is ^{15}N-alanine. Early results indicate that this compound labels both nitrogens of the uracil ring in liver and intestine. The fraction of the uracil nucleotide pool in liver and intestine that is formed by de novo synthesis during a two hour infusion of ^{15}N-L-alanine is independent of dose and is consistent with the fraction estimated by extrapolating the $^{15}NH_4Cl$ data to zero dose. A comparison of the contribution of the de novo vs. salvage pathway for pyrimidine nucleotide synthesis using this new method indicates that the salvage pathway is comparable to de novo in several mouse tissues; this finding supports our contention that inhibitors of pyrimidine salvage are necessary for use in combination with inhibitors of de novo synthesis.

Activation of pyrimidine synthesis in mitogen-stimulated quiescent 3T3 fibroblasts. Activation of uridine salvage is an early event in the mitogenic response, occurring within minutes of serum stimulation of quiescent fibroblasts, as compared with DNA synthesis which occurs 10 to 15 hours later. A goal of this project is to understand where the early activation of pyrimidine synthesis fits in the cascade of biochemical events that eventually converge to initiate DNA synthesis and cell proliferation. Data from our first series of experiments to study activation of pyrimidine synthesis indicate that the activation of uridine uptake is associated with hyaluronate (HA) formation. This observation was made possible by a new HPLC system we developed that separates most of the purine and pyrimidine nucleotides and nucleotide sugars. We found that serum stimulation of quiescent Swiss 3T3 cells results in an increase in the UDP-glucuronic acid (UDP-GA) peak that correlates in time course and magnitude with the early increase in uridine uptake. After 30 min, UDP-GA becomes the predominate uracil-containing species in the cell, exceeding the concentration of UTP. Since UDP-GA is a precursor for HA synthesis and a substrate and activator of HA-synthase, we postulate that the activation of pyrimidine nucleotide synthesis is a direct result of an increased requirement for HA synthesis. HA has been implicated in tumor cell invasion and

metastasis. We propose to develop agents to decrease HA production and to evaluate the effect of this inhibition on the growth, invasion, and metastasis of tumors in mice.

Publications

Moyer JD, Malinowski N, Treanor S, Marquez VE. Antitumor activity and biochemical effects of cyclopentyl cytosine, Cancer Res 1986;46:3325-29.

Zimm S, Strong JM. A clinically useful ion-pairing high-performance liquid chromatographic assay for monophosphate metabolites of thioguanine and mercaptopurine in human neoplastic cells, Anal Biochem 1987;163:1-4.

Anderson LW, Zaharevitz DW, Strong JM. Glutamine and glutamate: automated quantification and isotopic enrichments by gas chromatography/mass spectrometry, Anal Biochem 1987;163:358-68.

Hiraga S, Klubes P, Owens E, Cysyk RL, Blasberg RG, Brain tumor and cerebral blood flow is increased by blood-perfluorocarbon emulsion (fluosol-DA) exchange, Cancer Res 1987;47:3296-302.

Klubes P, Hiraga S, Cysyk RL, Owens E, Blasberg RG. Attempts to increase intratumoral blood flow in the rat solid Walker 256 tumor by the use of the perfluorocarbon emulsion fluosol-DA, Eur J Cancer Clin Oncol 1987;23:1859-67.

Zaharevitz DW, Napier EA, Anderson LW, Strong JM, Cysyk RL. Stimulation of de novo pyrimidine synthesis in liver and intestine by ammonium chloride infusion, Eur J Biochem, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06164-04 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Inhibitors of Phospholipid Metabolism as Potential Chemotherapeutic Agents

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

PI:	J. Moyer	Senior Staff Fellow	LBC, NCI
Others:	S. Ahir	Staff Fellow	LBC, NCI
	N. Malinowski	Chemist	LBC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.8	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Agents which block the formation of second messengers that mediate growth factor action may be of value in cancer chemotherapy. Recently the hydrolysis of phosphatidylinositol-bisphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol triphosphate (IP_3) has been implicated in the action of a number of growth factors including platelet derived growth factor and bombesin. We are therefore examining the chemotherapeutic effects of inhibition of this process and attempting to develop inhibitors of number of the enzymes involved in phosphatidylinositol (PI) metabolism. Specific areas of investigation are as follows. A series of inositol analogs and phospholipids incorporating these analogs are under investigation as inhibitors of phospholipase C. The cellular requirements for myo-inositol and the regulation of myo-inositol metabolism are being studied in collaboration with Dr. John Strong of this laboratory. In collaboration with Dr. E. Sausville of Naval Med. Center we are characterizing the mechanisms of transmembrane signalling by the putative autocrine growth factor bombesin in human small cell lung cancer cells (SCLC).

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

Objectives.

Inositol phosphates formed from phosphatidylinositides may be the second messengers that mediate the action of many growth factors. In addition, numerous alterations in phosphoinositide metabolism are associated with transformation by specific oncogenes. The synthesis of phosphatidylinositides and their subsequent hydrolysis therefore provide attractive, well-defined targets for the design of inhibitors for use in chemotherapy. This project has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell proliferation. Secondly, to design, synthesize, and evaluate drugs to inhibit this process, specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme that produces inositol phosphates.

Major Findings.

1. The metabolism of inositol phosphates by homogenates of GH3 rat pituitary cells has been characterized in detail and the results have been published. These studies established that independent routes of dephosphorylation are followed for $I(1,3,4,5)P_4$ and $I(1,4,5)P_3$.

2. In collaboration with Dr. E. Sausville of Naval Medical Center we have published results showing an activation of phospholipase C by bombesin in human SCLC cells. We also found that the activation of phospholipase C by bombesin is attenuated by pretreatment with agents that activate protein kinase C such as phorbol esters and diacylglycerols. This effect seems to be mediated by protein kinase C because dow regulation of this enzyme by prolonged exposure to phorbol esters eliminates the ability of the phorbol esters to attenuate phospholipase C activation. A role for a G-protein in the activation of phospholipase C was implicate by the demonstration of increased accumulation of inositol phosphates in the presence of ALF. The particular G-protein involved is uncertain, and results with cholera toxin and pertussis toxin were inconclusive. We demonstrated a potent inhibition of the bombesin-induced activation of phospholipase C by a newly synthesized peptide antagonist of the bombesin receptor synthesized by Dr. Coy of Tulane Univ.

3. We published results showing that murine L1210 cells can grow without addition of _myo_-inositol to the medium and synthesize _myo_-inositol from glucose. The intracellular concentration of _myo_-inositol and its synthesis were measured by a mass-spectrometric method in a collaboration with Dr. J. Strong of this laboratory. The uptake of _myo_-inositol from the medium was shown to occur by a mechanism not saturated even at 2 mM _myo_-inositol, but partially inhabitable by the isomeric scyllo-inositol.

4. We published the synthesis and evaluation of a series of analogs of _myo_-inositol. We demonstrated that 5-deoxy-5-fluoro-inositol was incorporated into cellular phospholipid and phosphorylated to a compound similar to PIP but not further phosphorylated to the corresponding PIP_2 derivative. The product formed by PI synthetase from the 5-deoxy-5-fluoro-_myo_-inositol was partially characterized.

Proposed Course.

1. The metabolism and effects of the newly synthesized inositol analogs will be further explored to determine if they effectively inhibit PI turnover and Ca^{++} mobilization in intact cells.

2. New inhibitors of phospholipase C and PI kinase will be synthesized and evaluated, including phospholipid analogs incorporating modified inositols.

3. An inhibitor of PI synthetase now being synthesized will be evaluated.

4. The metabolism of phosphoinositides in small cell lung cancer cells will be further characterized. In particular, the effect of the inositol phosphates on Ca^{++} metabolism, the role of G-proteins in the regulation of this process, and the mechanism of the attenuation of phospholipase C activation by activators of protein kinase C will be studied.

5. Receptor antagonists of the bombesin receptor will be examined as potential chemotherapeutic agents.

Publications.

Jiang C, Moyer JD, Baker DC. Synthesis of deoxy- and deoxyhalogeno analogs of myo-inositol, J Carbohydrate Chem;1987 6:319-55.

Cunha-Melo JR, Dean N, Moyer JD, Maeyama K, Beaven MA. The pattern of phosphoinositide hydrolysis in rat basophilic leukemia (RBL-2H3) cells varies with the type of IgE receptor cross-linking reagent used, J Biol Chem 1987;262:1145-63.

Moyer JD, Reizes O, Dean MN, Malinowski N. D-myo-inositol(1,4) bisphosphate 1-phosphatase. Partial purification from rat liver and characterization. Biochem Biophys Res Commun;1987 146:1018-26.

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Sausville EA, Moyer JD, Heikkila R, Neckers LM, Trepel J. A correlation of bombesin-responsiveness with myc-family gene expression in small cell lung carcinoma lines, Ann NY Acad Sci;1988, in press.

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Moyer JD, Reizes O, Malinowski N, Ahir S, Jiang C, Baker DC. Substrate properties of analogs of myo-inositol, Mol Pharmacol;1988, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06166-04 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Mechanism of Multidrug Resistance

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

PI:	Ronald L. Felsted	Research Chemist	LBC, NCI
Others:	Susan Arnold	Chemist	LBC, NCI
	Constance Glover	Microbiologist	LBC, NCI

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.5	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The overall goal of this project is to determine the suitability of P-glycoprotein (P-gp) drug binding as a specific target for the development of chemotherapeutic drugs. A radioactive photoactive analog of vinblastine has been used to identify a specific vinblastine binding P-gp in multidrug-resistant cell lines. P-gp vinblastine photolabeling was blocked by a number of indole alkaloids previously shown to increase anticancer drug cytotoxicity and increase drug retained by cancer cells. It was also found that there is a correlation between the level of vinblastine photolabeling of P-gp and the cellular collateral sensitivity of CEM/VLB cells to verapamil up to about 40-fold vinblastine resistance. At higher levels of resistance this relationship was not maintained suggesting that collateral sensitivity of these cells to verapamil may be mediated in part my mechanisms other than P-gp. P-gp has also been identified in HL60/Vinc but not HL60/Adr cell lines by photolabeling with vinblastine or the calcium channel blocker, azidopine. The results suggest that mechanisms of drug resistance in HL60/Adr sublines have features which are distinct from cells containing P-gp and may represent a new model for investigating experimental and clinical drug resistance. Overall, the results suggest a complex relationship between the ability of a compound to modulate multidrug resistance and its ability to compete for binding to P-gp.

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

The exposure of malignant cell lines to natural product cytotoxic drugs such as vinblastine, actinomycin D, adriamycin or colchicine frequently results in the isolation of populations of cells with resistance to the selecting agent as well as a collateral resistance to other mechanistically distinct and structurally unrelated compounds. The mechanism(s) by which these cell lines become multidrug-resistant is unknown, but it is thought to be related to a parallel reduction in the cellular accumulation of those drugs to which the cells are resistant. The multidrug-resistant phenotype also is characterized by the presence of a 150-180 kDa surface membrane glycoprotein P-gp which occurs in multidrug-resistant cells in direct proportion to the degree of their acquired drug-resistance. The relationship of P-gp to multi-drug resistance is not known. It may accumulate only as a secondary consequence of the multidrug-resistant phenotype. Alternatively, P-gp could promote multidrug resistance by direct or indirect effects on membrane permeability, drug transport, or drug binding.

Objectives.

This project involves the identification of specific drug interactions with macromolecules in normal and multidrug-resistant cell lines. The relationship of specific drug binding macromolecules to multidrug-resistance mechanisms will be examined. New chemotherapeutic agents designed to circumvent multidrug-resistance will be designed, synthesized and tested.

Major Findings.

Collateral Sensitivity of Multidrug Resistant Human Leukemic Cell Lines to Verapamil. Verapamil has been shown in many experimental multidrug resistance (MDR) systems to enhance the cytotoxic activity of "natural product" anticancer drugs, especially the Vinca alkaloids and anthracyclines. We show here that verapamil caused a marked enhancement of vinblastine (VLB) cytotoxicity, ranging from ~20- to 80-fold, in four progressively VLB-resistant human leukemic cell lines that were ~14- to 700- to 1100-fold resistant. We also found that verapamil alone was increasingly toxic to these multidrug-resistant cell lines in rough proportion to their degree of VLB resistance. This effect of verapamil was not great, however, ranging between 6 and 27% inhibition of cell growth in the drug-sensitive (CEM) and most resistant (CEM/VLB_{1K}) cell lines, respectively. Moreover, there was a narrow range for this effect, between 5- and 20 M verapamil; lower concentrations had no effect and higher concentrations were too toxic to discriminate any resistance-related response. This verapamil effect did not appear to be due to a non-specific detergent-like action, since Tween 80 was equally toxic to all cell lines, resistant and sensitive alike. As measured by photoaffinity labeling with N(p-azido-[3-^{125}I]salicyl)-N'-ß-aminoethylvindesine ([^{125}I]NASV), the CEM/VLB cells expressed more of the mdrl gene product, P-glycoprotein (Pgp), in proportion to their degree of resistance. This labeling could be blocked by excesses of either unlabeled VLB or by verapamil. The CEM/VLB cell lines were also found to have increased expression of the mdrl gene, as measured by RNA dot-blots with the pMDR1 probe. Gene expression increased with increasing resistance of the cell lines up to ~130-fold; no further increases in mdrl gene expression and the collateral sensitivity to verapamil in CEM/VLB cells up to ~40-fold VLB resistance. At higher VLB resistance, however, this relationship was not

maintained. In support of this, 50 M verapamil did not completely block [^{125}I]NASV labeling of Pgp in the two most highly resistant lines. Our data suggest that the collateral sensitivity of the CEM/VLB cell lines to verapamil may be mediated in part by mechanisms other than Pgp.

Analysis of Mechanisms of Multidrug-resistance in HL60 Cells Which Do Not Contain Detectable Levels of P-glycoprotein.

Previous studies have shown that HL60 cells isolated for resistance to adriamycin (HL60/Adr) do not contain detectable levels of P-glycoprotein. These cells are however multidrug resistant and are defective in the intracellular accumulation of drug. In view of these findings we have examined in greater detail certain properties of this isolate and have compared these properties to an HL60 drug resistant isolate (HL60/Vinc) which contains high levels of P-glycoprotein. Studies with the calcium channel blocker verapamil show that this agent is capable of inducing a major increase in cellular drug accumulation in both HL60/Adr and HL60/Vinc isolates. With sensitive cells verapamil at high concentrations induces only a slight increase in drug uptake. Additional studies demonstrate that with membranes prepared from HL60/Vinc cells the photoactive dihydropyridine calcium channel blocker [^{3}H]azidopine labels two major proteins of 180 (P180) and 210 (P210) kilodaltons. There is no detectable labeling of P180 or P210 in membranes from sensitive cells or the HL60 Adr isolate. Furthermore, the pattern of proteins which are labeled with [^{3}H]azidopine is essentially the same in membranes of sensitive and HL60/Adr cells.

Additional studies have been carried out to examine the binding of [^{3}H]vincristine to membranes from sensitive and resistant cells and the photoaffinity labeling of sensitive and resistant membranes by [^{125}I]NASV. The results show that [^{3}H]vincristine binds to membranes of the HL60/vinc isolate whereas binding of this drug to membranes from sensitive cells or the HL60/Adr isolate is greatly reduced. Studies with [^{125}I]NASV show that this agent specifically labels a 150-180 (P180) kilodalton P-glycoprotein in membranes of HL60/Vinc cells. There is no detectable labeling by [^{125}I]NASV of a similar protein in membranes of sensitive cells or the HL60/Adr isolate. Furthermore with sensitive or HL60/Adr membranes the proteins that [^{125}I]NASV labels are the same.

The results of this study suggest that mechanisms of drug resistance in the HL60/Adr isolate have certain major features which are distinct from those exhibited by cell lines containing P-glycoprotein. The HL60/Adr isolate may thus represent a new model system for investigating experimental and clinical drug resistance.

Effects of Indole Alkaloids on Multidrug Resistance and Labeling of P-Glycoprotein by a Photoaffinity Analog of Vinblastine. Multidrug resistant cells are characterized by decreased drug accumulation and retention, thought to be mediated by a high molecular weight glycoprotein, P-glycoprotein (Pgp). Agents such as verapamil have been shown to increase anticancer drug cytotoxicity and increase the amount of drug accumulated and retained by such sells. We show that in addition to verapamil, reserpine, chloroquine, quine, quinacrine, yohimbine, vindoline, and catharanthine also enhance the

cytotoxicity of vinblastine (VLB) in a multidrug resistant, human leukemic cell line, CEM/VLB_{1K}, described for the first time. These cells express Pgp as a doublet that is photoaffinity labeled by the analog of VLB, N(p-azido-[3-^{125}I]salicyl)-N'-ß-aminoethylvindesine ([^{125}I]NASV). Both reserpine and, to a lesser extent, verapamil, compete with [^{125}I]NASV for binding to Pgp. We also found that chloroquine, quinacrine, vindoline, and catharanthine, each of which enhanced VLB cytotoxicity in CEM/VLB_{1K} cells by 10- to 15-fold, similarly inhibited [^{125}I]NASV labeling of Pgp. However, neither quinine nor yohimbine inhibited this labeling, and the inhibition produced by catharanthine and vindoline was the greatest or exclusively on the lower band of the Pgp doublet. Our results suggest a complex relationship between the ability of a compound to modulate MDR and its ability to compete for binding to Pgp.

Proposed Course.

The above data confirm the usefulness for identifying drug binding proteins by photoaffinity labeling. A major P-gp *Vinca* alkaloid acceptor has been identified in multidrug-resistant cells. Other drugs will be used to establish the multidrug specificity of P-gp. The role of P-gp in drug resistance will be examined using agents previously shown to counteract multidrug resistance. The effect of photolabeling on cellular drug uptake and efflux will be examined. The subcellular distribution of gp150-180 with time will be monitored. The P-gp will be purified using immunoabsorption and affinity chromatographic methods. Partial structural analysis will be used to identify drug binding sites. Ultimately, gp150-180 will be inserted into artificial lipid vesicles for functional reconstitution experiments. From this understanding of the multidrug-resistance mechanism, we will synthesize and test new compounds designed to reverse the multidrug-resistant state.

Publications:

1. Safa AR, Glover CJ, Sewell JL, Meyers MB, Biedler JL, Felsted RL. Identification of the multidrug resistance-related membrane glycoprotein as an acceptor for calcium channel blockers, J Biol Chem 1987; 262:7884-88.

2. Safa AR, Glover CJ, Felsted RL. Identification of *Vinca* alkaloid acceptors in P388 murine leukemia cells with a photoactive analogue of vinblastine, Cancer Res 1987; 47:5149-54.

3. Safa AR, Glover CJ, Felsted RL. Pharmacological, molecular and cytogenetic analysis of "atypical" and multidrug-resistant human leukemic cells, 1987;47:5455-60.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06167-04 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Inhib. of Myristoylation-Dependent Cell. Transformation & Retroviral Replication

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

PI:	R.L. Felsted	Research Chemist	LBC, NCI
Other:	C. Glover	Microbiologist	LBC, NCI
	C. Goddard	Visiting Fellow	LBC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

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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 modification of onc-proteins with the fatty acid myristate is an early step associated with the transformation of normal to neoplastic cells and mammalian retroviral reproduction and it is thought to be part of the mechanism by which cytoplasmic oncogene protein kinases or viral _gag_ structural proteins are localized to the inner plasma membrane surface. Since the transforming activity of onc-kinases and viral replication is dependent upon this membrane binding, this project will investigate the role of myristoylation as it relates to the mechanism of this subcellular localization. N-Myristoyl transferase has been partially purified from bovine brain. Several potential inhibitors of the enzyme have been synthesized and are being tested for their _in vitro_ effects on the purified enzyme. N-Myristoyl and nonmyristoyl peptides homologous to the N-terminus of $p60s^{rc}$ have been used to identify high affinity protein acceptors in red cell membrane vesicles. The results suggest the existence of _src_-specific acceptors in the plasma membrane which might be involved in the normal cellular regulation and transforming activity of _c-src_ and _v-src_, respectively. A new method for identifying N-myristoyl-proteins has been developed. The N-myristoylglycine from myristoylated proteins is released by mild acid hydrolysis, derivitized to p-nitrobenzylazlactone, and identified at pmole levels by reversed phase HPLC. This procedure has been used to identify the alpha subunit of the GTP-binding protein, Go, as a myristoylated protein. Information on the enzymology of myristoylation and the role of myristic acid in membrane binding will be used to design and synthesize specific inhibitors of myristoylation and membrane association with the goal of developing chemotherapeutic agents specific for critical early steps of tyrosine kinase mediated malignant transformation and mammalian retroviral reproduction.

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Tyrosine-specific protein kinase activity is associated with several known oncogenes and is an appealing target for the chemical manipulation of kinase associated cellular transformation. The transforming activity of onc-kinases depends upon their association with the inner plasma membrane surface. It has been proposed that the mechanism by which viral encoded onc-kinases such as p60src become membrane bound is through a co-translational addition of myristic acid to their NH2-terminal glycine via an amide linkage. Myristoylation is also an essential step in the replication of type B,C & D retroviruses and is typified by the covalent attachment of myristic acid to the NH_2-terminal glycine of viral gag structural proteins via an amide linkage. These myristoylated gag proteins are then localized to the inner plasma membrane where viral assembly and maturation occurs. The importance of myristoylation to onc kinase transformation and retroviral replication has been illustrated by oligonucleotide-directed mutagenesis, resulting in mutant gene proteins in which NH2-terminal glycines were either absent or replaced with alanine. These mutant proteins are no longer localized to the membrane but are found in the cytoplasm. Most pertinent to this proposal, these mutant proteins no longer transformed cells nor were viral particles formed. Presumably, myristoylation is critical to onc-kinase transformation and retroviral reproduction.

Objectives.

In this project we will examine the role of myristoylation of onc-tyrosine kinases and retroviral gag structural proteins as targets for the chemotherapeutic inhibition of cellular transformation and viral reproduction. Specifically, we will study two mechanisms for blocking myristoylation dependent transformation by p60src: these include, (i) inhibition of the myristoyl transferase(s), and (iii) inhibition of binding of myristoyl-proteins to potential membrane receptors.

Major Findings.

Myristoyl Dependent High Affinity Binding of Peptides Homologous to the N-terminus of pp60src with Red Cell Membrane Vesicles. N-myristoylation is essential to the plasma membrane association and transforming properties of the oncogenic tyrosine kinase pp60src. The existence of non-membrane bound myristoylated proteins suggested that myristoylation was not serving as a bilayer anchor. We have used myristoyl and non-myristoyl peptides homologous to the N-terminus of pp60src to test a model in which myristoylation facilitates exposure of src to specific protein binding sites at the plasma membrane. We report here the existence of high affinity protein acceptor sites (K_D <12 nM) to a 15 amino acid myristoylated src peptide in red cell membrane vesicles. This binding is not effectively competed by the non-myristoylated homolog of the peptide nor by shorter N-myristoyl src peptides and other non-src related peptides. The results suggest a role for myristoylation in enhancing interactions between N-myristoyl-proteins and membrane bound proteins and infer the existence of specific acceptors for pp60src in the plasma membrane.

Identification of N-myristoylated Proteins by Reverse-phase High Performance Liquid Chromatography of an Azlactone Derivative of N-myristoylglycine. A method for the identification of N-myristoylated proteins was developed. N-Myristoyl transferases have an absolute requirement for a free N-terminal

glycine. N-Myristoylglycine is released upon mild acid hydrolysis of myristoylated peptides and proteins and its derivitization to a p-nitrobenzyl-azlactone with subsequent analysis by reverse phase HPLC has enabled its detection to pmole levels. This has facilitated the identification of N-terminal myristate in nanomole quantities of purified proteins. Using this method we demonstrate that the alpha subunit of the GTP-binding protein Go is N-terminally myristoylated.

Proposed Course

Myristoylation is thought to be an important cellular phenomenon. The fact that such a rare fatty acid is utilized with such absolute specificity by cellular enzymes with broad regulatory effects as well as by transforming onc-kinases suggest it has a central role in the control of cellular growth and differentiation. Its involvement in mammalian retrovirus replication utilizes the basic myristoylation pathways. The work in this project is designed to clarify several specific aspects concerning the process of myristoylation. From these studies, it may be possible to design novel new compounds for specifically blocking myristoylation. These agents may then provide us with pharmacologic tools to specifically inhibit myristoylation dependent cellular transformation and mammalian retroviral related disorders.

At least two key aspects of myristoylation dependent membrane binding of transforming oncogene and retroviral gag structural proteins may be susceptible to direct chemotherapeutic manipulation. These include, (i) inhibition of the myristoyl transferase(s), and (ii) inhibition of binding of myristoyl-proteins to potential membrane receptors. Most of the current work on the myristoylation phenomena is directed toward the identification of new N-myristoyl-proteins and the defining of their fatty acid attachment site amino acid sequences. Little is known about N-myristoyl transferase(s) from mammalian sources nor is there any information about hypothetical membrane receptor sites. We would predict that both steps are essential to the overall myristic acid dependent transforming mechanism and therefore, each represents an opportunity to block malignancy that results from this type of transformation and viral reproduction.

(i) N-Myristoyl Transferase(s). We are continuing our characterization of mammalian myristoyl transferases using peptide acceptors corresponding to the NH_2-terminal sequence of $p60^{src}$, $p24^{gag}$ and cAMP dependent protein kinase. The enzyme(s) is being purified from bovine brain and the enzymatic mechanism characterized. We have designed several new possible, irreversible and competitive inhibitors. These have been synthesized and are now being tested as inhibitors of the enzyme. Their ability to inhibit oncogene-kinase mediated transformation and retroviral replication in tissue culture cells will also be examined.

(ii) Myristoyl Protein Membrane Binding. Membrane binding sites for $p60^{src}$ N-myristoyl-proteins have been described in red cells. We will also look for similar binding sites in platelets and transformed cell lines. N-Myristoyl protein receptors will be characterized and purified by affinity absorption to peptide affinity resins. Specific agents will be designed to block N-myristoyl-protein receptor association. These will be tested as

inhibitors of oncogene-kinase mediated transformation and/or retroviral replication in tissue culture cells.

Publications:

1. Glover CJ, Goddard C, Felsted RL. Myristoylation of $p60^{src}$: Identification of a myristoyl-CoA: glycylpeptide N-myristoyltransferase in rat tissues, Biochem J 1988;250:485-91.

2. Goddard C, Felsted RL. Identification of N-myristoylated proteins by reserve-phase high performance liquid chromatography of a azlactone derivative of N-myristoylglycine, Biochem J 1988, in press.

3. Felsted RL, Goddard C, Glover CJ. N-Myristoylation as a novel molecular target for the design of chemotherapeutic drugs, Biochem J 1988, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06179-03 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Proto-oncogene Tyrosine Kinase Activity in Myeloid Differentiation

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

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Yu Gang	Visiting Fellow	LBC, NCI
	Thomas Smithgall	PRAT Fellow	LBC, NCI
	Marian Knode	Biologist	LBC, NCI

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Victor E. Marquez Visiting Scientist LMC, NCI

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	2.3	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.)

The aims of this proposal are to characterize the role of proto-oncogene tyrosine protein kinase activities in myeloid differentiation. p93c-fes tyrosine protein kinase has been purified from DMSO-differentiated HL-60 leukemia cells. Therefore one goal of this proposal is to assess the relationship between the expression of p93c-fes and myeloid differentiation in various myelomonocytic cell lines which are sensitive and resistant to differentiating agents or colony stimulating factors. This will be accomplished by protein blotting with antibodies to specific antigenic determinants of p93c-fes, by determining the effects of tyrosine protein kinase inhibitors and by using antisense oligodeoxynucleotides to the initiation codon of p93c-fes mRNA. A second goal of this proposal is to determine the effects of overexpression of the c-*fes* gene in myeloid cell lines deficient in such expression. This will be accomplished by transfecting the genomic DNA for c-*fes* into HL-60, KG-1a and K562 cells. The regulation of expression of the mRNA and c-*fes* protein will be analyzed by hybridization analysis using a cDNA restriction fragment as well as by protein blotting using polyclonal antibodies to various regions of the recombinant p93^{c}-fes. A third goal of these studies will be to determine the cellular protein substrates of p93^{c}-fes by using antibodies against phosphotyrosine to detect phosphoproteins radiolabeled with 32P *in vivo* or *in vitro* in over-expressing cells.

Objectives:

The aims of this proposal are to characterize the role of tyrosine protein kinase (PKT) activities in myeloid differentiation. Recently, we reported the purification and characterization of 60 and 93 kDa PKT's from DMSO-differentiated HL-60 leukemia cells, which were antigenically related to the cellular proto-oncogenes c-src and c-fes, respectfully. The latter PKT has now been identified as the cellular proto-oncogene product of c-fes, while the identity of the 60 kDa PKT has not been established. One goal of this proposal is to assess the relationship between the expression of p60 and p93 and myeloid differentiation. This will be accomplished by the measurements of these PKT activities in various myelomonocytic cell lines which are sensitive and resistant to differentiating agents or colony stimulating factors, and by determining the effects of PKT inhibitors and antisense oligodeoxynucleotides to the initiation codon of $p93^{c-fes}$ mRNA on myeloid differentiation. A second goal of this proposal is to determine the effects of overexpression of the c-fes gene in myeloid cell lines deficient in such expression. This will be accomplished by transfecting the genomic DNA for c-fes into HL-60, KG-1a and K562 cells. The regulation of expression of the mRNA and c-fes protein will be analyzed by hybridization analysis using a cDNA restriction fragment as well as by Western blotting using polyclonal antibodies to various regions of recombinant p93. A third goal of these studies will be to determine the cellular protein substrates of $p93^{c-fes}$ by using antibodies against phosphotyrosine to detect phosphoproteins radiolabeled with ^{32}P in vivo or in vitro in over-expressing cells.

Significance:

The regulation of viral oncogene and proto-oncogene expression is an area of investigation which should lead to a clearer understanding of viral transformation as well as normal cellular proliferation and differentiation. Among the 20 viral oncogenes detected thus far, approximately 50% produce a PKT activity as their gene product. An equal number of PKT activities have been detected in normal tissues and there is a close homology between the viral oncogene PKT and the normal cellular enzyme in many instances. PKT activity is associated with the membrane receptors for epidermal growth factor, platelet-derived growth factor, insulin-like growth factor, insulin and macrophage colony stimulating factor (M-CSF). In the latter case, the PKT is the gene product of the c-fms proto-oncogene. This enzymatic activity provides a functional relationship between many growth factors and proto-oncogene products and thus, undoubtedly plays a central role in the signal transduction process. In a similar context, there is evidence that PKT activity may play a role in differentiation. The dependence on M-CSF for macrophage colony formation from normal chicken macrophage progenitor cells was abrogated in v-fps-infected cells which formed macrophage colonies independently of colony stimulating factors. The v-fps oncogene is the avian counterpart of v-fes. The expression of proto-oncogene c-fms mRNA was increased during differentiation of promyelocytic leukemia cell line HL-60 to the macrophage phenotype by the phorbol ester TPA. The c-fms gene product was shown to be a 140-170 kDa PKT activity and it appears to be the receptor for M-CSF. Differentiation of HL-60 cells to the granulocytic or monocytic/macrophage phenotypes by DMSO or TPA, respectively, resulted in the increased appearance of $pp60^{c-src}$ PKT. PKT activity was also demonstrated in the particulate fraction of the monocytic

cell line U-937 and to be stimulated in vitro by TPA. Uncharacterized PKT activities which increase slightly during granulocytic or macrophage differentiation of HL-60 cells have also been reported. A new hematopoietic cell kinase (hck) gene that is especially prominent in cells of the myeloid lineage and codes for a 57 kDa product similar to $pp60^{c-src}$ was recently reported by two laboratories. The expression of the c-fes gene product is also associated with cells of the myeloid lineage and contains PKT activity. Our laboratory recently reported the identification of p60 and p93 PKT activities in HL-60 cells, the latter of which was associated with granulocytic or monocytic differentiation. p93 was antigenically related to residues 739-768 and 790-805 of the v-fes gene product, a region adjacent to the autophosphorylation site of this protein. We have now demonstrated using polyclonal antibodies against various domains of the cloned c-fes protein that p93 is the c-fes proto-oncogene product. This study was the first reported purification of a human proto-oncogene product with PKT activity.

Cellular studies using antiphosphotyrosine antibodies to detect changes in phosphotyrosine-containing proteins in response to growth factors, viral transformation or differentiation are useful adjuncts for defining the intracellular targets of PKT activities such as p93. Using azobenzylphosphonate (ABP) as the hapten, and coupling the ABP to keyhole limpet hemocyanin, Frackleton et al. first described the use of these antibodies to identify phosphotyrosine-containing proteins in retrovirus-transformed and growth-stimulated cells. We have used this approach to purify p93 by coupling polyclonal anti-ABP immunoglobulins to an epoxy-activated HPLC column. Similar HPLC procedures or immunoprecipitation methods should be useful for identifying phosphotyrosine-containing proteins labeled _in vivo_ or _in vitro_ with ^{32}P.

Major Findings:

We first discovered a differentiation-associated PKT (p93) activity while we were developing a nondenaturing polyacrylamide gel electrophoresis (PAGE) assay for this class of enzymes. We determined that p93 was closely associated with either granulocytic (treatment with DMSO or retinoic acid) or monocytic (treatment with IFN-γ, tumor necrosis factor, or $1,25(OH)_2$vitamin D_3) differentiation of HL-60 cells. Purification of p93 was subsequently accomplished by hydrophobic and anti-phosphotyrosine chromatography. We identified p93 as the c-fes gene product on the basis of its antigenicity to polyclonal antibodies against cloned human c-fes gene products.

At the cellular level, we have studied the relationship of p93 to the differentiation of HL-60 cells using a DMSO-resistant subline of HL-60 cells (HL-60/DMSO). DMSO alone neither induced differentiation in HL-60/DMSO cells nor induced expression of p93. HL-60/DMSO cells partially resensitized to respond to DMSO by cotreatment with IFN-ãA and DMSO expressed p93. A second approach was to determine whether treatment of wild type cells with the p93 substrates such as $poly(Glu,Tyr)_{4:1}$, angiotensin and vasoactive intestinal peptide would prevent differentiation by saturating the PKT and rendering it unable to phosphorylate its intracellular target(s). Indeed, the ability of various p93 substrates to block the appearance of p93 and the differentiated phenotype correlated with their K_m values for p93. Thus, these results appear

to indicate that there is an obligatory association between the expression of p93 and granulocyte/monocyte differentiation in this cell line.

Experimental Design and Methods:

HL-60, U-937, THP-1, KG-1, KG-1a, COS-1, and CHO cell lines will be obtained from the American Type Culture Collection. HL-60/DMSO and HL-60/TPA cells will be provided by Dr. Steven Grant, Medical College of Virginia. Human peripheral blood monocytes and granulocytes will be obtained from the NIH Blood Center. Cell culture conditions and cytochemical determinations of differentiation have been described previously.

Tyrosine kinase inhibitors that are currently available are genistein (Extrasynthese, France), erbstatin (Drug Synthesis and Chemistry Branch, DTP, NCI) and ST-638 (Kanegafuchi Chemical Industry Co., Japan). These have been obtained from the appropriate sources and will be used to test the feasibility of whether PKT activity can be inhibited *in vivo*, and if so, whether such an effect can block myeloid differentiation.

PKT assays will be performed by the nondenaturing PAGE assay or solution assay using angiotensin II as substrate. Extraction and purification of p93 and p60 from HL-60 cells and other cells will be carried out as described previously.

NH_2-terminal sequencing will be performed in collaboration with Dr. John E. Shively, Beckman Research Institute of the City of Hope, Duarte, CA. In this procedure, the protein of interest is characterized by SDS-PAGE, blotted onto the membrane Immobilon (Millipore), located by staining with Coomassie blue, cut out and destained. The membrane is directly inserted into the holder of the sequencing apparatus and the analysis is performed. This procedure gives high yields (70%) with no diphenylthiourea background interference as well as high sensitivity (about 20 pmoles). The sequence of the autophosphorylation sites in c-*fes* and p60 will be determined in a similar manner following isolation of the tryptic phosphopeptides separated by reverse-phase HPLC.

Polyclonal antibodies to the c-*fes* protein will be provided by Dr. Dennis J. Slamon, UCLA. Monoclonal antibodies to c-*fes* sequences 565-576 (IGRGNFGEVFSG), 739-768 (SDVWSFGILLWETFSLGA- SPYPNLSNQQTR) and 790-805 (LMEQCWAYEPGQRPSF) will be provided by the Division of Cancer Etiology, NCI. Recombinant granulocyte-macrophage colony stimulating factor (GM-CSF), ^{125}I-GM-CSF and polyclonal antibodies to GM-CSF will be provided by Dr. Judith C. Gasson, UCLA. The amino terminal sequence 2-17 (GFSSELCSPQGHGLQ) will be synthesized by Dr. Chien-Hua Niu, NCI, for use in preparing polyclonal antibodies after coupling of the peptide to keyhole limpet hemocyanin with glutaraldehyde. Anti-ABP antibodies will be prepared as described previously.

Tissue culture conditions for inducing differentiation of HL-60 cells have been described. The conditions for the other myeloid cell lines will be determined on an individual basis. Cells will be used following induction of differentiation with DMSO or IFN-gamma (HL-60 cells), GM-CSF, IFN-gamma and 1,25$(OH)_2$vitamin D_3 (U-937), or retinoic acid (THP-1) for the appropriate time interval required for maximum induction of nitroblue tetrazolium (NBT) positive cells (granulocytic and monocytic cells) and nonspecific esterase (monocytic

cells). Fc receptors will be determined visually by microscopic examination using anti-sheep red blood cell coated sheep erythrocytes and erythrophagocytosis will be determined under similar conditions after lysing extracellular erythrocytes. Macrophage-like cells will be characterized by their morphology and adherence to the plastic flask. KG-1 (GM-CSF-sensitive) and KG-1a (GM-CSF-resistant) cells will be used after treatment with GM-CSF.

Cells will be extracted with Triton X-100 in a buffer containing protease inhibitors and sodium vanadate to inhibit phosphotyrosine phosphatase. Anti-ABP antibodies will be used for immunoprecipitation using previously published procedures.

Cloning of p93 will employ three approaches. One procedure will utilize a cDNA library constructed in lambda gt11 from poly(A)RNA of HL-60 or U-937 cells (Clontech). Initial screening will rely on polyclonal antibodies to p93 peptides. If this procedure is unable to detect p93, then we will construct our own cDNA library in lambda gt11 using poly(A)RNA from DMSO-differentiated HL-60 cells. The second procedure will use the 14 kb EcoRI genomic sequence of c-fes (American Type Culture Collection). A vector for the inducible and stable expression of p93 will be constructed using pMAM-neo (Clontech). pMAM-neo contains the long terminal repeat (LTR) promoter of RSV and MMTV and its expression in transfected cells is regulated by a dexamethasone-inducible transcription promoter. The EcoRI cDNA sequence of p93, if obtained, or the EcoRI 14 kb genomic fragment of c-fes will be inserted into the Xho I site in pMAM-neo. A third approach will utilize the expression vector pECE which has been used to express the insulin receptor tyrosine kinase. This vector will be obtained from Dr. William J. Rutter, University of California San Francisco and the EcoRI c-fes fragment will be inserted into the EcoRI restriction site in the polylinker region. For selection of stable transformants, cotransfection with pSV2NEO (Clontech) is necessary followed by selection in G418.

Transient expression will be carried out with COS-1 and CHO cells in order to test whether or not the c-fes constructs can be transcribed to yield p93. The expression of p93 will be determined by Western blotting, autophosphorylation, and non-denaturing gel assays. Stable expression of the c-fes vectors will be performed by transfecting the GM-CSF-independent cell line KG-1a and the DMSO-resistant cell line HL-60/DMSO using the protoplast fusion technique. Cells successfully transfected with either pECE + pSV2NEO or pMAM-neo containing the p93 gene will be selected for resistance to the neomycin analog G418 (Gibco) since the neo gene codes for a phosphotransferase with inactivates the antibiotic. Since KG-1a, K562 and HL-60/DMSO do not express p93, they would be the most suitable choices for determining whether the expression of p93 is linked to myeloid differentiation. Stably transfected cells will be examined for their ability to undergo differentiation in response to differentiating agents (HL-60/DMSO and K562) and GM-CSF (KG-1a). Oligodeoxynucleotides against the initiation region of the c-fes mRNA will be synthesized on an Applied Biosystems Model 380B DNA synthesizer using the individual blocked deoxynucleotide methylphosphonates from Applied Bionuclear. These analogs will be characterized by NMR, SDS-PAGE and ion-exchange HPLC using Nucleopore columns (Nest Group). The 12-21 mer complementary sequences will encompass the start codon and adjacent sequences starting 10 bases downstream from the 5' end of exon 2 of c-fes. This sequence is: 5'-ATG GGC TTC TCT TCC GAG CTG.

Transfection with the antisense analogs will be performed by a modified calcium phosphate precipitation procedure. Assessment of the activity of these compounds will be by Northern or dot blots of the c-*fes* mRNA with a restriction fragment of the c-*fes* cDNA.

Publications:

1. Glazer RI, Yu G, Knode MC. Analysis of tyrosine kinase activity in cell extracts using nondenaturing polyacrylamide gel electrophoresis, Anal Biochem 1987;264:214-20.

2. Yu G, Glazer RI. Purification and characterization of $p93^{fps}$- and $p60^{src}$-related tyrosine protein kinase activities in differentiated HL-60 leukemia cells, J Biol Chem 1987;262:17543-48.

3. Yu G, Grant S, Glazer RI. Association of $p93^{c\text{-}fes}$ tyrosine protein kinase with granulocytic/monocytic differentiation and resistance to differentiating agents in HL-60 leukemia cells, Mol Pharmacol 1988;33:384-88.

4. Chapekar MS, Glazer RI. The synergistic cytocidal effect produced by immune interferon and tumor necrosis factor in HT-29 cells is associated with inhibition of rRNA processing and (2',5')oligo(A) activation of RNase L, Biochem Biophys Res Commun 1988;151:1180-87.

5. Glazer RI. Differentiation of malignant cells as a new mode of chemotherapy. In: Elsebai I, ed. Current treatment of cancer. Heidelberg:UICC-Springer-Verlag, in press.

6. Glazer RI. Cellular oncogenes and their gene products as potential therapeutic targets for the differentiation and inhibition of cancer cells. In: Kuo, JF, ed. CRC Critical Reviews in Pharmacological Sciences. Boca Raton:CRC Press, in press.

7. Glazer RI, Aquino A, Yu G. Cellular oncogenes as biotherapeutic targets for the differentiation and inhibition of cancer cells. In: Glazer RI, ed. Developments in cancer chemotherapy, volume II. Boca Raton:CRC Press, in press.

8. Glazer RI. Cyclopentenyl nucleoside analogs as antiviral and anticancer drugs. In: Calio R, Nistico G, eds. Proceedings of the international symposium: basic and therapeutic aspects of antiviral drugs. Milan:Pythagora Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06180-03 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Sulfhydryl Group in Cancer Cell Growth, Metastasis and Chemotherapy

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

PI: Marco Rabinovitz Research Chemist LBC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1	0.1

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

☐ (a1) Minors

☐ (a2) Interviews

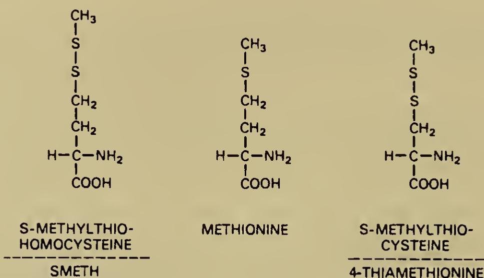
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new derivative of homocysteine, its mixed disulfide with methyl mercaptan, S-(methylthio-L-homocysteine (L-SMETH) was synthesized and found to be cytotoxic to L1210 leukemia cells in culture at micromolar concentrations. The inhibition is markedly promoted by added cupric ion, but not by ions of other metals, is stereospecific, and is competitive with glutamine. For example, at 10 M each of L-SMETH and Cu++, complete growth inhibition was observed if cells were incubated in 1 mM glutamine, 50% inhibition at 2 mM glutamine and none at 4 mM glutamine. The inhibition is also completely relieved by cytidine in a non-competitive manner, but not by guanosine or uridine, indicating that the principal damage to the cellular economy resides in the amination of uridine to cytidine. This was confirmed by HPLC analysis of cell extracts, which showed a marked decrease in CTP with <u>increases</u> in the levels of UTP, GTP, and ATP. A major swelling of cells leading to lysis accompanies the inhibition and increases in DNA and protein per cell confirms this unbalanced growth. High concentrations of SMETH-copper which are more than adequate to completely inhibit growth when left in the medium do not kill cells if they are washed free of inhibitor after 4 hours. The SMETH-copper compound is not toxic, and at dosages and schedules attempted decreased morbidity and peritoneal disease but did not increase the life span of tumor-bearing mice. It thus may require more extensive dose and schedule evaluation as observed with other glutamine amido-transferase inhibitors. Both the chemical and biochemical bases for activity of the novel bio-reactive compound are presented.

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

Objectives

The general goals of this project are directed toward an understanding of the factors influencing the sulfhydryl-disulfide status of the cell and cellular milieu, and the application of this knowledge to the development of selectively toxic regimens for chemotherapy. This portion of the project was originally designed to improve the delivery of homocysteine to tumor cells via its methylthio derivative, followed by reduction of the disulfide bond in a manner similar to that of its lower homolog, S-(methylthio)cysteine, also referred to as 4-thiamethionine.



Experimental evidence, however, indicated that these two compounds are metabolized by entirely different pathways.

Major Findings

SMETH Toxicity and Copper Potentiation. Smeth was cytotoxic to L1210 cells in culture when present at a broad range of micromolar concentrations. The range of inhibitory concentrations was both reduced and narrowed in the presence of copper ion. This ion was very effective at micromolar concentrations in bringing a threshold level of SMETH (25 μM) to a completely inhibitory concentration while being non-toxic itself at much higher levels. A concentration of 10 μM was more than adequate as a potentiating dose, but at this concentration other metal ions (Zn^{++}, Mn^{++}, Co^{++}, Ni^{++}, Fe^{++}, Cr^{+++}) were ineffective. Of interest is the fact that the lower homolog of SMETH, S-(methylthio)-L-cysteine or 4-thiamethionine did not become cytotoxic in the presence of copper, but actually protected L1210 cells in primary culture from copper dependent growth inhibition which is due to depletion of cysteine. Thus both the organic and inorganic moieties of this combination show high specificity in this inhibition.

Stereospecificity

The racemic form of SMETH was half as active as L-SMETH and D-SMETH was completely inactive at 50 μM, with 50 μM copper ion.

SMETH toxicity is not due to Homocysteine Delivery. Although SMETH was originally synthesized as a prodrug to deliver homocysteine to cells, inhibition analysis indicated that cytotoxicity was not due to this mechanism. Such toxicity has been reported for homocysteine thiolactone, which in

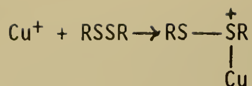
combination with added adenosine and an adenosine deaminase inhibitor, such as deoxycoformycin, can block growth due to adenosylhomocysteine formation (Kredich NM & Hershfield MS. Proc Natl Acad Sci 1979;76:2450-54). Adenosylhomocysteine is a potent inhibitor of cellular methylation processes and, as an endogenous product of methionine metabolism trapped intracellularly by added adenosine, is the reported basis for adenosine toxicity. We have evaluated this toxicity of adenosine across a concentration range for 5 to 40 μM. At 10 μM it was not toxic to L1210 cells, but was when added together with a non-toxic concentration of L-homocysteine thiolactone. Adenosine, however, did not increase the potency of a toxic dose of SMETH. The failure of adenosine to promote SMETH toxicity suggested that such toxicity was not due to adenosylhomocysteine formation.

Glutamine Protection against SMETH Toxicity. Glutamine, at millimolar concentrations which supported growth, protected cells against SMETH and SMETH plus Cu^{++} inhibition. At concentrations of 1 to 4 mM the full range for complete inhibition to complete protection is evident. This type of protection was not seen with other amino acids, some having a closer structural resemblance to SMETH. In fact, such amino acids promoted SMETH toxicity. This promotion of inhibitory activity may be due to the phenomenon termed "trans-stimulation of uptake" which is common in the amino acid series. Such increased uptake would increase cytotoxicity; further analysis of this problem would require radioactive material.

Amination of Uridine to Cytidine as Site of Inhibition. Among the several biochemical roles of glutamine, that involving the amination of uridine-5′-triphosphate (UTP) to cytidine-5′-triphosphate (CTP) was the only locus blocked by copper-SMETH. This conclusion was sustained by two observations. 1) Cytidine alone protected the cells from growth inhibition, and this protection was non-competitive, equivalent concentrations of cytidine being equally effective at two concentrations of copper-SMETH which gave maximal growth inhibition. Uridine and guanosine were ineffective in such protection. 2) HPLC analysis of cells inhibited in growth showed greater than a 1/3 diminution of CTP content but a two-fold elevation of UTP, ATP and GTP. Other uncharacterized peaks were also elevated in the inhibited sample. Such elevated levels of cellular constituents may be due to the increased volume of inhibited cells as described below.

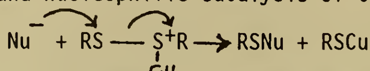
Cell Expansion and Unbalanced Growth. Characteristic of SMETH and copper-SMETH growth inhibition is the progressive increase in cell volume observed over a two day period. Ultimately the cells burst, as indicated by the accumulation of debris. Flow cytometric analysis of control and inhibited, swollen cells showed increases in fluorescein isothiocyanate staining and propidium iodide staining in the latter, which are indicative of increases in protein and DNA per cell, respectively. The cause of such unbalanced growth leading to a defect in cellular composition brought about a CTP deficiency and resulting in swelling and lysis is currently under investigation.

Molecular Mechanism of Action. The coordination between the cuprous ion and a symmetrical disulfide as described by Ottersen et al. (Inorg Chem 1974;13:1904) involves a lengthening of the disulfide bond and thus its weakening.

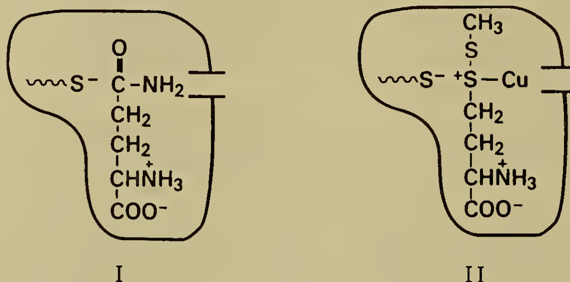


The -$\overset{+}{S}$-R moiety thus becomes a much better leaving group than the original -SR.
(with Cu attached to S)

The sulfur-sulfur bond becomes more susceptible to nucleophilic attack, and the structure may be considered an example of an intermediate in concomitant electrophilic and nucleophilic catalysis of the scission of this bond.



The positioning of glutamine in CTP synthetase relative to the enzyme's reactive thiolate anion can be represented as indicated below.



Diagrammatic Representation of Glutamine (I) and copper-SMETH (II) at the active site of CTP synthetase.

In this representation the glutamine subsequently loses its amide group and reacts to form a thio-ester. A corresponding positioning of copper-SMETH is shown in the right panel. This positioning is dependent upon the "natural" L-configuration of SMETH and places the nucleophilic thiolate ion in close proximity to the sulfonium moiety of the copper disulfide function. In accordance with the reactions described above, the cuprous sulfide of homocysteine would act as the leaving group and the enzyme would be methylthiolated.

Proposed Course

I. Chemical Synthetic Studies

Synthesis of the S-(t-butyl)-, S-(trifluoromethyl)- and related analogs as well as their corresponding selenium congeners, and elucidation of their mode of action both with respect to activity in normal and tumor CTP synthetase and in cell culture and tumor bearing animals. Attempts will be made to extend these

structural characteristics to the purine nucleoside series, with the formation of 6-methyldithio- and 6-homocysteyldithio-adducts.

II. Biochemical Studies

A marked swelling of cells leading to lysis is a characteristic of SMETH cytotoxicity. Since the sole inhibition observed is that of CTP formation, and in view of central role of CTP in phospholipid biosynthesis, it appears plausible that the biochemical lesion leads to the physical lesion. This is particularly underscored because of the high Km of the enzymatic reactions for CTP in phospholipid biosynthesis compared to those of nucleic acid biosynthesis. Accordingly, an unbalanced growth would take place with insufficient phospholipid generation to maintain membrane stability. Attempts will be made to overcome at least a portion of SMETH cytotoxicity by phospholipids added to cell cultures. Such intervention by exogenous phospholipids has been reported and would require adaptation of cells to phospholipid free medium so that the effectiveness of added material could be evaluated.

III. Chemotherapeutic Studies

Attempts will be made to develop binary chemotherapeutic schedules where copper and the organic moieties are delivered at different times. Evidence that delivery of copper by transporting chelates has chemotherapeutic potential is well recognized and introduces the possibility of independent administration of SMETH (or one of its analogs) and a carcinostatic copper delivery agent for specific, synergistic cytotoxicity and resultant chemotherapy. KTS or one of its analogous bis-thiosemicarbazones are candidates for this proposal.

Publications and Patent

Rabinovitz M. The structure of flavone-8-acetic acid, a chemotherapeutic agent, and its application to drug design, J Enzyme Inhibition 1988;2:151-52.

Rabinovitz M. Emerging evidence for control of monovalent cation homeostasis as a critical target in alkylating agent resistance. In: Kessel D, ed. Resistance to anti-neoplastic drugs. CRC Press, 1988; in press.

Rabinovitz M. U.S. Patent, Notice of Allowance: Chemotherapeutic 1-(2-chloroethyl)-4-(3-chloropropyl)piperazine, dihydrochloride. January 14, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06181-03 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Interaction of GTP-binding Proteins with Cellular Components

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

PI:	Richard A. Kahn	Senior Staff Fellow	LBC, NCI
Others:	Janet Holden	Visiting Fellow	LBC, NCI

COOPERATING UNITS (if any)

None

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Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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

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

☐ (a1) Minors

☐ (a2) Interviews

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

Activation of the ras oncogene has been implicated as the causative agent in as many as 30% of all human tumors. Yet in spite of extensive work on the ras gene, almost nothing is known about the biochemical function of the ras proteins. A recently characterized protein, the ADP-ribosylation factor or ARF, is a component of the adenylate cyclase system and shares several features with ras, p21 including size, location, and the ability to bind GTP. Characterization of the binding and hydrolysis of guanine nucleotides by ras and ARF will be undertaken. A systematic search will then be conducted to identify factor(s) which increase either the exchange or hydrolysis of guanine nucleotides by these regulatory proteins. The assays developed in these studies will also be used to help define the function of specific genes, identified in yeast as suppressors or activators of ARF disruption. These studies should help locate cellular targets for these regulatory proteins and may identify the physiological role of these proteins in cellular metabolism or proliferation.

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

In a series of related studies we have recently demonstrated that the binding of ARF to cellular plasma membranes is regulated by the binding of GTP. We will be expanding these studies to see if this is a general phenomena related to G-proteins, if there is a specific membrane receptor, and how this localization affects the functioning of ARF.

Introduction

Research on the regulation of the adenylate cyclase system has led to the discovery of a family of homologous GTP-binding, regulatory proteins (G-proteins). More than twenty distinct members have been identified and purified, though only two have defined roles in the regulation of adenylate cyclase activity. Although implicated as regulators of K^+, Ca^{++}, and other ion channels, phosphoinositide metabolism, and cellular proliferation, the roles of the other G-proteins are not yet known.

A novel G-protein has been purified and characterized by the PI. This protein, termed ARF, interacts with the adenylate cyclase system and shares many of the GTP-binding characteristics with the oncogene, p21 ras. Both proteins have a molecular mass of 21,000 daltons.

Objectives

In all cases where a cellular role is known the activity of a guanine nucleotide binding protein is controlled by the binding of GTP. Hydrolysis of the bound GTP results in deactivation. Thus, knowledge of the factors which control the binding and hydrolysis of GTP will also identify elements upstream and downstream of the regulatory proteins. The objective of this work is to utilize the nucleotide binding properties of ARF and identify cellular targets and pathways controlled by this protein. Another factor known to be critical to G-protein function is localization of the regulatory proteins to the plasma membrane. The membrane association of the other G-proteins appears to be aided by covalent acylation and is irreversible.

Methods employed

The principal method utilized will be radioligand binding studies of nucleotide binding to purified ARF. Factors which increase either the off-rate of bound GDP or hydrolysis of bound GTP will be screened for in tissue extracts. Standard protein purification methods will be utilized for further characterize any activity present. Other genes in the ARf pathway are being identified in yeast and will be assessed for these activities as they become available.

Membrane association is determined by monitoring the amount of ARF in 100,000xg pellets after incubating with nucleotides and purified plasma membranes.

Major findings

The binding of the activating ligand, GTP, to ARF promotes its association with plasma membranes. GDP-bound ARF displays little or no affinity for membranes under the same conditions. This result strongly suggests that the membrane is the site of action of ARF. The binding to membranes appears to be non-

saturable and temperature dependent, suggesting an association with membrane lipid rather than a specific protein acceptor. We have also recently shown that purified bovine brain ARF is myristoylated at the amino terminal glycine. We are currently attempting to define the requirement for the myristic acid in membrane association as well as checking other G-proteins for guanine nucleotide dependent membrane association.

Publications

1. Kahn RA, Goddard C, Newkirk M. Chemical and Immunological Characterization of the 21 kDa ADP-ribosylation Factor (ARF) of Adenylate Cyclase, 1., J Biol Chem, 1988, in press.

2. Kahn RA. Regulators of signal transduction: families of GTP-binding proteins. In: Glazer R, (ed) Developments in Cancer Chemotherapy. Boca Raton: CRC Press, 1988; in press.

3. Kahn RA. The ADP-ribosylation Factor of Adenylate Cyclase: a 21 kDa GTP-binding Protein. In: Birnbaumer L, Iyengar R, (eds) G-Proteins. Academic Press, 1988; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06182-03 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Genetic and Immunologic Analyses of the ADP-ribosylation Factor, ARF

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

PI:	Richard A. Kahn	Senior Staff Fellow	LBC, NCI
Others:	Jenny Sewell	Microbiologist	LBC, NCI
	Ofra Weiss	Visiting Fellow	LBC, NCI

COOPERATING UNITS *(if any)*

None

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Laboratory of Biological Chemistry

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

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☐ (a1) Minors

☐ (a2) Interviews

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

ARF is a recently characterized GTP-binding regulatory protein component of the adenylate cyclase system. These studies are aimed at the elucidation of the role of ARF in signal transduction, differentiation, and proliferation. We have used the bovine ARF gene to clone two homologous genes, ARF1 and ARF2, from the yeast, S. Cerevisiae. Disruption of ARF1 leads to slow growth, cold sensitivity, and supersensitivity to fluoride. We plan to investigate the consequences of ARF2 disruption both alone and in combination with disruption of ARF1. Suppressors of the arf1- phenotype have been obtained and will be analyzed. It is expected that some of these suppressor genes will code for other proteins in the ARF pathway. We will determine the ability of human ARF to rescue the arf1- phenotype to test for conservation f ARF function. In addition, we are cloning and sequencing ARF from both human and Xenopus laevis libraries. The human gene will be used to screen a variety of human tumors for the presence of abnormal ARF expression. The Xenopus gene will be used in studies of ARF function in Xenopus oocytes which allow microinjection of purified ARF proteins and biochemical assays of resultant changes in cellular metabolism.

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

Introduction

ARF has recently been identified as a 21,000 Da GTP-binding regulatory protein. Activated ARF binds to the stimulatory, regulatory component (Gs) of adenylate cyclase and allows cholera toxin to irreversibly activate the cyclase. Though apparently a component of the adenylate cyclase complex, the physiological role of ARf is unknown. ARF is present in the plasma membrane of every eukaryotic tissue or cell type examined. Attempts to isolate ARF deficient cell lines been unsuccessful in a number of laboratories. ARF has the same molecular mass and shares the GTP-binding characteristics of the 21 kDa ras oncogene. Ras proteins have been implicated as regulators of adenylate cyclase in yeast. The role of ras in mammalian cells is unknown.

Objectives

The overall objectives of this project include the elucidation of each component of the ARF pathway and determination of the role, if any, of ARF in transformation or oncogenesis. We will utilize many of the powerful genetic techniques to analyze the yeast ARF genes and use both DNA and antibody probes to address these questions. Thus, the cloning and sequencing of two ARF genes will be the starting place.

Methods Employed

We are currently finishing up work on the cloning and sequencing of two yeast ARF genes, ARF1 and ARF2, as well as human ARF and Xenopus ARF. Human ARF cDNA will be used to screen tissue obtained from human tumors for the levels of ARF mRNA and DNA in blots. The yeast genes will be used in disruption experiments as well as being used in inducible yeast expression vector, pBM272. Techniques for manipulation of yeast cells and genes can be found in the Cold Spring Harbor Manual for its Yeast Course. Synthetic peptides will be injected into rabbits for the production of mono-specific antibodies. Injection of ARF protein and antibodies into Xenopus oocytes will be performed in collaboration with Dr. Hsiang-fu Kung at FCRF, Frederick, MD.

Major Findings

Two ARF genes, ARF1 and ARF2 have been cloned from a genomic yeast library. Both genes have been mapped to chromosome IV. The amino acid sequences are more than 96% identical. Disruption of ARF1 results in three phenotypic changes: slow growth at 30°C, cold sensitivity, and supersensitivity to growth in the presence of fluoride ion. Suppressors of fluoride supersensitivity have been obtained and are currently being classed. There are at least three independent genes capable of suppressing the ARF1 disruption. Further characterization of these genes, both genetic and sequencing, is planned. Completion of the sequencing of ARF2 may yield further information on the relationship between these two genes. Disruption of ARF2 either alone or in conjunction with ARF1 will be of great interest to these studies and is currently in progress.

A human ARF gene has been cloned and partially sequenced. The coding region appears to be 100% identical to the bovine gene. Thus, the mono-specific

bovine antibodies already in hand will be excellent probes for the human protein as well. We are currently trying to determine if man has one or more ARF genes.

We have successfully put the bovine and yeast ARF1 genes into bacterial and yeast inducible expression vectors, respectively. Expression of bovine ARF in bacteria results in about 20% of cell protein being ARF and purification of this expressed protein is underway. The expressed protein appears similar to purified bovine brain ARF with respect to nucleotide binding.

Proposed Course

1) Finish sequencing the ARF2 gene. Map both ARF genes on chromosome IV.
2) Disrupt ARF2 and determine phenotype, compare to arf1. Also do the double disruption, $arf1^{-}arf2^{-}$. Prediction is that double disruption will be lethal.
3) Immunolocalization of ARF proteins in fixed yeast cells. If possible prepare better yeast ARF antibodies as well as antibodies specific to each of ARF1 and ARF2.
4) Put human ARF into inducible yeast expression vector. Put into WT, arf1, arf2, arf1arf2 cells. Does human ARF complement arf1 or arf2?
5) Clone and sequence the Xenopus ARF gene. Delete Xenopus ARF gene and determine phenotype. Microinject ARF proteins or antibodies into Xenopus oocytes and monitor development. Also monitor cellular levels of cAMP, phosphoinositides, both in control and hormone stimulated oocytes.

Publications

1. Zaremba T, Gierschik P, Pines M, Bray P, Carter A, Kahn R, Simons C, Vinitsky R, Goldsmith P, Spiegel A. Immunochemical Studies of the 36-kDa ß Subunit of Guanine Nucleotide-Binding Proteins: Identification of a Major Epitope. Mol. Pharm., 33:257-264, 1988.

2. Kahn RA. Regulators of signal transduction: families of GTP-binding proteins. In: Glazer R, ed. Developments in Cancer Chemotherapy. Boca Raton: CRC Press, 1988 in press.

3. Kahn RA. The ADP-ribosylation Factor of Adenylate Cyclase: a 21 kDa GTP-binding Protein. In: Birnbaumer L, Iyengar R, eds. G-Proteins, Academic Press, 1988, in press.

4. Sewell J, Kahn RA. Sequences of the Bovine and Yeast ADP-ribosylation Factor and Comparison to other GTP-binding Proteins, Proc Natl Acad Sci, USA 1988 in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06187-02 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Inhibitors of Protein Kinases as Potential Chemotherapeutic Agents for AIDS

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

PI:	Robert I. Glazer	Pharmacologist	LBC, NCI
Others:	Angelo Aquino	Visiting Fellow	LBC, NCI
	Kathleen Hartman	Chemist	LBC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	1.3	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.)

The aims of this proposal are to: 1) define the role of phosphorylation of the human immunodeficiency virus (HIV) antigen, p17gag, by cellular and/or viral serine/threonine and tyrosine kinases and 2) to design and test inhibitors of the protein kinases as potential inhibitors of HIV replication or its cytolytic activity. One goal will be to determine whether a viral protein kinases(s) exists, to what gene product it is related and whether it can utilize p56/p17 as a substrate *in vitro*. A second goal will be to determine whether cellular protein kinases such as protein kinase C and tyrosine protein kinases associated with monocytes and lymphocytes can utilize recombinant p56gag as a substrate *in vitro*. A third goal of these studies will be to determine whether there is a difference in the phosphoamino acid/phosphopeptide pattern of p56/p17 *in vivo* betwen H9 cells for which HIV is not cytolytic and ATH-8 cells and peripheral blood lymphocytes which are killed by the virus. The last goal of these studies will be to determine whether any known inhibitors of serine/threonine or tyrosine protein kinases can inhibit HIV replication in H9, ATH-8 or peripheral blood cells *in vitro*. If this aspect of the study proves fruitful, then computer modeling studies will be carried out to design and synthesize new inhibitors of protein kinases.

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Objectives:

The aims of this proposal are to: 1) define the role of phosphorylation of the human immunodeficiency virus (HIV) antigen, $p17^{gag}$, by cellular and/or viral serine/threonine and tyrosine kinases and 2) to design and test inhibitors of the protein kinases as potential inhibitors of HIV replication or its cytolytic activity. This proposal is based on the finding in our laboratory that one of the structural proteins known as the group specific antigen (gag), p17, is phosphorylated in vitro in viral lysates, and the presumption that this process is positively related to the replication or cytolytic activity of HIV. Whether the phosphorylation of p17 is accomplished by protein kinase(s) of viral origin and/or by the cellular kinases of the infected helper T-cell or monocyte is not known. Moreover, it is not known whether phosphoserine- and/or phosphotyrosine-containing sequences have differential effects on HIV replication. Therefore, **one goal** will be to determine whether a viral protein kinase(s) exists, to what gene product it is related and whether it can utilize p56/p17 as a substrate in vitro. A **second goal** will be to determine whether cellular protein kinases such as protein kinase C, protein kinase M, protein kinase A, p60 and p93 tyrosine protein kinases from HL-60 cells and monocytes and $p58^{T-cell}$ tyrosine protein kinase from T-cells can utilize recombinant $p56^{gag}$ as a substrate in vitro. A **third goal** of these studies will be to determine whether there is a difference in the phosphoamino acid/phosphopeptide pattern of p56/p17 in vivo between H9 cells for which HIV is not cytolytic, and ATH-8 cells and peripheral blood lymphocytes which are killed by the virus. The **last goal** of these studies will be to determine whether any known inhibitors of serine/threonine or tyrosine protein kinases can inhibit HIV replication in H9, ATH-8 or peripheral blood cells in vitro. If this aspect of the study proves fruitful, then computer modeling studies will be carried out to design and synthesize new inhibitors of protein kinases.

Significance:

There is a close association between HIV infection and the acquired immunodeficiency syndrome (AIDS) as determined by serological evidence of antibodies to HIV envelope (env) and gag antigens in patients with AIDS. In seropositive patients, there is a high titer to HIV antigens of 120, 65, 55, 41, 24 and 17 kDa. Among these proteins, p120, p65 and p41 are env glycoproteins, and p17, p24 and p55 are gag proteins with the latter being the precursor. Little or no information is available about the posttranslational modification, viz. the phosphorylation of any of the HIV antigens.

The importance of phosphorylation in transformation by the avian retrovirus, Rous sarcoma virus, is particularly well documented. It is known that transformation is dependent on a functionally competent $pp60^{v-src}$ tyrosine kinase which not only autophosphorylates on tyrosine but also phosphorylates cellular host proteins. Documentation of the phosphorylation of retroviral gag proteins stems from studies with the murine Rauscher leukemia virus whose $p65^{gag}$ precursor is phosphorylated in infected cells in contrast to the lack of phosphorylation of the env proteins of the virion. Long-term labeling of virus-infected cells with ^{32}P showed that one of the processed gag proteins,

p12, was the most highly labeled of the structural proteins. The phosphorylation of p12 influenced the extent of binding of the viral RNA to this structural protein, ie. the greater the phosphorylation of p12, the lower the affinity of viral RNA for this protein. Since this virus does not contain its own protein kinase, phosphorylation of p12 by cellular enzymes may serve as a means for the host cell to modulate the function of the virus. Other viral proteins may also serve as targets for phosphorylation. A protein kinase from Rous sarcoma virus-infected cells produced a 2-5-fold stimulation of the reverse transcriptase activity _in vitro_. Thus, the aforementioned studies with animal retroviruses indicate a regulatory role for viral protein phosphorylation, although the exact mechanism of this process is undefined. In addition, no information of this type exists for cells infected with human retroviruses. However, a recent publication has documented that the recombinant 27 kDa gene product of the 3' open reading frame (3'-_orf_) of HIV is phosphorylated _in vitro_ by purified protein kinase C as well as _in vivo_ by metabolic labeling of BHK21 cells transfected with a vaccinia virus vector containing the 3'-_orf_ gene.

If phosphorylation of _p17_ should have a positive influence on HIV replication, several candidate inhibitors of protein kinases would be useful for determining the relative importance of phosphoserine/phosphothreonine vs phosphotyrosine. The flavonoid, genistein, is a specific inhibitor of EGF receptor, $p110^{gag-fes}$ and $p60^{v-src}$ tyrosine protein kinases _in vitro_ and has no inhibitory activity towards protein kinase C, protein kinase A or phosphorylase kinase. Erbstatin is an antibiotic which possesses specific inhibitory activity against the epidermal growth factor receptor tyrosine kinase activity. ST-638 is a new 4-hydroxycinnamamide analog with specificity as an inhibitor of tyrosine kinase activities. K-252b is an antibiotic which is a highly potent and fairly specific inhibitor of protein kinase C. Thus, these compounds should provide us with some assessment of the role of protein kinases for the replication and/or cytolytic activity of HIV, and whether these inhibitors may have therapeutic potential as a new class of antiviral agents. If the latter proves to be true, then these inhibitors should provide structure-activity information for the design of new inhibitors of protein kinases.

Major Findings:

A sufficient quantity of $p56^{gag}$ for use as a substrate for various protein kinases has been obtained by expressing the recombinant protein in bacteria. _E. coli_ strain MC1061 transfected with pEV2-_gag_ was obtained from Dr. Herbert Weissbach, Roche Institute of Molecular Biology. This expression vector codes for a $p56^{gag}$ precursor and contains a truncated p17 and the entire p15 and p24 sequences. In collaboration with Dr. Rodney Levine, large scale production of recombinant $p56^{gag}$ has been performed. We have determined that the _gag_ proteins are expressed as three transcription products of 44, 53 and 56 kDa, all of which react with a monoclonal antibody to $p24^{gag}$, a result identical to that in the original report. We are now exploring ways to purify and separate the three proteins.

The phosphorylation of p17 _in vitro_ is being studied in Triton X-100 lysates of HIV strain B. Phosphorylation assays were conducted with [gamma^{32}P]ATP

and lysates from concentrated virus particles purified by two consecutive sucrose density gradient procedures. SDS polyacrylamide gel electrophoresis of the ^{32}P-labeled lysates followed by Western blotting with either monoclonal antibodies to p17 and p24 or serum from a patient with AIDS revealed that only p17 was phosphorylated in a strict Mn^{2+}-dependent manner. Control experiments using lysates of the ribosome fraction from uninfected H9 cells obtained by an identical sucrose density gradient procedure did not reveal any Mn^{2+}-dependent phosphorylation. Serum from an AIDS patient or anti-p17 blocked the phosphorylation of the p17 _gag_ proteins.

We have also carried out preliminary studies to determine whether the HIV itself contains a protein kinase. Triton X-100 lysates of HIV were chromatographed by gel filtration HPLC and the fractions were assayed for protein kinase activitiy by using the synthetic substrates, poly(Glu,Tyr)$_{4:1}$ or poly(Lys, Ser)$_{3:1}$. Our initial results indicate that HIV lysates contain a Mn^{2+}-dependent serine protein kinase. We plan to characterize this enzyme further chromatographically as well as by the use of deletion mutants of HIV, eg. HIV variant deltaX-C lacking the _3'-orf_ gene, to determine if the protein is absent. One candidate protein under consideration is the gene product of _3'-orf_. The _3'-orf_ protein shows structural homology with the phosphorylation domain of the interleukin-2 receptor and contains an ATP-binding site. Recently, it has been demonstrated that the recombinant gene product expressed in BHK21 cells is phosphorylated. Sera from AIDS patients contain antibodies to the 27 kDa _3'-orf_ protein and it has been detected in H9 cells infected with HIV as well as in viral lysates.

Experimental Design and Methods:

T cell lines H9 and ATH-8 will be obtained from Dr. Carlo Federico Perno, National Cancer Institute. HIV strain B and X-C will be obtained from Dr. Robert C. Gallo, National Cancer Institute. Monoclonal antibodies to p24 and p17 and serum from patients with AIDS will be obtained from Dr. Perno and Dr. Prem Sarin, National Cancer Institute. Antiserum to the _3'-orf_ protein will be obtained from Dr. Genoveffa Franchini, National Cancer Institute. Concentrated preparations of HIV will be purified by two consecutive sucrose density gradient procedures and will be obtained from Electro-Nucleonics. Virus preparations will be received frozen in dry ice and immediately lysed in our standard 1% Triton X-100 lysis buffer upon receipt. All procedures will be conducted under Biosafety Level 3 protocols. Use of HIV-infected cells in our tissue culture laboratory will also employ similar procedures. viz. restricted access to the room, wearing surgical gloves when handling HIV or HIV-infected cell cultures, using biological hoods and leaving lab coats in the room when exiting. Infection of the cell lines will be done by Dr. Perno in his laboratory and not in our own. Radiolabeling and extraction of HIV-infected cells as well as the purified virus will be done in our tissue culture laboratory.

Genistein will be obtained from Extrasynthese, erbstatin will be obtained from the Natural Products Branch, NCI, ST-638 will be obtained from Dr. T. Shiraishi, Kanegafuchi Chemical Industry Co. and K-252b will be obtained from Dr. S. Nakanishi, Kyowa Hakko Kogyo.

Protein kinase C and protein kinase M will be purified by published procedures. Monocytic tyrosine kinases p^{60} and $p93^{c-fes}$ will be purified by our previously published method. T-Cell tyrosine kinase p58 will be isolated from the ribosome fraction of H9 cells by gel filtration HPLC and characterized by the method of Earp et al. Initial characterization of the HIV protein kinase will entail gel filtration HPLC and assay of activity in the presence of Mn^{2+} with $p56^{gag}$ or poly(Lys, $Ser)_{3:1}$ as substrate. Incorporation of ^{32}P from [gamma^{32}P]ATP into trichloroacetic acid-precipitable material will be used to measure protein kinase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06190-01 LBC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Protein Phosphorylation in Multidrug Resistant Cells

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

PI:	Robert I. Glazer	Pharmacologist	LBC, NCI
Others:	Angelo Aquino	Visiting Fellow	LBC, NCI
	Marian Johnson	IPA	LBC, NCI
	Marian Knode	Biologist	LBC, NCI
	Kathleen Hartman	Chemist	LBC, NCI

COOPERATING UNITS *(if any)*

Kenneth Cowan, Medicine Branch, DCT, NCI

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Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	2.3	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.)*

The purpose of this project is to characterize protein phosphorylation and the associated Ca2+- and phospholipid-dependent protein kinase C (PKC) activities in multidrug resistant (mdr) cells. The overexpression of PKC is closely associated with the mdr phenotype in both leukemic and breast carcinoma cell lines. Since PKC is a family of enzymes with strikingly different responses to proteolysis and phorbol ester activation, the first goal of this proposal will be to determine the relative abundance of the three major isoforms of PKC in mdr cells in comparison to sensitive cells using chromatographic and protein blotting techniques. Secondly, mdr cells selectively activate proteolytically, PKC to the Ca2+_ and phospholipid-independent form termed protein kinase M (PKM). Therefore, the second goal of this study is to quantitate the levels and isoforms of PKM in mdr cells. The generation of PKM in mdr cells results in the utilization of the actin binding cytoskeletal protein, vinculin, as an endogenous substrate. Therefore, the third goal of this study is to assess vinculin phosphorylation both in vitro and in vivo. In a similar context, the last objective of this project is to assess the phosphorylation of the mdr-associated P-glycoproteins. This will be accomplished by determining: 1) whether specific antibodies to the isoforms of PKC block phosphorylation of P-glycoproteins in vitro, 2) whether phosphorylation of P-glycoproteins can be modulated in vivo or in vivo by TPA and 3) whether the use of inhibitors of PKC such as K252b block the phosphorylation of P-glycoproteins and the drug resistance-associated increases in drug binding and drug efflux.

Objectives:

The purpose of this project is to characterize protein phosphorylation and the associated Ca^{2+}- and phospholipid-dependent protein kinase C (PKC) activities in multidrug resistant (mdr) cells and in primary tumor samples from drug-resistant patients. We have discovered that the overexpression of PKC is closely associated with the mdr phenotype in both leukemic and breast carcinoma cell lines. Since PKC is a family of enzymes with strikingly different responses to proteolysis and phorbol ester activation, the first goal of this proposal will be to determine the relative abundance of the three major isoforms of PKC in mdr cells in comparison to sensitive cells, and in primary human tumors from drug-resistant patients. This parameter may be a particularly important feature of multidrug resistance since we have found that mdr cells selectively activate PKC to the Ca^{2+}- and phospholipid-independent form termed protein kinase M (PKM). Therefore, the second goal of this study are to quantitate the levels of PKM in mdr cells and to determine from which isoform of PKC they are derived. Since the proteolysis of PKC is associated with the utilization of the actin binding cytoskeletal protein, vinculin, as an endogenous substrate, vinculin may be a specific cellular marker (substrate) indicative of the presence of PKM generated via proteolysis of the overabundant PKC isoform(s) in the mdr cells. Therefore, the third goal of this study is to assess vinculin phosphorylation both in vitro and in vivo. Since the measurement of PKM is not feasible on the basis of enzymatic activity alone (PKM has no distinguishing characteristics like the Ca^{2+}- and phospholipid-dependence of PKC), antibodies will be prepared to a synthetic peptide encompassing a consensus sequence in the COOH terminal catalytic region of PKC and to a hypervariable catalytic region (V3) of PKC near the junction between the hydrophobic and catalytic domains. Using the technique of Western blotting, we will measure the presence of isoforms of PKM as well as PKC in extracts from various sensitive and mdr cell lines. The last objective of this study is to assess the phosphorylation of the mdr-associated P-glycoproteins. Since there is evidence that these proteins are phosphorylated in the Adriamycin-resistant chronic myelocytic leukemia cell line K562, it would be pertinent to this proposal to determine the role of PKC or PKM in this phenomenon. This will be accomplished by: 1) determining whether specific antibodies to the isoforms of PKC block phosphorylation of P-glycoproteins in vitro, 2) whether phosphorylation of P-glycoproteins can be modulated in vivo or in vivo by TPA and 3) whether the use of inhibitors of PKC such as K252b block the phosphorylation of P-glycoproteins and the drug resistance-associated increases in drug binding and drug efflux. Alterations in drug binding to P-glycoproteins will be assessed by photoaffinity labeling with vinblastine or Adriamycin which are highly specific functional probes for this mdr-associated protein.

Significance:

The cell biology of multidrug resistance has centered on the role of cell membrane-associated proteins termed collectively, P-glycoproteins. Previous studies on the development of drug resistance have focused on alterations in the properties of this cell membrane component. Amplified membrane glycoproteins have been identified in several cell lines exhibiting mdr. Recent transfection experiments with the genomic or complementary DNA encoding

an mdr-associated P-glycoprotein have established that this glycoprotein confers multidrug resistance to sensitive cells. In contrast, studies of the posttranslational modification, eg. phosphorylation, of P-glycoproteins have been limited. A 100-fold Adriamycin-resistant cell line of promyelocytic leukemia cell line HL-60 contained two membrane glycoproteins of 110 and 160 kDa which were not present in the parental cell line. This cell line also exhibited cross resistance to vinblastine and actinomycin D as well as enhanced drug efflux, characteristics typical of mdr cells. Similar phenotypic changes have been noted in other HL-60 cell lines that were 10-fold and 80-fold resistant to Adriamycin and which contained membrane proteins of 120-250 kDa that were not only absent in wild type cells but were also phosphorylated _in vitro_ in the resistant cell line. However, it has not been demonstrated whether the latter cell lines display true multidrug resistance. Similar studies in Chinese hamster lung cell cells resistant to Adriamycin revealed _in vitro_ phosphorylated proteins of 180 and 220 kDa. A recent report described the phosphorylation _in vivo_ of the 170-180 kDa glycoprotein in K-562 cells that were 150-fold resistant to Adriamycin. In the latter study, drugs such as verapamil and trifluoperazine which inhibit drug efflux and restore drug sensitivity to mdr cells increased phosphorylation of the membrane glycoproteins above its basal level. This implies that phosphorylation of transport or drug-binding proteins may regulate the efflux of drugs and other xenobiotics in mdr cells. Whether this phenomenon pertains to normal tissues containing P-glycoprotein is not known. However, a direct association between phosphorylation of any normal cellular or mdr-associated protein and the function of that protein in the multidrug resistance phenomenon has yet to be established.

Vinculin is a cytoskeletal protein of 130 kDa that is associated with actin filaments at adhesion plaque attachment sites along the inner surface of the plasma membrane. Vinculin inserts in the membrane lipid bilayer by interacting with acidic phospholipids such as phosphatidylinositol and is believed to be involved in the transmission of contact-dependent growth-related signals. Agents such as TPA and platelet-derived growth factor result in removal of vinculin from adhesion plaques with the subsequent disruption of microfilament stress fibers. More recently, a transmembrane linkage has been established between the plasma membrane fibronectin receptor and vinculin via another adhesion plaque protein of 220 kDa, talin, thus establishing a relationship between actin-containing microfilament bundles, the cytoskeleton and the extracellular matrix. The acylation of vinculin and its colocalization with $pp60^{src}$ tyrosine kinase as well as its phosphorylation by PKC suggests that phosphorylation of vinculin, and perhaps talin as well, may be a means of regulating cytoskeletal organization. Results from this laboratory indicate that vinculin is phosphorylated _in vitro_ in a Ca^{2+}- and phospholipid-independent manner in cell extracts prepared from TPA-treated wild type HL-60 cells. We have recently discovered that cell extracts from _untreated_ Adriamycin-resistant HL-60 cells (HL-60/ADR) exhibit the same phenomenon as TPA-treated wild type cells, viz. high levels of vinculin phosphorylation _in vitro_ as well as _in vivo._ Thus, these studies suggest that cytoskeletal organization may play an important role in drug resistance and that the phosphorylation of vinculin or perhaps talin, may be a ubiquitous feature of multidrug resistance.

Major Findings:

In vitro phosphorylation assays carried out with extracts of HL-60/ADR cells showed the Ca^{2+}-and phospholipid-independent phosphorylation of a 130 kDa protein which was not present in parental HL-60 cells except after treatment with TPA. The 130 kDa protein was antigenically related to vinculin. This phenomenon did not occur in the parental cell line. Phosphorylation of vinculin also occurred *in vivo* in HL-60/ADR cells, but not in the drug sensitive cell line. Since HL-60/ADR cells exhibit the ability to phosphorylate vinculin *in vitro* in the same manner as TPA-treated wild type cells, and since PKC is believed to be a receptor for phorbol esters, we examined the role of this enzyme in the ability of HL-60/ADR cells to phosphorylate vinculin. DEAE-Sepharose chromatography of cell extracts revealed that HL-60/ADR cells contained 2-fold more PKC than the parental cell line. PKC activity was found only in the cytosol of wild type HL-60 cells, whereas 85% of the PKC activity was cytosolic and 15% was membrane-bound in HL-60/ADR cells. After treatment for two days with 10 nM TPA, PKC activity was reduced 80-90% in both cell lines regardless of its intracellular distribution. Coincident with TPA treatment of HL-60/ADR cells was a reduction in Ca^{2+}- and phospholipid-independent phosphorylation *in vitro* of vinculin whereas, TPA-treated wild type cells exhibited elevated levels of this phosphoprotein. Similar results were achieved by metabolic labeling of HL-60/ADR cells with $H_3{}^{32}PO_4$. The phosphorylation of vinculin in TPA-treated HL-60 or untreated HL-60/ADR cells was blocked *in vitro* by goat polyclonal antibodies to PKC or rabbit polyclonal antibodies to vinculin. These results suggest that it is not only the absolute level of PKC but also the proteolytic activation of PKC to a Ca^{2+}-and phospholipid-independent form (PKM) which is associated with the utilization of vinculin as an endogenous substrate.

Quantitation of PKC and PKM by Western blotting using polyclonal antibodies to the consensus sequence AYQPYGKSVD in the catalytic domain of PKC has demonstrated that one of the two isoforms of PKC present in HL-60 cells is more abundant in HL-60/ADR cells and that substantially more PKM is generated in these cells.

Experimental Design and Methods:

Human promyelocytic cell line HL-60 will be obtained from the American Type Culture Collection. HL-60/ADR cells displaying multidrug resistance will be provided by Dr. Steven Grant, Medical College of Virginia. Human breast carcinoma cell line MCF-7 rendered resistant to Adriamycin or vinblastine and subcloned to generate several cell lines with varying degrees of multidrug resistance and human ovarian cell lines displaying similar characteristics of multidrug resistance, will be provided by Dr. Kenneth H. Cowan, National Cancer Institute. The conditions for cell culture have been described previously.

PKC assays and the immunoblotting and detection of PKC, PKM and vinculin will be performed as described previously. Monoclonal antibodies to vinculin will be purchased from ICN Immunochemicals. The preparation of polyclonal antibodies to vinculin and PKC have been described. Monoclonal antibodies to PKC type alpha, ß and gamma will be provided by Dr. Kuo-Ping Huang, NICHHD, NIH. The consensus peptide AYQPYGKSVD in the catalytic domain of PKC alpha, ß

and gamma will be synthesized using a Beckman amino acid synthesizer by Dr. Chien Niu, National Cancer Institute. The unique sequences, AGNKVISPSEDRRQ, GPKTPEEKTTNTIS, and LELYERVRTGPSSS, in the variable V3 region of PKC alpha, ß and gamma respectively, will also be synthesized by Dr. Chien Niu. Polyclonal antibodies will be prepared by coupling each peptide to keyhole limpet hemocyanin with glutaraldehyde and immunizing rabbits using the adjuvant MPL+TDM+CWS (Ribi Immunochem Research). The general experimental design will be similar to that outlined in ref. 1. Cell extracts will be prepared in a Triton X-100 buffer containing the protease inhibitors leupeptin, aprotinin, pepstatin and phenylmethylsulfonyl fluoride (leupeptin is omitted when Ca^{2+}-dependent protease effects are assessed). Phosphorylation assays will be performed in the presence and absence of Ca^{2+} and phosphatidylserine to quantitate PKC-dependent phosphorylation. The phosphorylated proteins will be separated by one- or two-dimensional polyacrylamide gel electrophoresis. Western blotting of the gels onto nitrocellulose will be carried out by the Towbin procedure as described (1) using the Proto Blot (Promega Biotec) procedure for the detection of the antigen. For analysis of phosphorylation _in vivo_, cells will be incubated with $H_3{}^{32}PO_4$ overnight in medium containing 0.1X phosphate. Other labeling times and pulse-chase analyses will also be used to measure the turnover of the phosphorylated proteins in question. In experiments where we wish to measure the effect of the phorbol ester, 12-_O_-tetradecanoylphorbol-13-acetate (TPA), 10 nM TPA will be added to the medium for varying periods of time. To measure PKC-dependent phosphorylation in cells labeled with $H_3{}^{32}PO_4$ _in vivo_, the inhibitor K252b will be used at varying concentrations.

Isoforms of PKC will be characterized chromatographically by DEAE Sepharose, phenyl-Sepharose, polylysine-Sepharose and hydroxyapatite chromatography as described by Huang et al.

Photoaffinity labeling of P-glycoproteins in membrane preparations of sensitive and mdr cells will be carried out by Dr. R.L. Felsted of this laboratory. Preparations will be phosphorylated _in vitro_ as described above and then analyzed for their capacity to bind the photoaffinity probes. Similar studies will be carried out following treatment of the cells with TPA for 2 days in order to modulate the level of PKC and PKM.

Publications:

1. Aquino A, Hartman KD, Knode MC, Grant S, Huang KP, Niu C-H, Glazer RI. The role of protein kinase C in the phosphorylation of vinculin in Adriamycin-resistant HL-60 leukemia cells, Cancer Res 1988; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

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PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Differentiation of Human Leukemia Cells

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

	Name	Title	Affiliation
PI:	Theodore R. Breitman	Chemist	LBC, NCI
Others:	Noriko Takahashi	Visiting Fellow	LBC, NCI
	Yuya Abe	Visiting Fellow	LBC, NCI
	He Ruyi	Guest Worker	LBC, NCI
	Cherrie Rulka	Biologist	LBC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	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.)

Recent clinical results support the possibility that induction of differentiation is an alternative approach for the treatment of some malignancies. However, a greater understanding is needed of both the process of terminal differentiation and the metabolism of known inducers. To aid in this search, studies were conducted to: a) measure changes in the levels of oncogene proteins during differentiation and to correlate changes in the levels of oncogene proteins with other changes associated with differentiation; and b) study the metabolism of known inducers of differentiation. The human myeloblastoid cell line, HL60, has been a useful model system for studying terminal differentiation. A method was developed to fix and permeabilize HL60 cells so that intracellular antigens could be detected with specific antibodies and measured with a fluorescence activated cell sorter (FACS). We examined the level of c-*myc* oncogene protein during retinoic acid- and dimethylsulfoxide-induced differentiation and found that both the rate and the extent of decreases in c-*myc* protein during differentiation are much less than what would be expected from the decreases in c-*myc* mRNA levels under similar conditions. Although many biological effects of retinoic acid have been described the mechanism for these actions is unknown. We have now discovered that in HL60, a covalent bond is formed between retinoic acid and protein. Based on sensitivity to hydrolysis with hydroxylamine, about 80% of the retinoic acid moiety is linked to protein *via* either an oxygen-ester or a thio-ester bond. Fractionation of cells labeled with radioactive retinoic acid indicates that greater than 80% of the retinoylated protein is associated with the membrane fraction.

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

Objectives

This project seeks an understanding of the process of terminal differentiation of human myeloid cells. It is now generally agreed that some leukemias, as well as other malignancies, are diseases resulting from a block in terminal differentiation. This view suggests that viable treatment may be possible with agents that induce differentiation. To aid in this search, studies are conducted to: a) better understand the mechanism(s) of terminal differentiation; b) study the metabolism of known inducers of differentiation, e.g., retinoic acid; c) screen known and newly synthesized compounds for their differentiation inducing activity either alone or in combination with differentiation-inducers.

Recent studies have been elucidating the mechanism of cell proliferation promoted by oncogenes and the relationship between oncogene expression and differentiation. Several oncogene products are related to growth factors or their receptors. Most studies on oncogene expression are based on measurements of transcriptional changes in a large population of cells. There have been relatively few studies on the translational products. Furthermore, there are relatively few studies in which oncogene proteins have been measured in individual cells as opposed to measurements on the population. Most of these later studies have been conducted with immunofluorescence microcopy. While this technique is primarily qualitative, important information has been obtained on both the percentage of cells positive for an antigen and localization of the antigen in the cell. However, it is difficult with immunofluorescent microscopy to estimate relative levels of specific antigenic proteins in individual cells and even more difficult (or at least laborious) to determine what percentage of a small population may be positive or negative. Alternative techniques to measure oncogene proteins include immunoprecipitation and Western blotting. While these techniques give fairly good quantitation they measure changes in the population and therefore changes in a subpopulation of cells can be missed.

A fluorescence activated cell sorter (FACS) can measure the level of a specific molecule in individual cells of a large population. The ability to measure oncogene proteins with this instrument allows for a detailed examination of oncogene protein expression during growth and differentiation. In addition, the development of methods to measure intracellular antigens, a necessary prerequisite of FACS analysis of intracellular oncogene protein, should also find applicability in studies on non-oncogene intracellular antigens. Because other parameters such as cell size, granularity, and other antigens can also be measured on the same cells correlations of changes in oncogene protein expression with other parameters can be made. In addition, cells of interest can be isolated for further analysis.

Although the actions of retinoic acid (RA) have been investigated by many, the mechanism for these actions is unknown. RA supports growth in animals and maintains epithelial tissues and bone, but does not function in vision and mammalian reproduction. The importance of RA is underscored by evidence that it is a potent inducer of differentiation of some cell types and that it has utility in the treatment of patients with various malignancies. _In vivo_ and in some cell types there is an isomerization equilibrium between all-trans- and

13-cis-RA and also a conversion of the water-insoluble RA to the water-soluble retinoyl glucuronide (1-O-retinoyl-ß-D-glucopyranuronic acid). Both 13-cis-RA and retinoyl glucuronide are active in some systems *in vitro*. However, there is essentially no information on reactions involving RA that are directly correlated with the effects of RA. We have speculated that RA is activated in a CoA-SH mediated reaction to form retinoyl-CoA. This high energy intermediate could then react with susceptible groups on a macromolecule *e.g.*, an hydroxy group, to form a low energy covalent ester bond. We now have preliminary results that RA is covalently linked to HL60 protein through either an oxygen-ester or a thio-ester bond. Thus, the amino acids serine, threonine, tyrosine, and cysteine may be sites for this acylation. If this acylation competes with or modulates other modifications (phosphorylation, methylation, palmitoylation) at the same or closely associated sites, it could lead to a better understanding of the mechanism of action of RA as well as throw new light on the function(s) of these other post-translational modifications. Furthermore, if retinoylation is essential for the response of a cell to RA, then a measurement of retinoylation could be the basis for a predictive test for the potential clinical utility of RA.

Methods Employed

The principal methods employed involve measurement of differentiation of human leukemia cell lines in cell culture. Most studies are conducted with the HL60 human myeloblastoid cell line. Differentiation is assessed primarily by morphology, the ability of cells to reduce nitroblue tetrazolium to a formazan, and with other cell-type specific assays. Measurement of proto-oncogene expression is by immunological techniques using flow cytometry, Western blotting, and immunoprecipitation.

Major Findings

1. Employing FACS we have obtained data showing that c-*myc* protein decreases during differentiation of HL60 at a much slower rate than expected from the c-*myc* mRNA level. Immunofluorescence microscopy of the same cells that were analyzed on the FACS confirms the relatively slow rate of decrease in c-*myc* protein and the localization of c-*myc* protein in the nucleus. c-*myc* Protein is lost from the cells after treatment with DNase but not after treatment with RNase. These results are consistent with the reported DNA-binding properties of c-*myc* protein.

2. We now have evidence that a covalent bond is formed between RA and proteins in growing HL60 cells. The extent of the retinoylation of HL60 protein is dependent on both time and RA concentration. Radioactive RA is released from labeled HL60 cells in a trichloroacetic acid soluble form after digestion with proteinase K and hydrolysis with hydroxylamine under mild conditions. These results are consistent with retinoylation of protein with the formation of thio- or oxygen-ester bonds.

Proposed Course

1. We are presently attempting to further support the findings with FACS by measuring c-*myc* protein by immunoprecipitation and Western blot techniques.

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Proposed Course

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Results from these techniques will allow us: to confirm if c-myc protein is being measured; do pulse and pulse-chase experiments to measure synthesis and degradation of c-myc protein; and by using site-specific antibodies be prepared to make some judgments regarding portions of the c-myc protein that may still be in the cell.

2. In the retinoylation subproject we will: a) examine the subcellular distribution of retinoylated proteins; and b) run two-dimensional PAGE to determine how many proteins are retinoylated. Studies on retinoylation in intact cells, the subcellular distribution, and two-dimensional PAGE will be conducted on cells grown in the presence of various concentrations of RA (from the physiologic concentration of 10 nM to the pharmacologic concentration of 1 M). It is possible that the subcellular distribution and the two-dimensional PAGE pattern will be RA dose-dependent. Other studies will examine changes in subcellular distribution and two-dimensional PAGE patterns as a function of time.

3. Another aspect of this project is to identify the site(s) of retinoylation. Based on the chemical stability to hydroxylamine it is likely that RA is linked via an ester bond to either serine, cysteine, threonine, or tyrosine. An approach to identify the retinoylated-amino acid is to grow cells in the presence of ^{3}H-RA and ^{14}C-amino acid, digest the protein to free amino acids, and separate the amino acids by one of several techniques. Comigration of the two labels in more than one separation technique should give a strong indication of the amino acid that is retinoylated. Furthermore, excision of the radioactive area, treatment with hydroxylamine, and rechromatography should result in radioactive spots corresponding to the free amino acid and the hydroxamate of RA.

Publications

1. Breitman TR. Retinoic acid-induced differentiation of HL-60: Studies in vitro and in vivo. In: Aarbakke J, Chiang PK, Koeffler HP, eds. Tumor Cell Differentiation. Clifton NJ: The Humana Press, 1987:159-81.

2. Breitman TR. The role of prostaglandins and other arachidonic acid metabolites in the differentiation of HL-60. In: Garaci E, Paoletti R, Santoro MG. eds. Prostaglandins in Cancer Research. Berlin, Heidelberg, Springer-Verlag, 1987;161-71.

3. Spruce LW, Rajadhyaksha SN, Berlin KD, Gale JB, Miranda ET, Ford WT, Blossey EC, Verma AK, Hossain MB, van der Helm D, Breitman TR. Heteroarotinoids. Synthesis, characterization, and biological activity in terms of an assessment of these systems to inhibit the induction of ornithine decarboxylase activity and to induce terminal differentiation of HL-60 cells, J Med Chem 1987;30:1474-82.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Laboratory of Biochemical Pharmacology was established in January, 1986, by the division of the former Laboratory of Pharmacology and Experimental Therapeutics into two components which are concerned respectively with the design and synthesis of antitumor drugs (Laboratory of Medicinal Chemistry) and the mode of action of new antitumor drugs (Laboratory of Biochemical Pharmacology). The Laboratory studies new agents which have originated within the Developmental Therapeutics Program and also agents derived from extramural sources in whose preclinical development the Program is playing a major role. Over the past two years, the Laboratory has also participated actively in elucidation of the cellular pharmacology of compounds with anti-HIV activity, currently under development within the Program.

Further significant progress has been made on the elucidation of the cellular and molecular pharmacology of anti-HIV drugs of the 2′,3′-dideoxynucleoside type. A complete study of seven compounds of this class has allowed the development of a general hypothesis to explain the extremely wide variation (three orders of magnitude) in the antiretroviral activity of this group, with ddCyd (and 5-fluoroddCyd) to date being the most active and ddThd being the least active in the series examined. The most important factor appears to be the susceptibility of the individual ddN's to phosphorylation to the 5′-triphosphate level, with other factors such as affinity of the ddNTPs for HIV reverse transcriptase and the ability of the nucleotides to chain-terminate viral DNA, while essential for activity, appear to be of much lesser importance in determining potency. It should be noted however, that most of the compounds are similar in therapeutic index, although AZT and ddCyd are less favorable in this respect than some of the other nucleosides: an increase in retroviral potency within this class is also accompanied by a parallel increase in cytotoxicity toward host cells (e.g. T-cells and monocytes/macrophages).

Substantial further progress has been made on the difficult technical problem of the measurement of intracellular levels of ^{3}H-ddATP seen after exposure of human T-cells to ^{3}H-ddAdo. It has now been definitely established that ddAdo and ddIno both lead to the same anabolic end-product, ddATP, a result of considerable practical importance in view of the Phase I/II clinical trials of ddAdo and ddIno. In another area of ddAdo pharmacology we have made the empirical observation that the formation of ddATP in T-cells is enhanced several-fold by the co-administration of the deoxynucleosides 2′-deoxyadenosine and 2′-deoxyinosine, and also by low levels of the adenosine deaminase inhibitors 2′-deoxycoformycin and EHNA. The practical significance of these observations in the clinical use of ddAdo is still not established: it is felt, however, that these studies are of potential value because of the numerous structurally related compounds under consideration for clinical development (e.g., 2′-fluorodideoxy-adenosine, the carbocyclic ene analogue of ddAdo and the acyclic analogue adenallene) of all of which ddAdo can be considered a prototype, and to which the methodology developed for ddAdo can be transferred with little modification.

Studies continued on the biochemistry and pharmacology of tubulin, a protein critical for cell division which is the site of action of many antitumor agents. Copolymerization of tubulin-GDP and tubulin-GTP was examined to define their relative efficiencies in microtubule elongation and the minimum concentration of tubulin-GTP required for initiation of microtubule assembly. Nucleotide concentration and composition markedly affects microtubule stability, and possible explanations for these properties were evaluated. Work continued on developing methods to separate the tubulin subunits preparatively, and on reconstitution of active tubulin following denatuation. Purification continued on two microtubule-associated proteins, One causes the formation of massive bundles of microtubules, the other the degradation of GDP to GMP. Work to determine the location of the disulfide bridges in tubulin and to introduce antimitotic nucleotide analogs into cells began.

Structure-activity studies with the potent antimitotic drugs derived from the South African tree Combretum caffrum were completed, defining two agents (combretastatin A-2 and combretastatin A-4) as exceptionally promising drugs based on excellent cytotoxicity, potent antitubulin activity in vitro, and retained activity against multidrug resistant cell lines. Work was initiated with highly cytotoxic peptides derived from a marine organism, Dolabella auricularia, which potently inhibit microtubule assembly. The mechanism of action of 2,4-dichlorobenzyl thiocynate was defined, for it specifically alkylates cysteine 239 of β-tubulin. Collaborative efforts to produce potent, simplified colchicine analogs, 6-benzyl-1,3-benzodioxole derivatives, and 5,6-diphenylpyridazin-3-one derivatives continued.

In conjunction with its research output in fields related to the mode of action of new antitumor drugs, the Laboratory of Biochemical Pharmacology continued its active publication record in 1987-88. A total of 24 papers describing these and related studies appeared or were accepted for publication during the current year; these publications are listed in the following section of this report.

Publications:

1. Ahluwalia G, Cooney DA, Mitsuya H, Fridland A, Flora KP, Hao Z, Dalal M, Broder S, Johns DG. Initial studies on the cellular pharmacology of 2′,3′-dideoxyinosine, an inhibitor of HIV infectivity. Biochem Pharmacol 1987;36:3797-3800.

2. Ahluwalia GS, Jayaram HN, Cooney DA. Metabolites of tiazofurin as mediators of its biochemical and pharmacologic effects. In: Muggia F, ed. Concepts, clinical developments, and therapeutic advances in cancer chemotherapy. Boston: Martinus Nijhoff, 1987.

3. Ahmad S, Okine L, Wood R, Aljian J, Vistica DT. γ-Glutamyl Transpeptidase (γ-GT) and maintenance of thiol pools in tumor cells resistant to alkylating agents. J Cellular Physiol 1987;131:240-246.

4. Balzarini J, Cooney DA, Dalal M, Kang GJ, Cupp JE, DeClerq E, Broder S, Johns DG. 2′,3′-dideoxycytidine: Regulation of its metabolism and antiretroviral potency by natural pyrimidine nucleosides and by inhibitors of pyrimidine nucleotide synthesis. Molec Pharmacol 1987;32:798-806.

5. Balzarini J, Kang GJ, Dalal M, Herdewijn P, DeClercq E, Broder S, Johns DG. The anti-HIV-III (Anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides: A comparison with their parental 2′,3′-dideoxyribonucleosides. Molec Pharmacol 1987;32:162-167.

6. Balzarini J, Pauwels R, Baba M, Herdewijn P, De Clercq E, Broder S, Johns DG. The in vitro and in vivo anti-retrovirus activity, and intracellular metabolism of 3′-azido-2′,3′-dideoxythymidine and 2′,3′-dideoxycytidine are highly dependent on the cell species. Biochem Pharmacol 1988; 37:897-903.

7. Batra JK, Lin CM, Hamel E. Nucleotide-interconversions in microtubule protien preparations, a significant complication for the accurate measurement of GTP hydrolysis in the presence of adenosine 5′-[β,γ-imido]-triphosphate. Biochemistry 1987;26:5925-5931.

8. Brossi A, Yeh HJC, Chrzayowska M, Wolff J, Hamel E, Lin CM, Quinn F, Suttress M, Silverton J. Colchicine and its analogues: recent findings. Medic Res Rev 1988;8:77-94.

9. Cooney DA, Ahluwalia G, Mitsuya H, Fridland A, Johnson M, Hao Z, Dalal M, Balzarini J, Broder S, Johns DG. Initial studies on the cellular pharmacology of 2′,3′-dideoxyadenosine, an inhibitor of HTLV-III infectivity. Biochem Pharmacol 1987;36:1765-1768.

10. Cooney DA, Hamel E, Cohen M, Kang GJ, Dalal M, Marquez V. A simple method for the rapid determination of the stereospecificity of NAD-dependent dehydrogenases applied to mammalian IMP dehydrogenase and bacterial NADH peroxidase. Biochimica et Biophysica Acta 1987;916:89-93.

11. Glover A, Chun HG, Kleinman LM, Cooney DA, Plowman J, Grieshaber CK, Malspeis L, Leyland-Jones B. Merbarone: An antitumor agent entering clinical trials. Invest New Drugs 1987;5:137-143.

12. Johnson MA, Johns DG, Fridland A. 2′,3′-dideoxynucleoside phosphorylation by deoxycytidine kinase from normal human thymus extracts: activation of potential drugs for AIDS therapy. Biochem Biophys Res Commun 1987;148:1252-1258.

13. Kelley JA, Litterest CL, Vistica DT, Poplack DG, Cooney DA, Nadkarni M, Balis FM, Broder S, Johns DG. The disposition and metabolism of 2′,3′-dideoxycytidine, an in vitro inhibitor of human T-lymphotrophic virus type III infectivity in mice and monkeys. Drug Metab Disp 1987; 15:595-601.

14. Knight RD, Mangum J, Lucas DL, Cooney DA, Khan EC, Wright DG. Inosine monophosphate dehydrogenase and myeloid cell maturation. Blood 1987;69:634-639.

15. Lin CM, Hamel E. Interrelationships of tubulin-GDP and tubulin-GTP in microtubule assembly. Biochemistry 1987;26:7173-7182.

16. Batra JK, Kang GJ, Jurd L, Hamel E. Methylenedioxy-benzopyran analogs of podophyllotoxin, a new synthetic class of antimitotic agents which inhibit tubulin polymerization. Biochem Pharmacol, in press.

17. Bender RA, Hande KR, Hamel E. The plant alkaloids. In: Chabner B, ed. Pharmacologic Principles of Cancer Treatment, 2nd ed, Philadelphia: W B Saunders Co, in press.

18. Hamel E. Interactions of tubulin with small ligands. In: Avila J, ed. Microtube Proteins. Boca Raton, FL: CRC Press, in press.

19. Hamel E, Ho HH, Kang GJ, Lin CM. Carnigerine, a potent antimitotic _Colchicum_ alkaloid of unusual structure: interactions with tubulin. Biochem Pharmacol, in press.

20. Hao Z, Cooney DA, Hartman NR, Perno CF, Fridland A, DeVico Al, Sarngaharan MG, Broder S, Johns DG. Factors determining the anti-HIV activity of 2′,3′-dideoxynucleotides. Molec Pharmacol 1988, in press.

21. Johnson, MA, Ahluwalia G, Connelly MC, Cooney DA, Broder S, Johns DG, Fridland A. Three metabolic pathways for the activation of the antiretroviral agent, 2′,3′-dideoxyadenosine in human lymphoid cells. J Biol Chem 1988, in press.

22. Lin CM, Singh SB, Chu PS, Dempcy RO, Schmidt JM, Pettit GR, Hamel E. Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin, a structure-activity study. Mol Pharmacol, in press.

23. Perno C, Yarchoan R, Cooney DA, Hartman NR, Gartner S, Popovic M, Hao Z, Gerrard TL, Wilson YA, Johns DG, Broder S. Inhibition of human immunodeficiency Virus (HIV-I/HTLV-III_{Ba-L}) replication in fresh an cultured human peripheral blood monocyte/macrophages by AZT and related 2′,3′-dideoxynucleosides. J Exp Med, in press.

24. Pettit GR, Singh SB, Schmidt JM, Niven ML, Hamel E, Lin CM. Isolation structure, sysnthesis and antimitotic properties of combretastatins B-3 and B-4 from _Combretum caffrum_ (combretaceae). J Nat Prod, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07102-13 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Tubulin as a Site for Pharmacologic Attack

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

PI:	E. Hamel	Senior Investigator	LBP, NCI
OTHERS:	R.-L. Bai	Visiting Fellow	LBP, NCI
	Z. Getahun	Visiting Fellow	LBP, NCI
	G. J. Kang	Visiting Fellow	LBP, NCI
	C. M. Lin	Biologist	LBP, NCI

COOPERATING UNITS (if any)

1) G.R. Pettit, Arizona State University; 2) L. Jurd, Dept. of Agriculture; 3) L.J. Powers, Ricerca Corp., Painesville, OH; 4) A. Brossi, NIDDK; 5) M.G. Banwell, Univ. of Melbourne, Australia; 6) Dr. N.Y. Nyugen, FDA.

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	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.)

The goal of this project is the development of new antineoplastic agents directed against tubulin, a protein critical for cell division. Work was continued with combretastatin congeners, a series of newly isolated natural products, more active than combretastatin itself. Several members of the series are among the most potent microtubule inhibitors yet described. The mechanism of action of 2,4-dichlorobenzyl thiocyanate was examined. The drug specifically alkylates the β-tubulin polypeptide. Alkylated β-tubulin was cleaved with cyanogen bromide and the modified peptide purified and sequenced. The target amino acid for the drug was cysteine 239. Derivatives of 6-benzyl-1,3-benzodioxole continued to interest the laboratory because their facile synthesis permits a structure-function approach to the colchicine/podophyllotoxin binding site of tubulin. Derivatives of 5,6-diphenylpyridazin-3-one, which bind to a distinct site on tubulin (i.e., no competition with other drugs), continued to be evaluated in a search for maximally active agents. Alkyl carbamates of aromatic amines continued to be examined in a search for new anti-tubulin agents. Colchicine analogs with unusual structural features and/or biological properties were evaluated to quantitate their interactions with tubulin. A new class of peptide antimitotics was identified and description of their interaction with tubulin was initiated.

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

Microtubules, cellular organelles critical for cell division, are sensitive to a number of antineoplastic drugs. These drugs all cause cells to accumulate in metaphase, disrupting mitosis, for microtubules from the mitotic spindle. The major constituent of microtubules is an acidic protein known as tubulin, and it is the cellular target of virtually all antimitotic drugs. New antimitotic agents continue to be an active area of interest in the laboratory. We are currently studying the following classes of drugs:

1) Analogs of combretastatin. Combretastatin (NSC 348103) is a natural product isolated by G.R. Pettit of Arizona State University from the South African tree Combretum caffrum; and we have demonstrated that combretastatin is a potent inhibitor of tubulin polymerization and binds at the colchicine site of tubulin. Dr. Pettit's group has now purified and characterized about 20 additional compounds from Combretum caffrum and chemically synthesized an additional 40 compounds of related structure. Several analogs of combretastatin were also synthesized in our laboratory. At least six of these agents are considerably more potent than combretastatin itself as tubulin inhibitors. A detailed structure-activity study characterizing these drugs was performed.

2) The compound 2,4-dichlorobenzyl thiocyanate (NSC 145813) was previously shown to inhibit mitosis in murine leukemia cells and tubulin polymerization in vitro, and, in collaboration with other investigators, we demonstrated that certain lines of Chinese hamster ovary cells with a mutant ß-tubulin gene were resistant to NSC 145813.

Radiolabeled NSC 145813 was prepared and found to form covalent bonds with tubulin, particularly with ß-tubulin. Several lines of evidence demonstrate that NSC 145813 reacts with cysteine residues, forming disulfide bonds with the protein. No other class of antimitotic drug seems to interfere specifically with the interaction of NSC 145813 with tubulin. Radiolabeled DCBT was used to alkylate ß-tubulin. This was degraded with cyanogen bromide and the radiolabeled peptide purified by high-performance liquid chromatography. The peptide was sequenced and the modified amino acid was identified as cysteine 239 (in collaboration with Dr. N. Y. Nyugen).

3) A large number of derivatives of 6-benzyl-1,3-benzodioxole have been prepared by Dr. L. Jurd of the Department of Agriculture as potential insect sterilants. A significant number of these compounds have antineoplastic activity and inhibit tubulin polymerization. They are most analogous to podophyllotoxin structurally and, like podophyllotoxin, inhibit both tubulin-dependent GTP hydrolysis and the binding of colchicine to tubulin. Initial studies established minimal structural requirements for the simplest benzylbenzodioxole derivatives (a 1-3 carbon substituent at position 5, and a methoxy group at position 4′ in the benzene ring) which have either an unsubstituted one carbon bridge between the benzene and benzodioxole rings or one or two methyl groups at this bridge position. Additional methoxy substituents on the benzene ring at the 3′ and 5′ positions, which seeminglyincrease the structural analog to podophyllotoxin, resulted in almost complete loss of activity.

Dr. Jurd has also prepared a group of compounds with a third ring (of variable structure) fused to the benzodioxole moiety. The benzene ring is attached directly to this third ring. Several of these agents have strong antitubulin activity, and all of the compounds active against tubulin in vitro also cause mitotic arrest. All compounds have three methoxy groups, attached at positions 3′, 4′ and 5′, of the benzene ring. Analogs with a different methoxy substituent pattern have reduced activity. Although both the third fused ring and the trimethoxy structure appear to substantially increase their analogy to podophyllotoxin, these new agents are more comparable to colchicine in their effects on tubulin-dependent GTP hydrolysis; for, like colchicine, they stimulate rather than inhibit this reaction even while inhibiting the microtubule assembly reaction normally coupled to GTP hydrolysis.

4) Dr. L. J. Powers of Ricerca Corporation has prepared numerous derivatives of 5,6-diphenylpyridazin-3-one as potential antihypertensive agents. Some of these compounds were found to be potent herbicides as a consequence of inhibition of mitosis in plant tissues. Several members of this class were then submitted to the NCI for screening, and some of these were found to have antineoplastic activity. We have found that a number of these drugs inhibit mitosis in mammalian cells in culture and the polymerization of tubulin in vitro. They potently stimulate tubulin-dependent GTP hydrolysis; but they probably bind at a previously undescribed site on tubulin, for they do not inhibit the binding of either colchicine, vinblastine, maytansine or GTP to the protein. Active compounds possess a nitrile group at position 4; and in vitro interactions with tubulin are significantly enhanced by chloride substituents on the phenyl rings, both of which are required for antitubulin activity. There is little overlap between compounds most active against mammalian tubulin and those which are most active in inhibiting mitosis in plant cells. In collaboration with Dr. Powers we are continuing to study structure-activity correlations in this class of drugs to develop maximally active agents. Two active compounds have been prepared in a radiolabeled form, one with the label in the phenyl rings, the other with the radiolabel in the pyridazinone ring. Thus far we have been unable to demonstrate binding of either radiolabeled drug to tubulin.

5) A number of compounds with very different structures have been found to have antineoplastic and antimitotic properties and to inhibit tubulin polymerization. Their only common feature is that they are alkyl carbamates of aromatic amines. A computer search of the NCI drug collection produced over 140 compounds with promising structural features. These were screened for effects on tubulin-dependent GTP hydrolysis, and over fifty compounds were positive. These in turn were examined for effects on tubulin polymerization, and about a dozen drugs had significant inhibitory activity. These agents were studied in further detail.

6) Dr. A Brossi of the NIDDK has isolated and synthesized a large number of analogs of the classic microtubule inhibitor colchicine. We have undertaken a collaboration with Dr. Brossi to quantitate more precisely interactions of these analogs with tubulin to provide structure-activity insights into a number of the unique characteristics of the colchicine-tubulin interaction (e.g., temperature-dependent, relatively slow, and irreversible binding of the drug to the protein).

7) Dr. M.G. Banwell of the University of Melbourne synthesized two chlorinated derivatives of the model colchicine analog 2-methoxy-5-(2′,3′,4′-trimethoxyphenyl)-tropone. We found one chlorinated derivative had enhanced activity, and the other greatly reduced activity.

8) Dr. Pettit has isolated a series of novel cytotoxic peptides from the marine animal Dolabella auricularia. Initial experiments have demonstrated that some of these are potent microtubule inhibitors.

PUBLICATIONS

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5. Lin CM, Singh SB, Chu PS, Dempcy RO, Schmidt JM, Pettit GR, Hamel E. Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin, a structure-activity study, Mol Pharmacol, in press.

6. Bender RA, Hande KR, Hamel E. The plant alkaloids. In: Chabner B, ed. Pharmacologic Principles of Cancer Treatment, 2nd ed, Philadelphia: W B Saunders Co, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07179-03 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Protein-protein and Protein-nucleotide Interactions in Microtubule Assembly

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

PI:	E. Hamel	Senior Investigator	LBP, NCI
OTHERS:	Z. Getahun	Visiting Fellow	LBP, NCI
	G. J. Kang	Visiting Fellow	LBP, NCI
	C. M. Lin	Biologist	LBP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	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.)*

The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interaction between the polypeptide subunits of tubulin, its two tightly bound guanine nucleotides, and microtubule-associated proteins. Copolymerization of tubulin GDP and tubulin GTP was studied in further detail, to determine the relative efficiencies with which the two species entered elongating microtubules and to define the minimum concentration of tubulin-GTP required to initiate microtubule assembly. The effects of nucleotides on the stability of microtubules continued to be examined, as were conditions to optimize the separation of α-tubulin and β-tubulin on a preparative scale. The purification of a microtubule-associated protein which causes the formation of microtubule bundles continued to progress. Another microtubule-associated protein, which specifically degrades GDP to GMP, was partially purified. Projects to define the cystine bridges in tubulin and to introduce potentially antimitotic nucleotide analogs into cells were initiated.

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

Microtubules, cellular organelles critical for cell division, are sensitive to a number of antineoplastic drugs. Their major constituent is an acidic protein known as tubulin, which consists of two different polypeptide chains and two molar equivalents of guanine nucleotide. Half this nucleotide (the exchangeable nucleotide) is in the form of either GTP or GDP. If GTP, it is hydrolyzed to GDP during microtubule assembly from tubulin and microtubule-associated proteins (MAPs -- minor, but essential, components of the microtubule). The remainder of the nucleotide exists only as GTP (the nonexchangeable nucleotide). This GTP is not altered during tubulin polymerization and can only be removed from tubulin by destroying the protein. Its function is unknown.

We have continued our studies on nucleotide interactions at the exchangeable site, with particular emphasis on reaction parameters previously shown to alter the ratio in which tubulin bearing GDP in the exchangeable site copolymerized with tubulin bearing GTP. These are all reaction components which increase the relative amounts of tubulin-GDP at the expense of tubulin GTP--low GTP concentrations if the tubulin concentration is fixed; high tubulin concentrations if the GTP concentration is fixed; GDP in the reaction mixture from any source; low magnesium concentrations.

The tubulin GDP-tubulin-GTP equilibrium was examined under multiple reaction conditions, as a function of tubulin, GDP, GTP, and Mg^{2+} concentrations, to determine the relative deficiencies with which the two species entered elongating microtubules and to define the minimum concentration of tubulin GTP required for the initiation of microtubule assembly. The key parameter was found to be Mg^{2+}. Although the cation is required for the efficient binding of GTP (but not GDP) to tubulin, in its absence assembly could be initiated if as little as 20-25% of the tubulin was in the form of tubulin-GTP. In the absence of Mg^{2+}, however, tubulin-GDP was only half as efficient as tubulin GTP in entering elongating microtubules. At the highest Mg^{2+} concentration examined (4 mM), 80% of the tubulin had to be in the form of tubulin-GTP for initiation, but tubulin GDP was as efficient as tubulin-GTP in elongation. Quantitative analysis of Mg^{2+} effects on nucleotide exchange was initiated.

We have continued to study the stability of microtubules as a function of their nucleotide content and environment. In particular, we are trying to determine whether stability is affected by the proportion of microtubule nucleotide which has resulted from hydrolysis of GTP to GDP as opposed to the proportion of GDP incorporated directly into the polymer. A related issue is the observation that microtubule integrity requires some GTP in the reaction mixture, for microtubules rapidly disintegrate if GTP is totally degraded by transfer of the terminal phosphate to frucose-6-phosphate by phosphofructokinase. Although we have established that nonexchangeable GTP is not destroyed in this reaction, we have yet to localize unambiguously the essential triphosphate.

We plan to exploit the known ability of guanosine 5′-[α-β-methylene]triphosphate to disrupt microtubule organization when injected into cells and the similar in vitro behavior of this analog and 2′,3′-dideoxyguanosine 5′-triphosphate with tubulin. We are attempting to design

antimitotic GTP analogs able to penetrate cells. We plan the synthesis of analogs with reduced phosphate change and hydrophobic modifications at position 8 of the guanine ring. These will contain the dideoxyribose and/or α-β methylene modifications.

For many years we have been attempting to reproducibly and preparatively separate the two subunits of tubulin. Although we had achieved significant separation by hydrophobic chromatography, reproducibility has been a problem. Continued efforts in the past year have not been successful. One goal of this project is reconstitution of activity from the separated subunits plus small ligands (i.e., GTP and/or GDP and Mg^{2+}). Even in the absence of a totally successful separation of subunits, we have begun to search for conditions to reactivate denatured tubulin, using as a starting point previously determined reaction conditions in which tubulin is optimally stable.

We are utilizing the ability to separate the α- and β-tubulin subunits to locate the protein's cystine disulfide bridges. Our strategy is to alkylate with nonradiolabeled sulfhydryl alkylating reagent, reduce, and alkylate with radiolabeled reagent. The protein will then be degraded with cyanogen bromide and the radiolabeled peptides purified and sequenced.

We are continuing to devote a great deal of attention to MAPs. We are particularly interested in a MAP which causes the formation of microtubule bundles (distinct microtubules which aggregate laterally). The active component (termed MAP-TB) appears to be present in MAP preparations in extremely small amounts. Although it is highly stable, it has proven more difficult to purify than anticipate. Despite DEAE-cellulose chromatography, ammonium sulfate fractionation, heat-treatment, hydroxyapatite chromatography, and HPLC chromatography (ion-exchange and gel filtration), the purest preparations remain disappointingly heterogeneous on polyacrylamide gel electrophoresis. Tubulin affinity chromatography may be useful in its purification.

In the course of preparing MAP-TB, because of the limited amounts obtained from microtubule protein, we have prepared large amounts of starting material. This includes most of the other MAPs present in the microtubules, and in processing this material we have developed superior methods of resolving some of the other MAPs components by DEAE-cellulose chromatography. In analyzing the properties of different MAPs fractions, we observed a peak of GDPase activity. This enzyme generates GMP and inorganic phosphate from GDP, and it also digests UDP, but not CDP or ADP. The presence of this enzyme in microtubule protein is probably significant--GDP is an inhibitor of microtubule assembly, and it would be desirable to be able to rapidly eliminate it. In addition, an enzyme capable of specifically eliminating GDP from reaction mixtures should be a valuable tool for studying tubulin-nucleotide interactions, in particular in the studies of microtubule stability described above.

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2. Lin CM, Hamel E. Interrelationships of tubulin GDP and tubulin GTP in microtubule assembly, Biochemistry 1987;26:7173-82.

3. Hamel E. Interactions of tubulin with small ligands. In: Avila J, ed. Microtubule Proteins. Boca Raton,, FL: CRC Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07181-03

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Antiretroviral Activity of Dideoxynucleosides

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

	Name	Title	Affiliation
P.I.:	D. Cooney	Senior Investigator	LBP, NCI
Others:	Z. Hao	Visiting Scientist	LBP, NCI
	Y. Wilson	Chemist	LBP, NCI
	N. Hartman	Staff Fellow	LBP, NCI
	R. Masood	Visiting Fellow	LBP, NCI

COOPERATING UNITS (*if any*)

C. Perno and R. Yarchoan, COP, DCT, NCI, A. Fridland, St. Jude Childrens' Research Hospital, Memphis, TN and P.A. Weinhold, Univ. of Michigan.

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

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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.*)

Dideoxycytidine (ddC) and dideoxyadenosine are undergoing trials in patients with AIDS. Studies on the metabolism of these and allied dideoxynucleosides in human cells have continued. Although dideoxycytidine undergoes a metabolic fate which is principally anabolic, the half-life of its most important anabolite, the 5'-triphosphate, is comparatively brief, approximating 6 hours in Molt-4 and CEM lymphoblasts. By contrast, the metabolic fate of ddA is principally catabolic, but its 5'-triphosphate decays with a $t_{\frac{1}{2}}$ well in excess of 24 hours. Two potentially important metabolites of these agents have been identified. In addition to the phosphate esters and diesters reported previously, we have found that ddC is also converted to a molecule with all the properties of dideoxycytidine diphosphoethanolamine. Dideoxyadenosine, after deamination, is phosphorylyzed to yield hypoxanthine and the extremely labile dideoxy sugar, dideoxyribose-1-phosphate. Since it has been shown that this transitory catabolite can be utilized by other nucleoside phosphorylases (acting in the preferred, synthetic direction), the operation of this reaction offers the potential of allowing the interconversion of a comparatively inert agent (such as dideoxythymidine EC_{50}: 100 μM) to a potent species such as ddI (EC_{50}: 5 μM) or vice versa.

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

1. Cellular Pharmacologic Studies of Dideoxycytidine and Dideoxyadenosine

Since the last annual report, we have continued our studies on the cellular pharmacology of dideoxycytidine (ddC) and dideoxyadenosine (ddA) using cultured human lymphoblasts as our model system. Both of these drugs are undergoing Phase I trials against AIDS, at NIH and elsewhere, but, for reasons of safety are being administered at comparatively low doses, which in turn generate mean plasma concentrations of dideoxynucleoside in the low micromolar or submicromolar range. It therefore became relevant to determine whether exposure to these clinically achievable levels of ddC and ddA would permit the accumulation of concentrations of the proximate antiretroviral species - their 5′-triphosphates - adequate, on the basis of kinetic considerations, to inhibit the reverse transcriptase of HIV. Since patients with AIDS are lymphopenic as a general rule, these measurements are, at present, technically impractical in vivo. It is for this reason that we have resorted to cultured lymphoblasts for the majority of the studies being recapitulated here.

When human Molt-4 cells are incubated with 0.5 μM dideoxycytidine it takes between six and ten hours for the cells to achieve their half-maximal concentration of ddCTP. When ddC is removed from the medium and the cells washed, preformed ddCTP disappears with a half-life of approximately 6 hours. Thus, with this dideoxynucleoside, the rates of build-up and decay of the final antimetabolite are roughly comparable. (It is relevant to point out that the pattern of decay seen in our Molt-4 cells appears to be strictly monoexponential; other workers, however, have reported biexponential kinetics and attributed this biphasic pattern to progressive undersaturation of the enzymes which hydrolyze or utilize ddCTP). The clinical implications of these cellular studies are obvious: because ddCTP builds up rather rapidly in the presence of its precursor but decays equally rapidly in its absence, it would seem to be desirable to maintain a finite extracellular level of the drug at all times if sustained inhibition of the retroviral polymerase is to be achieved. The exact value of this finite concentration has not been determined, but it is known that 0.5 μM ddC (the EC_{50} in the ATH-8 system) will generate levels of ddCTP roughly - after a 24 hour exposure - ten times the K_i of this nucleotide for the HIV reverse transcriptase. Since, in kinetic circles, 10 x the K_i is predicted to produce nearly total inhibition of a target enzyme in competitive cases, the maintainance of this concentration of ddC would appear to be a clinical desideratum.

The cellular pharmacology of dideoxyadenosine is quite different that of ddC. When CEM cells are incubated with 5 μM ddA (its EC_{50} versus the HIV), the rate of generation of ddATP is surprisingly rapid, such that half-maximal levels are reached within 30 minutes, and maximal levels after 3 hours of incubation (these levels are, nevertheless only 20% of those reached with ddC as precursor, despite the fact that the ddA concentration is ten times that of ddC). After approximately 4 hours, the ddATP concentration begins a slow decline, despite the fact that the drug has not been removed from the medium. This again is at variance with the kinetics of ddCTP accumulation - a process which continues in an incremental way, throughout 24 hours of exposure to the drug. Surprisingly, when, after 24 hours of incubation, the CEM cells are washed and resuspended in fresh medium, the decline in ddATP continues at an identical rate, such that the half-life approximates 24 hours, a value 4 times longer than that of ddCTP. If

this kinetic behavior can be extrapolated to the clinic, it would seem likely that brief exposures (say 1 hr) to comparatively high concentrations of ddA (in the vicinity of 5 μM) would suffice to generate a long-lasting pool of ddATP at a level well in excess of the K_i of this nucleotide for the HIV reverse transcriptase. Indeed on the basis of these data, twice daily 60-90 minute infusions have been chosen for the initial Phase I trials with this dideoxynucleoside.

2. Studies On the Metabolism of Dideoxycytidine and Dideoxyadenosine

In last year's annual report, the conversion of dideoxycytidine into an analog of CDP-choline (namely ddCDP-choline) was documented in some detail.

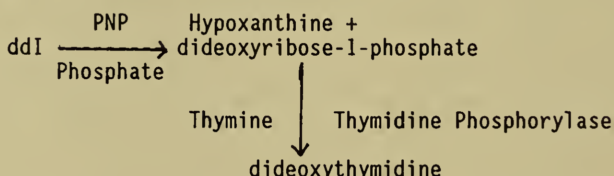
The identicication of this anabolite was considered to be provisional, however, until such time as it could be compared to an authentic chemically synthesized standard. Such a standard has now been prepared by Dr. David C. Baker of the University of Alabama: in four fundamentally different chromatographic systems (paper, ion exchange HPLC, ion-pairing HPLC, and reverse phase HPLC) this standard exactly coelutes with tritiated ddCDP choline prepared metabolically (in Molt-4 cells) or enzymatically (via choline phosphate/cytidylate transferase from rat liver). It is concluded that the provisional identification is correct.

In the past year, a second phosphodiester metabolite of dideoxycytidine has also been identified - as yet provisionally,i.e., ddCDP ethanolamine. On ion exchange HPLC on Partisil SAX, (used almost exclusively in our earliest experiments with ddC), this anabolite elutes along with ddCMP and so was overlooked. However, on reverse phase HPLC, using C-18 columns, an alkaline-phosphatase (AP) resistant metabolite is consistently observed to elute before, but fully separated form ddCDP choline; venom phosphodiesterase (VPDE) quantitatively decomposes this material, yielding ddCMP as the sole radioactive product. When cells are incubated with tritiated ddC and [^{14}C] ethanolamine, and the cellular extracts analyzed by a combined reverse-phase/ion-exchange system (courtesy of Ms C. Chisena in Dr. R. Cysysk's laboratory) an early eluting labeled diester is seen, widely separated from ddCDP choline. Dr. D. Baker has just completed the synthesis of a small sample of ddCDP ethanolamine. It will be the task of the next fiscal year to determine if the metabolite provisionally identified here, will correspond in all respects to this standard. Also projected, in collaboration with Dr. Douglas Feldman, are studies on the possibly deleterious interaction of these 'liponucleotides' of ddC with phospholipid metabolism. It is not impossible that such an interaction - if verified - could play a contributory role in the pathogenesis of the toxic (neurologic) side effects of dideoxycytidine.

In the last year, too, at the instance of Dr. Samuel Broder, we have again examined the possibility that the dideoxyribofuranosyl moiety of the antiretroviral dideoxynucleosides might be susceptible to metabolic neutralization. This could, in theory, be accomplished in several ways. After phosphorolysis, by a purine or (less likely) pyrimidine nucleoside phosphorylase, the resultant dideoxyribose-1-phosphate could act as a substrate for a second, different phosphorylase, operating in the synthetic direction (which is the favored route, from a thermodynamic standpoint).

In this way, at least in theory, a purine dideoxynucleoside could be converted into a pyrimidine dideoxynucleoside, a fate which could be envisioned as having therapeutic ramifications. It might also happen that dideoxyribose-1-phosphate would be converted to dideoxyribose-5'phosphate via a kind of phosphoglucomutase reaction, with the subsequent addition of pyrophosphate to the 1-hydroxyl, via the action of PRPP synthetase. The resultant dideoxy PRPP could in turn serve to activate a variety of purine or pyrimdine bases, in a typical phosphoribosyl transferase reaction. We have yet to examine this latter, more complicated case, in detail, but will now report on experiments which serve to establish the operation of the simpler 'direct phosphorylytic model.' In order to proceed with tests of this model, it was necessary first to verify that dideoxyribose-1-phosphate could, in fact, be generated from any of the 2'3'-dideoxynucleosides. Our earlier attempts to detect this cleavage product had failed, (in retrospect probably because of the acidic pH which prevailed during our chromatographies). When, however, we reverted to a simple, speedy and neutral system - namely ascending paper chromatography - we were successful in demonstrating that an abundant, alkaline-phosphatase-sensitive, material was in fact, cleaved from dideoxyinosine through the action of purine nucleoside phosphorylase. This product was not seen in acid extracts of cells and reaction mixtures, nor in neutral extracts (60% methanol) after even brief (24 h) storage on ice. Thus it is presumably of high lability.

We have additionally confirmed that a phosphorylated sugar can be cleaved from dideoxyinosine through the action of PNP, by means of an enzymatic trapping system. Thus, 2'3'-[^{3}H]- 2'3'dideoxyinosine was incubated with purine nucleoside phosphorylase in the presence of phosphate, xanthine oxidase (used to overcome the unfavorable equilibrium by consuming the product of phosphorolysis, hypoxanthine) thymine (or saline) and bacterial thymidine phosphorylase. We reasoned that if dideoxyribose-1-phosphate were in actuality, generated in this PNP reaction - however transitorily it might be utilized by an unrelated phosphorylase for nucleoside synthesis:



This result was in fact observed, albeit in modest yield. Whether an analogous conversion can occur in vivo, or intracelluarly is doubtful. For example, we have never seen the generation of dideoxypyrimidines in Molt-4 cells incubated with tritiated dideoxyinosine; this however maybe a result of the fact that the pyrimidine nucleoside phosphorylases exhibit poor catalytic efficiency with dideoxypyrimide nucleosides. On the other hand, dideoxyguanosine is seen in cells incubated with ddA or dd. Whether this represents salvage via purine nucleoside phosphorylase, or processing of ddIMP via the last steps of the de novo purine biosynthetic path, remains to be determined.

Publications

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2. Ahluwalia GS, Jayaram HN, Cooney DA. Metabolites of tiazofurin as mediators of its biochemical and pharmacologic effects. In: Muggia F, ed. Concepts, clinical developments, and therapeutic advances in cancer chemotherapy. Boston: Martinus Nijhoff, 1987.

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ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The Laboratory of Medicinal Chemistry (LMC) was established to give increased intramural emphasis to (1) the discovery and identification of new anticancer and antiviral drugs from both synthetic and natural sources and (2) the development of analytical methodology appropriate for the quantitation of new drugs in biological fluids and the identification of metabolites. Essentially all projects are collaborative in nature, either among the synthetic and analytical chemists within the Laboratory or between the LMC and other NIH or academic laboratories.

ANTITUMOR STUDIES

Arabinosyl-5-azacytosine (ara-AC), a compound synthesized a number of years ago in this laboratory, started Phase I clinical trials in three institutions including the NIH Clinical Center. The LMC has developed an analytical method for the quantitation of ara-AC in human body fluids and is applying the technique to pharmacokinetic studies in the COP clinical trial. Plasma, pleural and CSF samples from 20 patients have been analyzed.

LMC interest in the antitumor and antiviral activity of cyclopentenyl nucleosides continues to be high based on the DCT Decision Network Committee selection of one of our analogues, cyclopentenyl cytosine (CPE-C), as a potential clinical candidate. CPE-C is very active against a number of NCI *in vivo* tumor models as well as both RNA and DNA viruses. The 2′-deoxy and ara- analogues of CPE-C were synthesized and evaluated for cytotoxic and antiviral properties. Ara-CPE-C was active against the influenza A_2 virus. CPE-C-5′-triphosphate, the active metabolite of CPE-C, was synthesized and appears to be identical to the naturally occurring metabolite as an inhibitor of cytidine triphosphate synthetase.

An HPLC assay suitable for use in both preclinical and clinical CPE-C pharmacokinetic studies was developed using isocarbodine as an internal standard. With a limit of quantitation of 25 ng/ml, this method was used to show that the compound was stable for 24 hours in human plasma and that <5% protein binding occurred. The terminal half-life in the rat was 62 minutes.

3-Deazaneplanocin A (3DN), a compound synthesized by the LMC, is the most potent known inhibitor of the enzyme S-adenosylhomocysteine hydrolase. It has been found to have excellent antiviral activity against vesicular stomatitis virus, parainfluenza-3 and vaccinia virus. *In vivo*, 3DN is more active than ara-A against the vaccinia tail pox model. Because it is poorly phosphorylated, 3DN is much less cytotoxic than its parent compound, the fermentation product neplanocin A.

A phosphoramidite synthon was synthesized and used in an automated DNA synthesizer to prepare decamers containing dihydro-5-azacytidine and 5-azacytidine at defined nucleotide sites. These compounds will be used to investigate the role of 5-azacytosine-containing oligomers on the inhibition of DNA methylase and the consequent effects on gene expression.

Several inactive phosphonate nucleosides were prepared as possible inhibitors of purine nucleoside phosphorylase. The synthesis of a phosphonate-containing 2′,5′-oligoadenylate trimer is nearing completion. This compound is being prepared as an enzymatically stable interferon inducer.

The initial compounds have been prepared from a new project to synthesize protein kinase C inhibitors. These materials feature a rigid glycerol backbone and are designed to define the critical spatial relationships which exist between the polar and non-polar groups in activators of this enzyme.

A gas chromatographic method developed by the LMC to measure hexamethylenebisacetamide (HMBA) is being utilized in a collaborative Phase I study at Walter Reed Army Medical Center. Oral administration of HMBA is being compared with a previous infusion protocol. HMBA has been found to be 100% orally bioavailable and the LMC is using its analytical method to measure the concentration of both HMBA and its major human metabolites.

A major problem in the microscale mass spectral analysis of drugs and metabolites from human biological fluids is sample contamination with relatively large amounts of inorganic salts. A minicolumn desalting method has been developed to solve this problem. Complete NaCl removal from samples having a 10:1 :: NaCl:cytidine ratio has been achieved on a 1 micromolar scale. The method is now being extended to a semipreparative scale to facilitate purification of samples for elemental analysis.

ANTI-AIDS STUDIES

The oral bioavailability of 5-fluoro-2′3′-dideoxycytidine (5-F-ddC) was determined to be 70%, the same as the parent compound, ddC. In anti-HIV testing at FCRF, both 5-F and 5-Br-ddC were determined to be active. The 5-Br compound was not active when the ATH8 cell line was used.

2′-Fluoro-2′,3′-dideoxyarabinosyl adenine (2′-F-dd-ara-A) has been shown to be just as active and potent as ddA but completely acid stable which is desirable for an orally administered product. Its parent compound, ddA, is very unstable under acidic conditions. This difference was exemplified by 2′-F-dd-ara-A and ddA oral bioavailability values in the dog of 80% and 30%, respectively, when the compounds were administered in saline.

Two synthetic analogues of the unusual anti-HIV fermentation product, oxetanocin, were prepared for evaluation. The first was inactive and the second is currently on test.

CSF/plasma ratios were determined for ddC and its metabolite, ddU, in rhesus monkeys. The ratio for ddU (15%) was five times that for ddC and both ratios appear to be influx rather than efflux related.

Publications

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2. Cooney, D.A., Hamel, E., Cohen, M., Kang, G.J., Dalal, M., Marquez, V.E. A simple method for the rapid determination of the stereospecificity of NAD-dependent dehydrogenases applied to mammalian IMP dehydrogenase and bacterial NADH peroxidase. Biochim. Biophys. Acta. 916: 89-93, 1987.

3. Driscoll, J.S.: Cyclopentenyl Cytosine (CPE-C). A Neplanocin Analogue With Preclinical Antitumor and Antiviral Activity. Proceedings of the 1986 Beijing Symposium on Cancers in the Peoples Republic of China. (in press)

4. Goddard, A.J., Marquez, V.E. Synthesis of a Phosphoramidite of 2'-deoxy-5,6-dihydro-5-azacytidine. Its potential application in the synthesis of DNA containing dihydro-5-aza- and 5-azacytosine bases. Tetrahedron Lett., 29: 1767-1770, 1988.

5. Goldstein, B.M., Mao, D.T., Marquez, V.E. Ara-tiazofurin: Conservation of Structural features in an unusual thiazole nucleoside. J. Med. Chem. 31: 1026-1031, 1988.

6. Heideman, R.L, Kelley, J.A., Packer, R.J., Reaman, G.A., Roth, J.S., Balis, F., Ettinger, L.J., Doherty, K.M., Jeffries, S.L. and Poplack, D.G.: A pediatric Phase I and pharmacokinetic study of spirohydantoin mustard. Cancer Res., 48: 2292-2295, 1988.

7. Kelley, J.A., Litterst, C.L., Roth, J.S., Vistica, D.T., Poplack, D.G., Cooney, D.A., Nadkarni, M., Balis, F.M., Broder, S., and Johns, D.G.: The disposition and metabolism of 2',3'-dideoxycytidine, an in vitro inhibitor of human T-lymphotrophic virus type III infectivity, in mice and monkeys. Drug Metab. Dispos., 15: 595-601, 1987.

8. Kim, C.H., Marquez, V.E. Synthesis of ring-expanded cytidine: Homocytidine. J. Org. Chem. 52: 1979-1983, 1987.

9. Marquez, V.E., Goddard, A.J. A novel phosphoramidite reagent of 2'-deoxy-dihydro-5-azacytidine for chemical synthesis of modified DNA. NIH patent application E-112-88.

10. Marquez, V.E., Lim, M-I., Khan, M.S., Kaskar, B.: (4R,5R)-(-)-3-Benzyloxymethyl-4,5-O-isopropylidene-2-cyclopentenone. An optically active α, β-unsaturated cyclopentenone for the synthesis of Neplanocin A and other cyclopentene carbocyclic nucleosides. In Townsend, L.B. and Tipson, R.S. (Eds.). Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods and Techniques. Part 3. (in press)

11. Marquez, V.E., Lim, M-I., Markovac, A., Priest, M.A.: (-)-Neplanocin A. In Townsend, L.B. and Tipson, R.S. (Eds.): Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods and Techniques. Part 3 (in press)

12. Marquez, V.E., Lim, M-I., Treanor, S.P., Plowman, J., Priest, M.A., Markovac, A., Khan, S., Kaskar, B., Driscoll, J.S.: Cyclopentenyl cytosine. A Carbocyclic nucleoside with antitumor and antiviral properties. J. Med. Chem., (in press)

13. Marquez, V.E., Tseng, C.K-H., Kelley, J.A., Mitsuya, H., Broder, S., Roth, J.S. and Driscoll, J.S.: 2′,3′-dideoxy-2′-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). Biochem. Pharmacol., 36: 2719-2722, 1987.

14. Marquez, V.E., Tseng, C.K-H., Treanor, S.P., Driscoll, J.S.: Synthesis of 2′,3′-dideoxycyclopentenyl carbocyclic nucleosides as potential drugs for the treatment of AIDS. Nucleos. & Nucleot. 6: 239-244, 1987.

15. Moon, D.-C. and Kelley, J.A.: A Simple Desalting Procedure for Fast Atom Bombardment Mass Spectrometry. Biomed. Environ. Mass Spectrom. (in press).

16. Sutton, P.A., Cody, V., Marquez, V.E. Structures of two seven-membered ring pyrimidine nucleoside derivatives. Nucleos. & Nucleot. 6: 613-620, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03580-19 LMC

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chemical Research in the Development of New Anticancer Drugs

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

J. S. Driscoll	Chief	LMC, NCI
Others: V. E. Marquez	Visiting Scientist	LMC, NCI

COOPERATING UNITS (if any)
U.S. Army Antiviral Drug Testing Program, Fort Detrick, Frederick, Maryland
Southern Research Institute, Birmingham, Alabama

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

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.)

Ara-AC, a drug synthesized in this laboratory, has begun clinical trials in three institutions.

Cyclopentenyl cytosine (CPE-C) has excellent preclinical activity against NCI *in vivo* tumor models as well as viruses *in vitro*. CPE-C is active against the standard L1210 leukemia tumor in mice and produces "cures" in the ara-C-resistant L1210 model. It also shows excellent activity against solid human tumor xenograft models (A549 lung, MX-1 mammary, metastatic LOX melanoma) in athymic mice. The compound has outstanding antiviral activity against DNA viruses [HSV-1 (TK+ and TK-), HSV-2, vaccinia, cytomegalovirus, varicella-zoster] and RNA viruses [influenza A_2, vesicular stomatitis virus, Japanese encephalitis, Punta Toro, Rift Valley Fever] although, in several instances the antiviral therapeutic indices are low. Based on its antitumor activity, CPE-C has been selected as a potential clinical candidate by the DTP Decision Network Committee.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06173-03 LMC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Dideoxynucleosides as Potential Anti-AIDS Drugs

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

V. E. Marquez	Visiting Scientist	LMC, NCI
Others: J. S. Driscoll	Chief	LMC, NCI
C. K-H. Tseng	Senior Staff Fellow	LMC, NCI
R. W. Fuller	Chemist	LMC, NCI

COOPERATING UNITS (if any)

Toxicology Branch, DTP, DCT, NCI
DTP Anti-HIV Testing Program, FCRF
Office of the Associate Director, Clinical Oncology Program, DCT, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	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.)

5-Bromo and 5-fluoro-2′,3′-dideoxycytidine have been found to be active in the DTP anti-HIV screening program at FCRF.

2′-Fluoro-2′,3′-dideoxyarabinosyl adenine is not only as active and potent as ddA in anti-HIV tests, it is completely stable to the acid catalyzed glycosidic cleavage which occurs so rapidly with ddA. Oral studies in the dog showed 83% bioavailability for the fluoro analogue but only 30% for ddA.

Two five-member ring analogues of the fermentation product, oxetanocin, were prepared. The first had no cellular protective properties. The second is under evaluation.

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

Project Description:

General Objective:

The objective of this project is the discovery of 2′,3′-dideoxynucleoside analogs superior to known inhibitors of the AIDS virus.

Specific Objectives:

1. Synthesis of pyrimidine analogs

2. Synthesis of acid-stable purine analogs

MAJOR FINDINGS

Synthesis of Dideoxypyrimidine Nucleosides (Drs. Marquez, Driscoll):

2′,3′-Dideoxycytidine (ddC, 1a) is one of the most potent anti-HIV nucleosides known. In an earlier attempt to improve on the intracerebral activity of this compound, a number of 5-substituted ddC analogues were prepared. The 5-fluoro (1b), but not the 5-bromo (1c), compound proved to be active in the Broder and Mitsuya ATH8/HIV system. However, tests in the DTP screen at FCRF (MT-2, CEM-C/HIV) indicate that both compounds have anti-HIV activity.

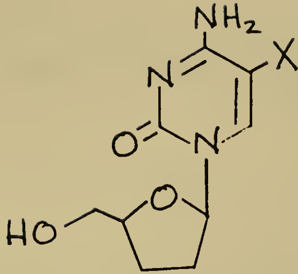
Synthesis of Acid-Stable Dideoxypurine Nucleosides (Drs. Marquez, Tseng, Driscoll):

2′,3′-Dideoxyadenosine (ddA, 2a) is an anti-HIV active nucleoside currently in clinical trial. This compound is very unstable in acid, a poor property for a drug which will eventually be administered orally. Last year we reported the synthesis of the 2′-fluoro analogue (2′-F-dd-ara-A, 2b). This compound is just as active and potent as ddA and is completely stable under acidic conditions (pH 1). Studies in dogs showed that the fluoro analogue was 83% orally bioavailable under conditions (administration in saline) which produced 30% bioavailability for ddA.

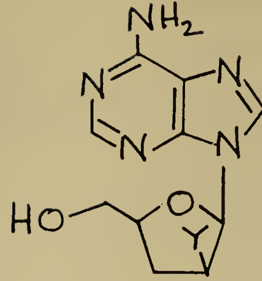
Oxetanocin (3) is a fermentation product discovered to have anti-HIV activity in the Broder and Mitsuya screen. The unique structural feature of this molecule is its four-member "sugar" ring and its extra hydroxymethyl group. In a project to explore structure-activity relationships in this series, two corresponding compounds (4,5) containing five-member glycon rings were prepared. Compound 4 was not active and 5 is being evaluated.

PUBLICATIONS

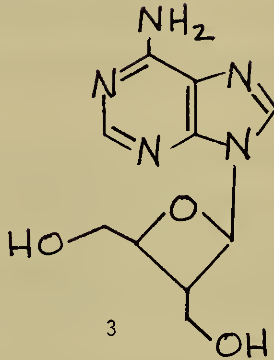
1. Marquez, V.E., Tseng, C.K-H., Driscoll, J.S., Mitsuya, H., Broder, S., Roth, J.S., Kelley, J.A. 2′,3′-Dideoxy-2-β-fluoroadenosine. An Acid-Stable Purine Nucleoside Active Against Human Immunodeficiency Virus (HIV). Biochem. Pharmacol 36, 2719-2722, 1987.



1a, X=H
1b, X=F
1c, X=Br



2a, Y=H
2b, Y=F



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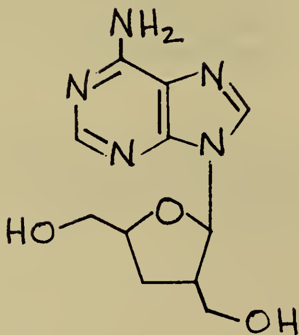

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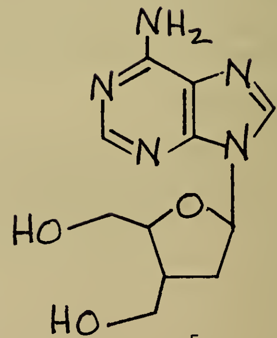


5

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06174-03 LMC

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclopentenyl Nucleoside Isosteres as Potential Antitumor and Antiviral Agents

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

	Name	Title	Affiliation
	Victor E. Marquez	Visiting Scientist	LMC, NCI
Others:	John S. Driscoll	Chief	LMC, NCI
	Christopher K-H. Tseng	Senior Staff Fellow	LMC, NCI
	Richard R. Copp	Visiting Fellow	LMC, NCI
	Richard W. Fuller	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biological Chemistry

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	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.)*

This work continues to generate a series of new cyclopentenyl (CPE) nucleoside isosteres that are being studied as antitumor or antiviral agents. The discovery of the remarkable antitumor and antiviral activity of the cytosine analogue (CPE-C) has prompted an extensive investigation into this type of carbocyclic nucleoside. During this endeavor, new improved methods of synthesis and protection-deprotection procedures have been investigated and adapted to the chemistry of the CPE series.

The ara and 2′-deoxy analogues of CPE-C were synthesized. Ara-CPE-C is active against the Influenza A_2 virus, but neither compound has antitumor activity. Additional amounts of CPE-U were synthesized for <u>in vivo</u> studies of the inhibition of the pyrimidine salvage pathway. The active metabolite of CPE-C (its 5′-triphosphate) also was synthesized and identified as a potent inhibitor of cytidine triphosphate synthetase.

Among the cyclopentenyl purine compounds, 3-deazaneplanocin, a potent inhibitor of S-adenosylhomocysteine hydrolase, has excellent antiviral activity against both RNA and DNA viruses without the high level of cytotoxicity normally associated with the parent fermentation product, neplanocin.

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

Objectives:

The objective of this work is the systematic evaluation of different structural modifications of cyclopentenyl carbocyclic nucleosides in terms of their antitumor and antiviral properties. These compounds are structurally related to the naturally occurring antibiotic neplanocin A.

Major Findings:

CPE-pyrimidine analogues. (Dr. Marquez, Dr. Copp and Mr. Fuller): Syntheses of 2′-deoxycyclopentenyl cytosine (2′dCPE-C, 2) and aracyclopentenyl cytosine (ara-CPE-C, 3) were repeated to obtain additional amounts of compounds for antitumor and antiviral testing. Both compounds were found to be non-cytotoxic to L1210 cells in culture indicating perhaps that the phosphorylated metabolite was not formed. When examined as antiviral agents against Herpes Simplex virus type 1 (HSV-1) and Influenza virus (Hong Kong) type 2, only ara-CPE-C showed significant antiviral activity against Influenza with an MIC_{50} of 173.2 μg/ml and a viral rating (VR) of 1.3 (ribavirin as positive control against influenza has a VR of 3.9 and an MIC_{50} of 3.1 μg/ml).

Syntheses of CPE-T (4) as well as the 2′- and 3′-deoxy analogues (5 and 6) were completed. These compounds were also noncytotoxic to L1210 cells in culture and were submitted recently for antiviral testing.

Additional amounts of CPE-U (7) were synthesized for in vivo studies of uridine salvage in mice. As reported earlier, CPE-U functions as an inhibitor of uridine-cytidine kinase. In vivo inhibition studies of uridine salvage were conducted by Dr. James D. Moyer (LBC, NCI) who determined that after a relatively high and nontoxic dose of 1000 mg/Kg of CPE-U, given twice at a 12 h interval, inhibition of uridine salvage ranged from 64 to 79% after nine hours in the various tissues examined (ascites, kidney, liver, spleen, and intestine). Combination studies of CPE-U administered under similar conditions but in the presence of PALA [N-(phosphonoacetyl)-L-aspartate] which functions as a de novo inhibitor of pyrimidine biosynthesis are underway. In these studies the consequences of a complete blockage of uridine synthesis will be ascertained.

The synthesis of the active metabolite of the antitumor and antiviral agent CPE-C (1) was completed. The triphosphostate metabolite of CPE-C (CPE-CTP) has been tentatively identified as the critical and potent inhibitor of cytidine triphosphate synthetase which is responsible for the biological activity of CPE-C. The synthetic material will be used for kinetic studies with this enzyme. Based on its antitumor data CPE-C was selected by the NCI for clinical trials.

Catalytic hydrogenation of CPE-C gave a mixture of carbodine (8) and isocarbodine (9). In comparison with CPE-C, the cytotoxicity profile against L1210 for these compounds was as follows: CPE-C ($IC_{50} = 3 \times 10^{-8}$ M), carbodine ($IC_{50} = 5 \times 10^{-7}$ M), isocarbodine ($IC_{50} = 8 \times 10^{-5}$ M).

CPE-purine analogues. (Dr. Marquez, and Dr. Tseng). The most important member of this group is 3-deazaneplanocin A (10). This compound was studied extensively as an antiviral agent in view of its earlier discovered properties of selectively inhibiting the enzyme S-adenosyl homocysteine hydrolase (SAH-ase). Consistently with its mechanism of action, 3-deazaneplanocin A exhibited very good antiviral activity against Parainfluenza-3 (VR = 3.6, MIC_{50} = 0.05 μg/ml), Vesicular Stomatitis (VR > 3.6, MIC_{50} = 0.07 μg/ml), and Vaccinia (VR = 3.1, MIC_{50} = 0.32 μg/ml) without the high level of cytotoxicity normally associated with the natural antibiotic neplanocin A. This selectivity towards viral infected cells is due to the poor substrate properties of 3-deazaneplanocin A towards cellular kinases that prevents the formation of the phosphorylated metabolite. In vivo studies against these viruses are underway.

The X-ray structure of 3-deazaneplanocin A was determined in collaboration with Dr. Barry M. Goldstein of the University of Rochester (Figure 1).

Antitumor and antiviral studies on other CPE-purine analogues such as CPE-guanine, CPE-hypoxanthine, and CPE-thioguanine did not reveal any outstanding antitumor or antiviral activities.

Publications

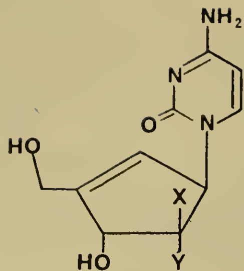
1. Marquez, V.E., Tseng, C.K-H., Treanor, S.P., Driscoll, J.S.: Synthesis of 2′,3′-dideoxycyclopentenyl carbocyclic nucleosides as potential drugs for the treatment of AIDS. Nucleos. & Nucleot. 6: 239-244, 1987.

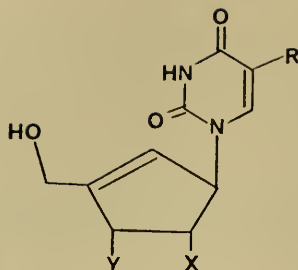
2. Driscoll, J.S.: Cyclopentenyl Cytosine (CPE-C). A Neplanocin Analogue With Preclinical Antitumor and Antiviral Activity. Proceedings of the 1986 Beijing Symposium on Cancers in the Peoples Republic of China. (in press)

3. Marquez, V.E., Lim, M-I., Treanor, S.P., Plowman, J., Priest, M.A., Markovac, A., Khan, S., Kaskar, B., Driscoll, J.S.: Cyclopentenyl cytosine. A Carbocyclic nucleoside with antitumor and antiviral properties. J. Med. Chem., (in press)

4. Marquez, V.E., Lim, M-I., Khan, M.S., Kaskar, B.: (4R,5R)-(-)-3-Benzyloxymethyl-4,5-O-isopropylidene-2-cyclopentenone. An optically active α, β-unsaturated cyclopentenone for the synthesis of Neplanocin A and other cyclopentene carbocyclic nucleosides. In Townsend, L.B. and Tipson, R.S. (Eds.). Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods and Techniques. Part 3. (in press)

5. Marquez, V.E., Lim, M-I., Markovac, A., Priest, M.A.: (-)-Neplanocin A. In Townsend, L.B. and Tipson, R.S. (Eds.): Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods and Techniques. Part 3 (in press)





4, X = Y = OH, R = Me

5, X = H, Y = OH, R = Me

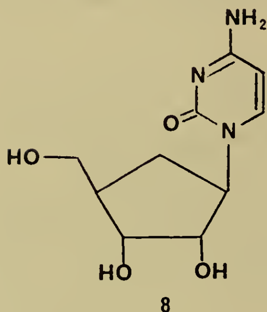
6, X = OH, Y = H, R = Me

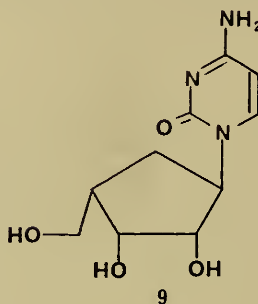
7, X = Y = OH, R = H

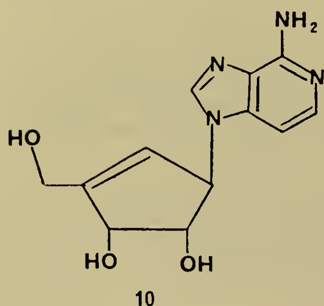
1, X = H, Y = OH (CPE-C)

2, X = Y = H

3, X = OH, Y = H







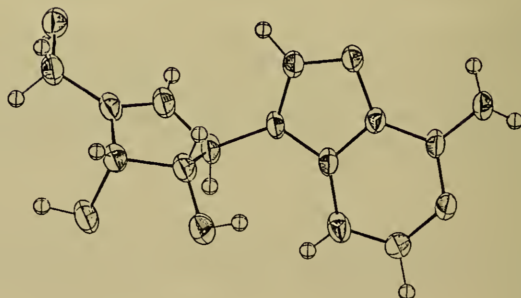


Fig.1. Crystal Structure of 3-Deazaneplanocin A

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06175-03 LMC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Synthesis and Properties of Oligonucleotides Containing 5-azacytosine Residues

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

Victor E. Marquez — Visiting Scientist — LMC, NCI

Others: Amanda Goddard — Visiting Fellow — LMC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	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.)

The main objective of this work is the development of techniques for the incorporation of 5-azacytosine (5-AC) and its reduced analogue 5,6-dihydro-5-azacytosine (DHAC) into specific sites of a synthetic oligonucleotide. The resulting modified oligonucleotides will be studied as specific DNA methylase inhibitors and as probes to study the relationship between DNA methylation and gene expression.

A phosphoramidite synthon was synthesized and used in an automated DNA synthesizer to prepare dimers and decamers containing DHAC and 5-AC. Reaction conditions were optimized. An efficient method was developed for the oxidation of DHAC to 5-AC in the oligomers.

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

Objectives:

The main objective of this work is to develop techniques for the incorporation of 5-azacytosine and its reduced analogue 5,6-dihydro-5-azacytosine into specific sites of a synthetic oligonucleotide. The resulting modified oligonucleotides will be studied as specific DNA methylase inhibitors and as probes to study the relationship between DNA methylation and gene expression.

Major Findings:

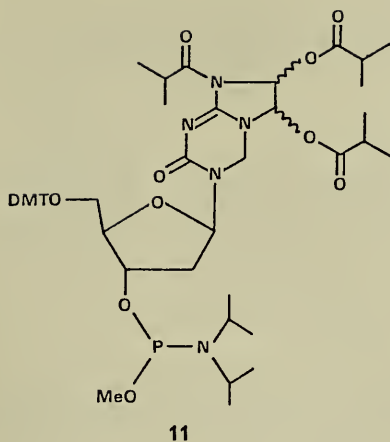
As reported last year, the phosphoramidite synthon 11 was synthesized and used in trial experiments to prepare the dimer DHAC-p-T (12) under various conditions. The reaction conditions for an efficient coupling have been optimized and the phosphoramidite 11 has been tested in the automated DNA synthesizer (Applied Biosystem model 380A) in the preparations of decamers containing 5,6-dihydro-5-azacytosine at different positions.

The modified decamers so far prepared are derived from the parent decamer (5′-TACGTCGCAG-3′) in which the third or sixth cytosine is replaced by 5,6-dihydro-5-azacytosine (Figure 2). Oxidation of 5,6-dihydro-5-azacytosine to 5-azacytosine in the DHAC-p-T dimer was studied in detail. Proper conditions to achieve >95% conversion have now been developed. A mixture of silicon-containing reagents including bis(trimethylsilyl)trifluoroacteamide, trimethylsilyl chloride and trimethylsilyl peroxide was developed and tested. These conditions are in the process of being tested for the oxidation of the 5,6-dihydro-5-azacytosine in the modified decamers. Other modified oligonucleotides synthesized were derived from the 26-mer (5′-CCGGCCAT-TACGGATCCGTCCTGGGC-3′) in which individually the second, the eleventh, and the seventeenth cytosine base was replaced by 5,6-dihydro-5-azacytosine. The selection of the parent 26-mer was based on its excellent substrate properties for DNA methylase. Evaluation of these and the corresponding oxidized substrates as substrates/inhibitors of DNA methylase will be initiated shortly. In addition to progress in the preparation of various modified oligonucleotides, we have improved our original synthesis of phosphoramidite 11 and the DHAC-p-T dimer 12. The modifications introduced in these syntheses have resulted in an overall reduction in the number of steps and in an increase in the overall yield.

Publications

1. Goddard, A.J., Marquez, V.E. Synthesis of a Phosphoramidite of 2′-deoxy-5,6-dihydro-5-azacytidine. Its potential application in the synthesis of DNA containing dihydro-5-aza- and 5-azacytosine bases. Tetrahedron Lett., 29: 1767-1770, 1988.

2. Marquez, V.E., Goddard, A.J. A novel phosphoramidite reagent of 2′-deoxy-dihydro-5-azacytidine for chemical synthesis of modified DNA. NIH patent application E-112-88.



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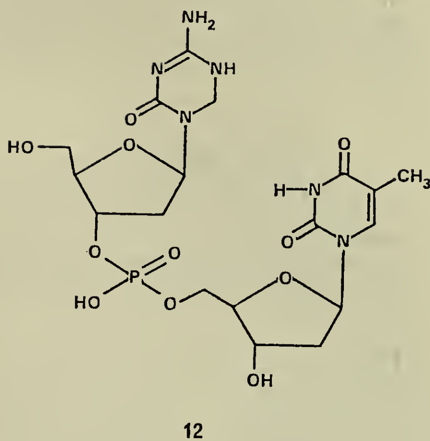

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Figure 2. Autoradiography of synthetic oligonucleotides obtained after 5′-end labeling and polyacrylamide gel electrophoresis.
Lane 1: $(CA)_3$, hexamer marker.
Lane 2: $(AT)_4$, octamer marker.
Lane 3: TACGTCGCAG, parent decamer.
Lane 4: TA**X**GTCGCAG, 3-modified decamer.
Lane 5: TACGT**X**GCAG, 6-modified decamer.
X = 5,6-dihydro-5-azacytosine

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06176-03 LMC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Enzyme Inhibitors as Potential Anticancer and Antiviral Drugs

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

	Name	Title	Affiliation
	Victor E. Marquez	Visiting Scientist	LMC, NCI
Others:	John S. Driscoll	Chief	LMC, NCI
	Joseph J. Barchi	Staff Fellow	LMC, NCI
	John J. McCormack	IPA	LMC, NCI
	Christopher K-H. Tseng	Staff Fellow	LMC, NCI
	Richard W. Fuller	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biochemical Pharmacology, NCI
Drug Synthesis and Chemistry Branch, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

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 work continues to explore the utility of mechanism-base inhibitors against enzymes critical for cancer chemotherapy. Successful inhibitors of inosine monophosphate dehydrogenase have been developed and future *in vivo* studies will be scheduled as soon as larger quantities of drugs become available.

Several phosphonate nucleosides were synthesized as possible inhibitors of purine nucleoside phosphorylase but the compounds were inactive. A phosphonate-containing 2′,5′-oligoadenylate trimer is being prepared as an enzymatically stable interferon inducer.

A project to prepare protein kinase C inhibitors containing a rigid glycerol backbone has been initiated. The work is intended to define the critical spatial relationships which exist between the polar and non-polar groups in known activators of this enzyme.

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

Objective:

The objective of this project is to design mechanism-based inhibitors of various enzymes of interest in antitumor or antiviral chemotherapy. Our current approach is based on the construction of a modified substrate which will either inactivate the enzyme, bind tighter to its active site, or resist enzymatic degradation.

Major Findings:

Dinucleotide Analogues of NAD as IMP Dehydrogenase Inhibitors. (Dr. Marquez, Dr. Tseng, and Mr. Fuller): As of May 5, 1988 significant amounts of the beta-methylene TAD analogue (13) have become available through a contractor (Stark Associates, Buffalo, N.Y.). Studies in cells naturally resistant to the action of tiazofurin will be studied *in vitro*. *In vivo* studies will follow. In addition, efforts to obtain a crystalline material suitable for X-ray analysis will be initiated.

Synthesis of Purine and Pyrimidine Phosphonates as Inhibitors of Nucleoside Phosphorylases (Dr. Tseng, Dr. Marquez, and Dr. McCormack): The syntheses of three target phosphonate nucleoside analogues (14, 15, 16) have been completed. Preliminary studies with purine nucleoside phosphorylase from either liver of red blood cells (compounds 14 and 15) and thymidine phosphorylase from *E. Coli* (compound 16) indicate that little of no inhibition of the enzymes occur in the presence of these compounds. The first two compounds show IC_{50}'s greater than 10^{-3} M against purine nucleoside phosphorylase, whereas compound 16 has an IC_{50} of 10^{-4} M against thymidine phosphorylase. Its was determined that the concentration of phosphate used in the assay buffers [low phosphate (1 mM) and high phosphate (50 mM)] has absolutely no effect in modulating the activity of these compounds.

Synthesis of Phosphonate Analogues of the 2',5'-Oligoadenylate Trimer as a Stable Inducers of Interferon Production (Dr. Tseng and Dr. Marquez): The major target of this project (compound 17) has not yet been achieved. Efforts towards the synthesis of the dimeric structure 18 are in progress.

Synthesis of Protein Kinase C Inhibitors. (Dr. Barchi and Dr. Marquez): This work intends to define the critical spatial relationships that exist between polar and non-polar groups in protein kinase C activators. It is proposed to accomplish this through the synthesis of molecules which are structurally simpler than the naturally occurring tumor promoters (i.e., phorbol esters) but more complex than the endogenous diacylglycerol activators. The proposed molecules are the gamma lactones of arabinonic, lyxonic, ribonic, and xylonic acid (both D and L series) all of which would provide a rigid scaffolding in which the chiral centers of the embedded backbone of diacylglycerol are varied. Starting with D-ribonolactone the target compounds 19 - 22 have been synthesized. Efforts to complete the D-ribo series are in progress with attempts to prepare the evasive 2-myristoylated derivative 23. With the exception of the latter compound the structures so far synthesized in the D-ribo series have been submitted for testing as protein kinase C activators or inhibitors. Work on the L-ribo series will begin shortly.

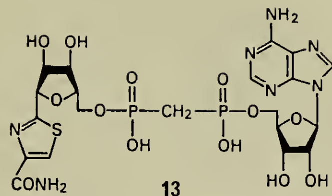
Publication:

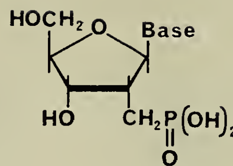
1. Kim, C.H., Marquez, V.E. Synthesis of ring-expanded cytidine: Homocytidine. J. Org. Chem. 52: 1979-1983, 1987.

2. Sutton, P.A., Cody, V., Marquez, V.E. Structures of two seven-membered ring pyrimidine nucleoside derivatives. Nucleos. & Nucleot. 6: 613-620, 1987.

3. Goldstein, B.M., Mao, D.T., Marquez, V.E. Ara-tiazofurin: Conservation of Structural features in an unusual thiazole nucleoside. J. Med. Chem. 31: 1026-1031, 1988.

4. Cooney, D.A., Hamel, E., Cohen, M., Kang, G.J., Dalal, M., Marquez, V.E. A simple method for the rapid determination of the stereospecificity of NAD-dependent dehydrogenases applied to mammalian IMP dehydrogenase and bacterial NADH peroxidase. Biochim. Biophys. Acta. 916: 89-93, 1987.



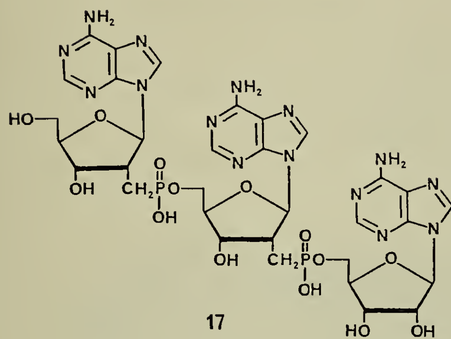


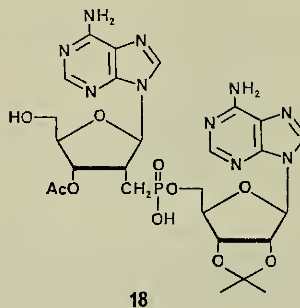
Base

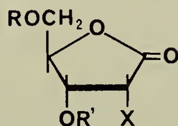
14, Ade

15, Hyp

16, Thy







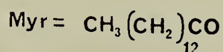
19, X = OH, R = Myr, R' = H

20, X = OH, R = H, R' = Myr

21, X = H, R = Myr, R' = H

22, X = H, R = H, R' = Myr

23, X = OMyr, R = R' = H



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJFCT	Z01 CM 03581-19 LMC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Analytical Chemistry of New Anticancer Drugs

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

	Name	Title	Affiliation
	James A. Kelley	Research Chemist	LMC, NCI
Others:	John S. Driscoll	Chief	LMC, NCI
	Jeri S. Roth	Chemist	LMC, NCI
	Harry Ford, Jr.	Biotechnology Fellow	LMC, NCI
	Lajos Hegedus	Visiting Fellow	LMC, NCI
	Dong-Cheul Moon	Visiting Fellow	LMC, NCI

COOPERATING UNITS (if any)

Laboratory of Biochemical Pharmacology, DTP, DCT; Clinical Pharmacology Branch, COP, DCT; Investigational Drug Branch, CTEP, DCT

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The objective of this project is the research and development of analytical methods which are used to: (1) establish the structure and purity of new antitumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs, (3) quantitate drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmacokinetics, and (4) study reaction mechanisms of potentially useful synthetic transformations. Mass spectrometry, gas chromatography and high-performance liquid chromatography, either alone or in combination, are emphasized techniques. Compounds of current interest are cytidine analogs, modified nucleosides, oligonucleotides and differentiating agents.

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

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques for establishing the structure and purity of new anticancer drug candidates, determining their important physical and chemical properties, elucidating structures of metabolites of new antitumor agents, measuring these drugs and their metabolites in biological samples, and studying synthetically useful reaction mechanisms. Gas Chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS) and the combination of these techniques are the emphasized methods. Other analytical methods such as NMR, UV and IR spectroscopy are also employed.

Specific Objectives:

1. Analytical methods development and preclinical pharmacology for cyclopentenyl cytosine (CPE-C).

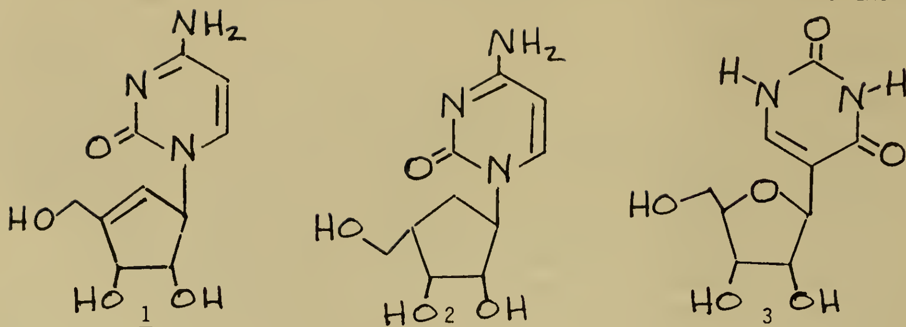
2. Plasma pharmacokinetics of arabinosyl-5-azacytosine (Ara-AC) in an adult Phase I clinical trial.

3. Bioavailability and metabolism of oral hexamethylene bisacetamide (HMBA) in an adult Phase I clinical trial.

4. Kinetic and mechanistic studies of silylation-mediated oxidation for the efficient synthesis of 5-azacytidine-containing oligonucleotides.

Major Findings:

1. Analytical Methods Development and Preclinical Pharmacology for Cyclopentenyl Cytosine (CPE-C) (Drs. Kelley, Hegedus, Ford): Cyclopentenyl cytosine (CPE-C, NSC 375575, 1) is a synthetic cytosine nucleoside containing the same unsaturated sugar as the antineoplastic and antiviral fermentation product neplanocin A. The NCI has designated CPE-C as a candidate for clinical development because of its antitumor activity in several model systems and its potency as an inhibitor of CTP synthetase. An isocratic reverse phase HPLC assay, which is suitable for both preclinical and clinical studies, has been developed for measuring CPE-C in biological samples. This assay employs solid phase extraction of CPE-C on phenylboronic acid cartridges and isocratic elution and automatic column switching of narrow-bore C_{18} columns to achieve a limit of quantitation of 0.1 μM (25 ng/ml) in plasma. Isocarbodine (2), synthesized by catalytic hydrogenation of CPE-C, is employed as an internal standard. The assay also allows simultaneous measurement of pseuodouridine (3), a C-nucleoside which may have some utility as a tumor marker in certain diseases and serve as an additional parameter for measuring the effectiveness of chemotherapy. CPE-C (0.5-20 μM) is stable in both human and rat plasma for 24 hr and exhibits minimal plasma protein binding (<5%) over this same concentration range. A preliminary pharmacokinetic study in the rat of CPE-C administered as a bolus dose suggests biphasic plasma elimination (Figure 1) with a terminal phase half-life of 62 min. More extensive pharmacokinetic studies are in progress.



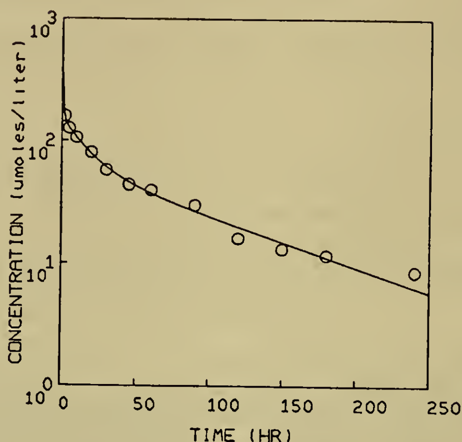
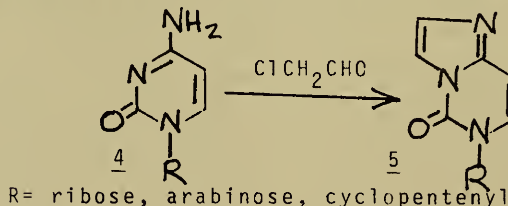


Figure 1. Concentration of CPE-C in the plasma of a male Sprague-Dawley rat after iv administration of 20 mg/kg. The indicated curve is the computer-generated best fit to a two-compartment open model.



Exploratory work has continued on the chloroacetaldehyde derivatization of cytosine nucleosides (4). Cytidine has been used a model compound to study the kinetics of the formation of 3,N^4-etheno derivative 5. At room temperature and pH 4.5, more than 24 hr are required for complete conversion to 5. This derivative exhibits increased retention on a reverse phase HPLC

column relative to the parent nucleoside. Although the UV absorption of 5 is not much different, its fluorescence should make possible the development of an even more sensitive and specific HPLC assay for CPE-C and other cytosine nucleosides.

2. Plasma Pharmacokinetics of Arabinosyl-5-azacytosine (Ara-AC) in an Adult Phase I Clinical Trial (Drs. Kelley, Ford, Cowan): Ara-AC (NSC 281272) is a new synthetic nucleoside which combines the structural elements of two established antitumor agents, Ara-C (the arabinose sugar) and 5-AC (the triazine base). This drug is currently being evaluated as a 72-hr continuous i.v. infusion in adult cancer patients. To date, twenty patients have been entered in this Phase I clinical trial. Plasma, pleural fluid and, in one case, CSF have been obtained from 27 cycles of therapy for Ara-AC analysis by our previously developed and recently refined HPLC assay. Ara-AC has been detected at i.v. infusion doses above 1.25 $mg/m^2/hr$ X 72 in the plasma of 5 patients (Table 1).

Table 1. Pharmacokinetic Summary of Ara-AC Phase I Clinical Trial

Patient	Dose ($mg/m^2/hr$)	C_{pss} (ng/ml)	Clearance ($ml/min/m^2$)
W. P.	1.25	32	651
	1.75	50	583
	2.44	57	713
	3.83	71	899
	4.75	108	733
Q. W.	1.95	74	439
J. F.	1.56	37	703
	1.95	72	451
R. D.	2.44	47	865
M. T.	1.95	62	524

3. Bioavailability and Metabolism of Oral Hexamethylene Bisacetamide in an Adult Phase I Clinical Trial (Drs. Kelley, Chun, Ward, Ms. Roth): Hexamethylene bisacetamide (HMBA, NSC 95580, 6), a potent *in vitro* differentiating agent, is currently being evaluated in a collaborative (NCI and Walter Reed Army Medical Center) Phase I clinical trial to compare a 5 day period of oral administration to a 120-hr continuous i.v. infusion in the same subjects. Since HMBA has a bitter taste which might inhibit direct oral intake, it is being given via nasogastric tube in equal doses every 4 hr. Oral bioavailability and dose equivalence are being evaluated by comparing plasma pharmacokinetics, overall drug exposure (area under the concentration *versus* time curve or AUC), metabolite plasma concentrations and urinary excretion of HMBA and its metabolites. A GC method employing selective derivatization

and solid phase extraction has been developed to measure HMBA and its major acidic (6-acetamidohexanoic acid, 7) and basic (N-acetyl-hexane diamine, 8) metabolites simultaneously. For the 120-hr continuous infusion, mean steady state plasma concentrations (C_{pss}) of 0.42, 1.65 and 2.36 mM at 12, 24 and 30 $gm/m^2/day$, respectively, are achieved within 24 hr. HMBA is rapidly absorbed from the gastrointestinal tract with complete oral bioavailability as calculated by comparing oral and i.v. AUC. Plasma elimination of parent drug is also monoexponential after either i.v. or oral administration. Other pharmacokinetic parameters are summarized in Table 2 for the patients who have been evaluated so far on this Phase I clinical trial which is still ongoing.

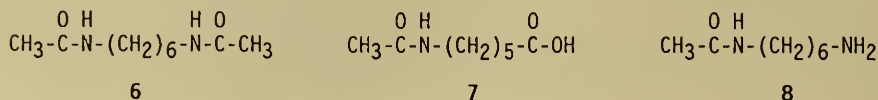


Table 2. Comparative Pharmacokinetics of HMBA

Patient	Daily Dose (gm/m^2)	Route	$t_{1/2}$ (hr)	Clearance ($ml/min/m^2$)	% Urinary Excretion HMBA	6
P. W.	12	NG[a]	2.46	78	44	-[b]
		IV[c]	1.94	84	36	-
L. S.	12	NG	2.09	89	37	-
		IV	2.20	94	27	-
R. R.	24	NG	2.97	74	45	-
		IV	2.76	66	35	-
E. O.	24	NG	3.64	47	28	37
		IV	3.59	59	36	38
R. S.	24	NG	3.58	51	43	36
		IV	5.97	39	42	38
R. C.	30	NG	2.65	61	61	23
		IV	2.35	54	53	22
C. H.	30	NG	3.93	39	31	27
		IV	3.44	41	38	32
Mean		NG	2.86	69	41	31
		IV	3.03	74	38	32

[a]via nasogastric tube every 4 hr for 5 days
[b]not determined
[c]via 120-hr i.v. continuous infusion

4. Kinetic and Mechanistic Studies of Silylation-Mediated Oxidation (Drs. Kelley, Moon): Trimethylsilylation of dihydropyrimidine and dihydro-*s*-triazine bases as well as their corresponding nucleosides under standard conditions sometimes results in their dehydrogenation to the corresponding aromatic derivatives. This reaction, which requires complete N- and O-silylation as well as molecular oxygen, appears to be free radical in nature. Since silylation-mediated oxidation has potential synthetic utility for the synthesis of 5-azacytosine-containing nucleosides and oligonucleotides, a kinetic and mechanistic study of this reaction by GC and GC/MS has been reinitiated for the purpose of improving the speed and yield of this transformation. The role of trimethylsilyl peroxide as a reagent or catalyst has been investigated kinetically. An excess of this peroxide in bis(trimethylsilyl)trifluoro-acetamide (BSTFA) and acetonitrile accelerates the microscale (3.5 μmole) oxidation of 5,6-dihydro-5-azacytidine to 5-azacytidine at 60-70°C, but reaction rates are comparable at higher temperatures. Trimethylsilyl peroxide can effect this oxidation in the absence of oxygen, but the rate is not comparable to that of oxygen and silylating reagent alone. Studies continue to define the mechanism and scope of this reaction.

5. Synthetic and Collaborative Project Support (Dr. Kelley): Numerous samples which cannot be categorized as coming from any one project area have been analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group are modified nucleosides, neplanocin analogs, carboacyclic nucleosides, dideoxyribose nucleosides, mono- and oligonucleotides, choline analogs, and sulfolipid and peroxide natural products.

PUBLICATION:

1. Heideman, R.L, Kelley, J.A., Packer, R.J., Reaman, G.A., Roth, J.S., Balis, F., Ettinger, L.J., Doherty, K.M., Jeffries, S.L. and Poplack, D.G.: A pediatric Phase I and pharmacokinetic study of spirohydantoin mustard. Cancer Res., 48: 2292-2295, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06177-03 LMC

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Analytical Chemistry of Anti-AIDS Agents

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

James A. Kelley	Research Chemist	LMC, NCI
Others: John. S. Driscoll	Chief	LMC, NCI
Jeri. S. Roth	Chemist	LMC, NCI
Harry Ford, Jr.	Biotechnology Fellow	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biochemical Pharmacology, DTP, DCT; Pediatric Branch, COP, DCT

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

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 objective of this project is the research and development of suitable analytical methods to: (1) establish the structure and purity of potential anti-AIDS agents and new antiviral drugs, (2) determine physical and chemical properties of these compounds and their metabolites, and (3) measure these drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmacokinetics. Gas chromatography, high-performance liquid chromatography and mass spectrometry are emphasized techniques. Compounds of current interest are dideoxycytidine, dideoxyadenosine, 2′,3′-dideoxy-5-fluorocytidine, 2′,3′-dideoxy-2′-β-fluoroadenosine and 2′,3′-dideoxy-2′-β-fluoroinosine.

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

Project Description:

General Objective:

The objective of this project is the research and development of suitable analytical methods for establishing the structure and purity of new anti-AIDS drug candidates, determining their important chemical and physical properties, elucidating structures of metabolites of these new agents, measuring these compounds and their metabolites in biological samples, and studying reaction kinetics and mechanisms of synthetically important transformations. High-performance liquid chromatography (HPLC) and mass spectrometry are the preferred techniques. Other analytical methods such as NMR, UV and IR spectroscopy, and ion exchange and affinity chromatography are also employed.

Specific Objectives:

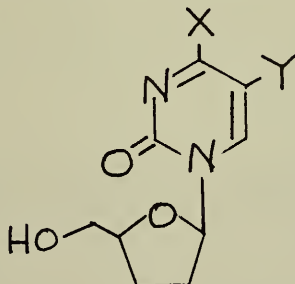
1. Comparative oral bioavailability of 2′,3′-dideoxycytidine and 2′,3′-dideoxy-5-fluorocytidine in mice.

2. Cerebrospinal fluid kinetics of 2′,3′-dideoxycytidine and 2′,3′-dideoxyuridine in rhesus monkeys.

3. Chemical and physical properties of sugar-modified dideoxynucleosides.

Major Findings:

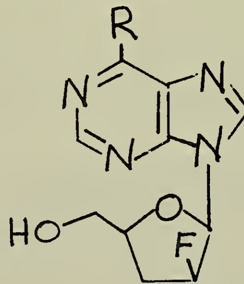
1. Comparative Oral Bioavailability of 2′,3′-Dideoxycytidine and 2′,3′-Dideoxy-5-fluorocytidine in Mice (Drs. Kelley, Ford, Litterst, Cooney and Ms. Roth): 2′,3′-Dideoxy-5-fluorocytidine (NSC 609067, 2, 5-F-ddC) is a synthetic analog of 2′,3′-dideoxycytidine (NSC 606170, 1, ddC), which possesses equivalent *in vitro* activity against HIV. Chemically, 5-F-ddC is slightly more lipophilic and its 4-amino group has a lower pK_a (2.5 *versus* 4.3) than ddC. A comparative pharmacology study of these two compounds in a rhesus monkey model showed that plasma elimination kinetics were similar and that little difference existed in the rate and magnitude of CNS penetration (as



1, X=NH_2;Y=H

2, X=NH_2;Y=F

3, X=OH ;Y=H



4, R=NH_2

5, R=OH

determined by cerebrospinal fluid (CSF) kinetics). Oral bioavailability studies in male BDF_1 mice suggested that 5-F-ddC was better absorbed than ddC, although a lower dose of 2 (33 mg/kg <u>versus</u> 100 mg/kg) was used. An oral bioavailability study was thus conducted at both doses for both compounds by determining the extent of urinary excretion (Table 1). No significant difference was found between ddC and 5-F-ddC in BDF_1 mice.

Table 1. Oral Bioavailability of 5-F-ddC and ddC in BDF_1 Mice

Drug	Dose (mg/kg)	Bioavailability[a] (%)	Total Drug Recovery (Urine and Feces) IV	Oral
5-F-ddC	33	73	100	77
	100	66	91	72
ddC	33	88	83	90
	100	60	93	75

[a]Determined from cumulative 24-hr urinary excretion of parent drug.

2. <u>Cerebrospinal Fluid (CSF) Kinetics of 2′,3′-Dideoxycytidine and 2′,3′-Dideoxyuridine in Rhesus Monkeys (Drs. Kelley, Poplack, Collins, Ms. Roth)</u>: Our previous result of about 3% CSF penetration for ddC in rhesus monkeys was much lower than the CSF:plasma ratio of more than 20% observed for 3′-azido-2′,3′-dideoxythimidine (AZT) in human patients. This difference prompted a more comprehensive collaborative study of the CSF penetration of other pyrimidine dideoxyribonucleosides in the above rhesus monkey model.The CSF elimination kinetics of 2′,3′-dideoxycytidine (ddC) administered as an intrathecal bolus dose of 0.25 mg was determined in the same set of monkeys for which plasma and CSF kinetics had been determined after i.v. bolus administration. The CSF clearance of 0.087 ± 0.010 ml/min for ddC was similar to that observed for an equivalent intrathecal bolus dose of AZT. This suggests that influx, rather than efflux, is the controlling process for accumulation of these compounds in the CSF. Retrospective analysis of the ddC i.v. bolus HPLC data allowed calculation of both plasma and CSF 2′,3′-dideoxyuridine (ddU, 3), which was formed to a small extent when ddC was deaminated <u>in vivo</u>. A CSF:plasma ratio of 15%, approximately 5 times greater than that of ddC, was estimated for ddU.

3. <u>Chemical and Physical Properties of Sugar-Modified Dideoxynucleosides (Drs. Kelley, Ford, Marquez, Tseng, Ms. Roth)</u>: Further studies were carried out to characterize chemically the acid-stable series of purine dideoxynucleosides incorporating a fluorine atom in the 2′-β-position of the sugar. Both 2′,3′-Dideoxy-2′-β-fluoroadenosine (4, β-F-ddA) and 2′,3′-dideoxy-2′-β-fluoroinosine (5, β-F-ddI) were found to be completely stable in the acidity range of the human stomach (pH 1-2). For both compounds, intact drug could be quantitatively recovered under conditions in which ddA and ddI were completely decomposed. Microscale purification using reverse phase mini

columns was employed to desalt and purify **4** and **5** for accurate mass analysis by fast atom bombardment mass spectrometry. Measured molecular weights were within 4 ppm of the calculated accurate masses. The purity and structure confirmation of several other sugar modified dideoxynucleosides submitted by sources outside the LMC was also determined.

Publications:

1. Kelley, J.A., Litterst, C.L., Roth, J.S., Vistica, D.T., Poplack, D.G., Cooney, D.A., Nadkarni, M., Balis, F.M., Broder, S., and Johns, D.G.: The disposition and metabolism of 2',3'-dideoxycytidine, an *in vitro* inhibitor of human T-lymphotrophic virus type III infectivity, in mice and monkeys. *Drug Metab. Dispos.*, 15: 595-601, 1987.

2. Marquez, V.E., Tseng, C.K-H., Kelley, J.A., Mitsuya, H., Broder, S., Roth, J.S. and Driscoll, J.S.: 2',3'-dideoxy-2'-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem. Pharmacol.*, 36: 2719-2722, 1987.

3. Collins, J.M., Klecker, R.W.,Jr., Kelley, J.A., Roth, J.S., McCully, C.L., Balis, F.M. and Poplack, D.G.: Pyrimidine dideoxyribonucleosides: selectivity of penetration into cerebrospinal fluid. *J. Pharmacol. Exp. Ther.* (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06178-03 LMC

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Applications of New Mass Spectral Techniques

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

James A. Kelley	Research Chemist	LMC, NCI
Others: Dong-Cheul Moon	Visiting Fellow	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Chemistry, NHLBI

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	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.)*

The objective of this project is research on and development of new mass spectral techniques in order to provide new and/or more rapid solutions to problems involving (1) chemical structure determination, (2) complex mixture analysis and (3) measurement of trace components in biological systems. The scope and utility of these mass spectral methods are determined, and a comparison to other types of analyses, both new and established, is carried out. Fast atom bombardment mass spectrometry and combined liquid chromatography-mass spectrometry are the techniques of current interest. Fast atom bombardment mass spectrometry in both the positive and negative ion mode continues to be applied for the rapid structure determination of nucleosides, nucleotides and natural products. A microscale desalting procedure has been developed, evaluated and applied to nucleosides, nucleotides and peptides. This desalting procedure is also suitable for the semipreparative scale purification of synthetic nucleosides and nucleotides.

Project Description:

General Objective:

The objective of this project is the development and application of new mass spectral techniques for the rapid analysis of complex mixtures, measurement of trace components in biological systems and chemical structure determination. Fast atom bombardment mass spectrometry and combined liquid chromatography-mass spectrometry are the new techniques of current interest. The advantages and limitations of these new methods to already established techniques is also an area of concern.

Specific Objectives:

1. Rapid structural analysis of nucleosides, nucleotides and natural products by fast atom bombardment mass spectrometry.

2. Evaluation and application of a microscale minicolumn desalting procedure for fast atom bombardment mass spectrometry and for semipreparative purification of synthetic samples.

Major Findings:

1. Rapid Structural Analysis of Nucleosides, Nucleotides and Natural Products by Fast Atom Bombardment (FAB) Mass Spectrometry (Drs. Kelley, Fales): The LMC continues to conduct an extensive program in the synthesis of nucleosides and nucleotides of novel structure. Rapid and simple methods employing FAB mass spectrometry are employed to characterize these nucleic acid constituents without derivatization. Negative ion FAB mass spectrometry has been applied to determine the structure and purity of chemically and enzymatically synthesized nucleotides using previously developed structural correlations. A variety of nucleotide mono- and triphosphates, 2′-phosphonate analogs and oligonucleotide dimers incorporating either dihydro-5-azacytosine or 5-azacytosine have been analyzed. A study comparing FAB, californium-252 plasma desorption and chemical ionization for the analysis of peroxide natural products has also been completed.

2. Evaluation and Application of Minicolumn Deasalting and Purification Procedures (Drs. Kelley, Moon): A commonly encountered problem in the analysis of samples of biological origin by FAB mass spectrometry is the suppression of structurally diagnostic ions by cationized species derived from contaminating salts. For biologically isolated samples, this ionic contamination most often arises from sodium salts, which are ubiquitous, but it can also result from the buffers employed in sample isolation and preparation or from ion leaching from glassware and silica-based chromatography media. Development and evaluation of a simple, rapid and efficient microscale (0.1 -1 μmole) procedure for removing contaminating salts from samples before FAB analysis has been completed. This method employs low pressure, high capacity reverse phase minicolumns and is applicable to nucleosides, peptides and some nucleotides. For cytidine, which is the most extensively studied model compound, essentially complete salt removal ($98.8 \pm 0.2\%$, n=3) can be achieved with high analyte recovery ($94 \pm 3\%$) from samples

(1 μmole, 243 μg) containing a 10-fold molar excess of NaCl. The positive and negative ion FAB spectral quality of even grossly contaminated samples after desalting is comparable to that of pure standards. Even for very small amounts of grossly contaminated sample (e.g. 0.1 μmole cytidine in 1.0 μmole NaCl) significant improvement (>20X) in the signal-to-background ratio of structurally important sample ions is achieved.

The suitability of minicolumn desalting for the semipreparative purification of nucleosides and nucleotides was investigated. Cytidine was again chosen as a model compound because it is the least retained of the pyrimidine ribosides upon reverse phase chromatography and therefore the most difficult case. A mandatory test of compound purity for the synthetic chemist is a satisfactory elemental analysis. Since a commercial carbon, hydrogen and nitrogen analysis usually requires 5-10 milligrams of compound, desalting an equimolar mixture of cytidine and NaCl at the 40 μmole level was evaluated. Greater than 96% of the salt could be removed in one chromatography cycle. After overnight drying at 100°C to completely remove water, elemental analysis results were within 0.08% of calculated values for all three elements. Semipreparative scale desalting of AMP (30 μmole, 10 mg) was also effective, being faster yet just as efficient as gel filtration or semipreparative HPLC. This method was also successfully applied to the milligram scale purification of a dihydro-5-azacytosine-thymine nucleotide dimer.

Publication:

Moon, D.-C. and Kelley, J.A.: A Simple Desalting Procedure for Fast Atom Bombardment Mass Spectrometry. Biomed. Environ. Mass Spectrom. (in press).

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1987 to September 30, 1988

The major goal of the Laboratory of Molecular Pharmacology is to obtain basic knowledge that could be applicable to the development of new strategies for the selective killing of human tumor cell types.

DNA Topoisomerases as Targets for Anticancer Drugs

Previous work in our Laboratory has developed the concept of DNA topoisomerases as targets for the antitumor actions of a variety of drugs, such as the DNA intercalating agents, anthracyclines, amsacrines and ellipticines, and the epipodophyllotoxins, VP-16 and VM-26. It is now well established that these drugs trap or stabilize covalent cleavable complexes between DNA and topoisomerase II, and there is considerable evidence that actions on topo II are causative in cytotoxicity. We have continued intensive investigation of drug actions on topoisomerases.

In order to determine the role of topoisomerases in drug sensitivity, we are studying an unusual topoisomerase which we have found in a drug-resistant cell line. Although the cells are resistant to topo II inhibitors, topo II is present at near normal levels. Instead we find the presence a large amount of an unusual form of topo I, unusual in having a molecular weight of 68 kd, rather than the usual 95 kd, and in having a greater tendency to form stable complexes with DNA. We have purified the enzyme to homogeneity and are investigating its properties. The enzyme has some properties that are more characteristic of topo II than of topo I: it is stimulated by ATP, and it has the ability to unlink catenated DNA circles. However it is unaffected by topo II inhibitors, and is sensitive to the topo I inhibitor, camptothecin, which causes the enzyme to cleave DNA at sites identical to those cleaved by bona fide topo I. Its molecular weight is much less than that of bona fide topo I, whose molecular weight in turn is much less than that of topo II.

We have been investigating the effects of polyamines and distamycin on DNA cleavage by topo II at specific nucleotide sequence sites. Evidence was obtained for the existence of a form of topo II-DNA complex that is tight but mobile and non-covalent. We refer to these as 'bracelet complexes' because of the apparent ability of the topo II in such complexes to glide along the DNA and to dissociate at strand ends. If the DNA is circular (relaxed circles) and has no ends, then the complex has increased stability. The polyamines, spermine and spermidine, increase the level of such complexes, perhaps by blocking the migration of the bracelet complexes towards dissociation sites along the DNA. The polyamines and distamycin tend to bind selectively to AT-rich sequence motifs which were localized by the footprinting technique. The compounds blocked topo II cleavage sites near the AT-rich binding sites, but enhanced cleavage at certain other sites.

Evidence was obtained in support of the hypothesis that the cytotoxic effect of drug-stabilized topo II-DNA complexes may be due to irreversible

consequences which may occur when a drug-stabilized complex is encountered by a replication fork. It was found that if the movement of replication forks is inhibited with aphidicolin, cell survival is increased, even though the level of drug-stabilized complexes remains unaltered.

A possible drawback of topo II as target for the treatment of slow growing solid tumors is that topo II is greatly reduced in non-proliferating cells. The possibility of topo I as target is of interest because this topoisomerase is present at substantial levels in non-proliferating cells. Like topo II, topo I forms covalent cleavable DNA complexes as intermediates in their normal DNA strand passage reactions, and abnormal stabilization of such complexes by drugs could produce cytotoxic events that could be the basis for antitumor activity. We have confirmed that treatment of cells with camptothecin produces protein-associated DNA strand breaks, the characteristic topoisomerase-DNA lesions that are observed by DNA filter elution assays. The characteristic 1:1 relationship between DNA single-strand breaks and DNA-protein links was demonstrated, as well as the association between the two types of lesions. The protein-associated DNA strand breaks of topo I are however more readily reversible, even in the cold or in the presence of mild detergents, than are the analogous topo II drug-induced DNA lesions.

We have carried out a structure-activity study of 22 derivatives of camptothecin, the only antitumor drug that has so far been found to stabilize cleavable complexes of mammalian topo I. The effects of the compounds on purified topo I were investigated, using assays for relaxation of supercoiled DNA, nicking of supercoiled DNA and induced cleavage at specific nucleotide sequence sites in a linear DNA fragment. We find that the ability of the drug to inhibit topo I requires the proper stereochemistry of the single asymmetric carbon in the structure, and is sensitive to conservative chemical substitutions on the molecule: whether activity is enhanced or abolished depends upon the exact site of substitution. Inhibition of the enzyme was correlated with the production or stabilization of cleavable complexes, and DNA strand cleavage always occurred at the same specific nucleotide sequence sites. On the basis of the structure-activity results, it was possible to infer the existence of an asymmetric receptor site for camptothecin on the topo I enzyme or enzyme-DNA complex. Moreover, the inhibition of topo I agreed well with previously published antitumor data, with only 3 discrepancies among 21 compounds compared. Thus topo I (or conceivably another enzyme bearing the same stereospecific receptor site) is the target for the antitumor action of camptothecin.

DNA Sequence-selective Alkylating Agents

It is known that alkylating agents and platinum complexes react selectively with guanine residues in DNA. It is also known that DNA sequence regions unusually rich in G's occur in the mammalian genome, including certain parts of oncogene sequences. This suggested to us that selective reaction in G-rich regions may somehow be an important factor in antitumor activity. Therefore we have been working towards an understanding of the factors that govern the sequence selectivity of DNA alkylation. We are also asking the question of whether hot spots for alkylation do in fact occur in the mammalian genome, and whether their presence affects drug sensitivity. Our previously reported findings showed that nitrogen mustards react preferentially with

G's that are flanked by other G's, and especially within runs of several successive G's. Runs of adjacent G's are statistically more frequent in G-rich regions, and adjacent G's can be sites where bifunctional alkylating agents can form cytotoxic intrastrand crosslinks. We found that the sequence selectivity of nitrogen mustards could be explained in part, in terms of the electrostatic environment in the immediate vicinity of the DNA bases. We have also shown that this sequence selectivity is specifically altered in certain nitrogen mustard structures. Our current work is aimed towards the design of new alkylating agents optimized for preferential reaction with G-rich regions, or other specific sequences, and towards the possibility that selective targeting of certain genomic regions could lead to more specific antitumor drugs.

We have now developed hypotheses using computer aided molecular modelling to account for the observed specific sequence preferences of uracil mustard and quinacrine mustard, and we have begun to investigate compounds designed by molecular modelling to test these hypotheses. Methods are also being established to investigate preferential reactivities in intact cells and to relate them to drug sensitivity.

DNA Damage and Repair in Specific Regions of the Genome

Previous work had shown that mammalian cells have the capability to repair DNA selectively in regions that are transcriptionally active, and that this capability helps the cells to survive DNA damaging treatments.

A significant step was taken towards an understanding of the factors which govern the repair of DNA lesions preferentially in essential genes. DNA repair deficient Chinese hamster cells were transfected with a prokaryotic or a human repair gene in order to determine whether DNA repair function introduced by these genes would discriminate between active and inactive genes. It was found that the transfected phage T4 repair endonuclease (denV gene) conferred DNA repair equally in a transcriptionally active and an inactive genome region. However transfection with the human ERCC-1 gene conferred repair capability preferentially to the transcriptionally active region. Human cells of the severe DNA repair deficiency form of xeroderma pigmentosum have recently been transfected with the denV gene; studies are in progress to determine whether the restored repair capacity is preferential for active gene regions.

Cells from patients with cancer-prone and DNA repair deficiency syndromes, such as Cockaynes syndrome, dysplastic nevus syndrome, Bloom's syndrome and Gardiner's syndrome, are being studied to determine whether these cells are deficient in the preferential repair of active genes, including proto-oncogenes.

Although these studies have mainly concerned ultraviolet light damage of DNA, which has been very extensively studied and is relatively well understood, we are doing the necessary groundwork for analogous investigations of DNA damage produced by anticancer drugs, especially alkylating agents and platinum complexes. Since the techniques used in the previous studies relied upon T4 endonuclease V which specifically cleaves DNA only at sites of UV dimer lesions, the planned studies of alkylation and platination lesions will

require other means to reveal the sites of damage. Although chemical means of cleaving DNA at some alkylation sites are available, extensive attempts to develope such a method for platinum complexes have failed. Therefore, the ABC endonuclease complex of E.coli, which is known to cleave DNA at a variety of adducts, is being investigated for its applicability to this problem.

Histone H2A Gene Family

An understanding of the abnormalities in the regulation of cell proliferation in malignant cells may open new possibilities for therapy. The study of oncogenes is a promising approach being pursued in many laboratories. Our Laboratory is concentrating on another, possibly complementary aspect, the regulation of histone variant genes. Although the synthesis of the major nucleosomal histones is regulated in synchrony with DNA synthesis, certain variant histones are regulated differently. Previous work in our Laboratory identified and characterized a minor histone variant, which we designated H2A.z, and showed that synthesis of this protein is regulated according to the proliferative state of the cells, ie G0 state versus cycling state. It was therefore undertaken to isolate the H2A.z gene, so that its manner of regulation can be investigated. We have cloned and sequenced the cDNA of H2A.z genes from human, cow and rat, and found the gene to be highly conserved in these species. The cDNA sequences are being used to isolate and investigate the genomic sequences so as to disclose the regulatory regions of the gene. Work is also in progress to look for clones of the H2A.x variant which has a different pattern of regulation.

Several findings from the nucleotide sequence of the H2A.z cDNA sequence, which is essentially the sequence to the mRNA, are noteworthy in regard to the regulation of the gene. The general structure of the message was found to differ markedly from that of the histones that are regulated in synchrony with DNA synthesis, in that it lacks some of the unusual features of these histones: it has a long 3'untranslated region and a poly-A tail. These feature differences may well be relevant to the differences in regulation; in this regard, it is noteworthy that the 3' untranslated regions of the H2A.z sequences were even more highly conserved than the part of the sequence homologous to the major H2A genes. Aspects of the sequence structure are consistent with the possibility that the differences in regulation could in part be due to differences in message stability. This bears on the studies of the kinetic behavior of the histone pools which are in progress.

Regulation of Histone Biosynthesis and the Control of Cell Proliferation

Since DNA replication in mammalian cells can be limited by the availability of histones, our Laboratory has been investigating the factors affecting the kinetics of histone biosynthesis, degradation and incorporation into chromatin. This was made possible by new methodology recently developed in our Laboratory to measure the low concentrations of histones in soluble pools in the cell. The previous work led to the hypothesis that the levels of soluble histones regulate the concentration of histone mRNA by stabilizing or destabilizing the mRNA, and in particular that there is a phenomenon of chromosome cycle compensation in which histone mRNA levels rise to maintain the rate of histone synthesis when overall protein synthesis is partially

inhibited. The hypothesis accounts for the observed effects of inhibitors of protein or DNA synthesis on the level of histone mRNA and on histone pool kinetics in proliferating cells. H2a and H2b were found to behave differently from H3 and H4, in that the former may move in both directions between cytoplasm and chromatin, whereas the latter move only one way: into chromatin. The different histone classes therefore may have individual influences on the state of chromatin. These studies are being extended to include quiescent cells.

The events during the transition from proliferation to quiescence are being investigated. Failure to carry out this transition normally, may be essentially what makes cells malignant. It was found in non-malignant cells that when the cells are put into serum-free medium, which causes the cells to become quiescent after completing the current S-phase, histone synthesis remains undiminished even though overall protein synthesis immediately drops to the level characteristic of quiescent cells. This at first puzzling behavior can be understood in terms of the above-mentioned chromosome cycle compensation model.

The mechanism of the transition to quiescence may be further elucidated by the study of the synthesis of histone H2A.z, because H2A.z synthesis is unusual in that it does not vary much during the cell cycle, but drops 20-fold when cells go into quiescence. The availability of H2A.z mRNA now makes it possible to look for transcriptional regulator molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06140-12 LMPH

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Histone Biosynthesis

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

PI:	William Bonner	Sr. Investigator	LMPH NCI
Others:	Concepcion Muneses	Chemist	LMPH NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NIH, NCI
Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.6	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our major objective is to elucidate factors involved in the interaction of the histone proteins with chromatin both in concert with DNA replication as in S phase cells and when DNA replication is absent as in G1 and quiescent or G0 cells. We have developed methodology which allows us to study soluble histone not bound to chromatin and alterations in the level of soluble histone in different cell growth states and during changes in the rates of protein or DNA synthesis. Recently we published a model which suggested that level of soluble histone was involved in the balancing of histone and DNA synthesis. In contrast to earlier ideas, this model viewed the level of soluble histone as instrumental in the stabilization of histone mRNA when protein synthesis was inhibited as well as in its destabilization when DNA synthesis was inhibited. Results of kinetic studies of soluble histone currently in press show that there are multiple kinetic components. Overall the results are consistent with the previously proposed model. Presently, these kinetic studies are being extended to include quiescent cells.

Two other directions are being pursued. The first direction is the characterization of soluble histone H1; this study has required some changes in our methodology. H1 is of interest because it binds to the outside of the nucleosome and has been implicated in the transcriptional repression of certain genes.

The second direction, which should lead to the next major phase of our investigations of histone metabolism, is to isolate and characterize factors which interact with soluble histone using typical biochemical techniques and assays developed during the kinetic studies.

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

Project Description

Introduction:

Histone protein synthesis and DNA synthesis are closely co-ordinated; inhibition of one leads to inhibition of the other. However, when DNA synthesis is inhibited, histone mRNA levels fall and when protein synthesis is inhibited, histone mRNA levels rise. Recently we published a model which suggested that inhibition of protein synthesis led to the inhibition of DNA synthesis by the depletion of histone from the soluble cellular fraction. In contrast to earlier models, this one viewed the resulting stabilization of histone mRNA as part of the same process that led to its destabilization when DNA synthesis was inhibited.

Methodology has been developed which allows one to study the flux of histone through the soluble cellular fraction and alterations in that rate of flux when protein or DNA synthesis has been inhibited. These methods can also be applied to cells in different states of growth (G1, G0, and S).

Objectives:

1) The development of greater understanding of the molecular mechanisms regulating chromatin biosynthesis and metabolism during the cellular states of proliferation and nonproliferation.

2) The characterization of chromatin or cellular components that may be involved in these regulatory mechanisms. At present soluble histone is receiving most of our attention.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional eletrophoresis. (Methods developed in this laboratory).

(2) Peptide analyses on acrylamide gels to determine the relationship of proteins to each other. (Method developed in this laboratory).

(3) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(4) Maintenance of cells and nuclei in viable non dividing states using modified and defined media.

(5) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells. Cell free translation of mRNA.

(6) Biochemical techniques such as sucrose and glycerol gradient centrifugation, isoelectric focusing, agarose gel electrophoresis.

Major Findings and Accomplishments:

1. Development of Methodology to Study Soluble Histone

Soluble histone has not been studied in any systematic way because of several technical difficulties. These include the small amount of material, the problems of purifying histone proteins from cytoplasmic supernatants, and the problem of distinguishing soluble histone from possibly contaminating chromatin histone.

We have adapted our methodology for the analysis of histone variants in chromatin to the analysis of histones in the cytoplasm and have overcome most of these problems. Newly synthesized H4 in the cytoplasm is doubly modified, by an acetate and a phosphate. When cytoplasm is prepared by gentle lysis of cells with a nonionic detergent, then extracted with HCl and the extract freeze dried for electrophoresis on AUT-AUC gels, the pattern of cytoplasmic histone shows doubly modified H4 as well as the absence of ubiquitin adducts of the H2A's. Thus cytoplasmic histone can be analyzed with little or no contamination from nuclear histone.

2. Investigations of the Cytoplasmic Histone Pool

We plan to use these newly developed methods to study several aspects of soluble histone. The first set of questions concern the relationship of soluble histone to chromatin histone during normal S-phase. Do histones flux in one direction only from polyribosomes, through the soluble fraction to chromatin, or do histones flux out of chromatin into the soluble fraction? Are soluble histones degraded? The second set of questions concern how the level of soluble histone reacts to the inhibition of DNA and/or protein synthesis. Do the levels of soluble histone change in such a way as to be consistent with autoregulation. The third set of questions concern the level and flux of histone in G1 and G0 as well as in S, and the transition between these states.

Results currently being prepared for publication show that when protein synthesis is inhibited, the rate of DNA synthesis falls in a biphasic manner. The initial fall has a half time of about 1 min. When the rate of DNA synthesis has fallen to 25% of the initial rate, the rate then falls more slowly with a half time of about 40 min. The rate of soluble histone depletion is also biphasic. The initial decrease in the rate of DNA synthesis coincides with the initial rate of soluble histone depletion. When soluble histone is depleted to about 50%, the rate of depletion slows to a rate similar to the slower decrease in the rate of DNA synthesis. These results are completely consistent with our model that DNA synthesis can be physiologically limited by the availability of histone.

When DNA synthesis is inhibited, the soluble histone level is elevated, with H4 and H3 being elevated more than H2B and H2A. The latter two histones continue to be incorporated into chromatin for sometime in the absence of DNA synthesis. We have also been able to demonstrate that H2B and H2A from prelabeled nuclei can be found in the cytoplasm while H4 and H3 are not. This result suggests that H2B and H2A can exchange between the nucleus and

cytoplasm in both directions.

Further experiments with G1 cultures have shown that H4 and H3 are elevated in the cytoplasm, while H2B and H2A are not detectable. This finding suggests that the same mechanism is operating in G1 cells as in S cells with inhibited DNA synthesis. Experiments with G0 cells are in progress.

Manuscripts for these findings are currently in press.

3. Biochemical Studies with Soluble Histone

The purpose of this project is to isolate and characterize factors which interact with soluble histone using typical biochemical techniques and assays developed during the kinetic studies. After trying isoelectric focusing and agarose gel electrophoresis, we have been using centrifugation through sucrose or glycerol gradients as a means of characterizing complexes. Most of the newly-labeled soluble histone sediments as a 10s particle or particles. We are currently engaged in further characterization of these particles.

Significance to Biomedical Research and Program of the Institute:

Cancer at one level is the inappropriate multiplication of cells. Our findings during the last few years have suggested that analysis of histone variant synthesis and the histone variant genes may yield some insight into the relationship of different cell states in normal and neoplastic cells.

Proposed Course:

1. To characterize soluble histone and to compare the predictions of the published model to the behavior of soluble histone.

2. To develop methodology to study the selective sensitivity of histone mRNA.

3. To isolate and characterize factors which interact with soluble histone using typical biochemical techniques and assays developed during the kinetic studies.

4. To investigate the biochemical aspects of the exchange of H2B and H2A from nuclei to cytoplasm.

Publications:

Bonner WM, Wu RS, Panusz HT, Muneses C. Kinetics of accumulation and depletion of soluble newly synthesized histone in the reciprocal regulation of histone and DNA synthesis, Biochemistry, in press.

Bonner WM, Wu RS, Panusz HT, Muneses C. Qualitative and kinetic characterization of soluble histone pools: linkage between protein and DNA synthesis during the cell cycle, Cancer Cells, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06150-07 LMPH

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Protein-associated DNA Breaks as Indicator of Topoisomerase Inhibition (modified title)

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

PI:	Yves Pommier	Visiting Associate	LMPH NCI
Others:	Joseph Covey	Sr. Staff Fellow	LMPH NCI
	Donna Kerrigan	Chemist	LMPH NCI
	Christine Jaxel	Visiting Fellow	LMPH NCI
	Kurt W. Kohn	Lab Chief	LMPH NCI

COOPERATING UNITS *(if any)*

Lata Dusre (Visiting Fellow) and Birandra Sinha (Sr. Investigator) Laboratory of Clinical Pharmacology, DCT, NCI

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION NIH, NCI
Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	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.)*

DNA topoisomerases have been identified as major targets for cancer chemotherapy. Two types of topoisomerases have been isolated from eukaryotic cell nuclei. Both topoisomerases I & II relax DNA supercoiling and play a crucial role in DNA replication and transcription. However, topoisomerase II is the only enzyme able to anchor DNA loops to the nuclear matrix and to decatenate newly replicated DNA circles. Two families of anticancer drugs inhibit topoisomerase II: DNA intercalators, such as amsacrine (m-AMSA), adriamycin and ellipticine derivatives, and demethylepipodophyllotoxins, such as etoposide (VP-16) and teniposide (VM-26). Drug-induced topoisomerase II inhibition results in the formation of protein-associated DNA breaks, which can be detected by alkaline elution. We have shown now, that the specific topoisomerase I inhibitor, camptothecin produces also protein-associated DNA breaks. Our recent finding that the DNA breaks induced by topoisomerase inhibitors are associated with strong and poorly reversible DNA synthesis inhibition, and that aphidicolin prevents the cytotoxicity of topoisomerase inhibitors suggest that drug-induced topoisomerase complexes could exert their cytotoxic action by producing stable topoisomerase-DNA complexes which would destabilize DNA replication complexes. Finally, we have found that a human breast cancer cell line (MCF-7) resistant to adriamycin has topoisomerase II modifications, which are associated with the drug uptake reduction usually encountered in pleiotropic resistant cell lines.

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

Objectives:

1. Characterize the production of protein-linked DNA strand breaks by camptothecin, an inhibitor of topoisomerase I, in mammalian cells in culture.

2. Study the role of DNA topoisomerases in DNA replication, and the cellular events leading to cell death upon exposure to antitumor topoisomerase inhibitors.

3. Determine whether topoisomerase II modifications play a role in drug resistance in pleiotropic resistant cells.

4. Isolate topoisomerases from resistant cells in order to determine the molecular alterations responsible for drug resistance.

Methods:

1. Standard cell culture and synchronization methods.

2. Clonogenic assays to measure drug-induced cytotoxicity.

3. Alkaline elution methodology to determine drug-induced topoisomerase-mediated DNA strand breaks and DNA-protein crosslinks.

4. Isolation and preparation of cell nuclei and nuclear extracts. Purification of nuclear extracts by FPLC and glycerol gradient centrifugation from DNA.

5. DNA topoisomerase assays with SV40 DNA: filter binding and agarose gel electrophoresis to identify enzyme-DNA links and DNA topoisomers and cleavage; autoradiography to determine drug-induced topoisomerase-mediated DNA cleavage patterns.

Major Findings:

1. Protein-linked DNA Single-Strand Breaks Induced in Mammalian Cells by Camptothecin, an Inhibitor of Topoisomerase I

Camptothecin has been reported to inhibit purified mammalian topoisomerase I. We have examined camptothecin-induced SSB and DPC in Chinese hamster DC3F cells and their isolated nuclei using the DNA alkaline elution technique. It was found that SSB and DPC frequencies depend upon the conditions used for lysis. When lysis was with sodium dodecyl sulfate, the observed frequencies of SSB and DPC were 2-to 3-fold greater than when sodium dodecyl sarkosinate was used. In either case, the SSB:DPC ratio was close to one. All of the camptothecin-induced SSB were protein-linked, as indicated by the absence of DNA elution under non-deproteinizing conditions. DNA cleavage assays with purified topoisomerase I gave comparable results. In contrast, lysis conditions had little effect on levels of SSB or DPC produced by *m*-AMSA, suggesting that trapping of topoisomerase II complexes occurs equally well with either detergent. In experiments using isolated nuclei, it was found that the camptothecin-induced SSB can form and reverse within minutes at 4°C. The activity of camptothecin at low temperature was also seen with purified topoisomerase I. We conclude that camptothecin-induced DNA lesions are entirely attributable to protein-linked strand breaks, representing trapped topoisomerase I complexes, and that these complexes tend to be more labile than the analogous topoisomerase II complexes.

2. Role of DNA Replication in the Cytotoxicity of Topoisomerase Inhibitors

We had found that: 1) the frequency of drug-induced topoisomerase II-mediated DNA breaks is greater in proliferative and S-phase cells than in quiescent and G_1 cells; 2) drug-induced cytotoxicities are also greater in proliferative than in quiescent cells (Markovits et al., Cancer Res. 47: 2050, 1987); 3) m-AMSA and VP-16 produce more protein-associated DNA breaks in replicating than in template DNA, and 4) drug-induced topoisomerase II DNA linking sites are approximately at replicon-size distance from each other (unpublished results). These findings prompted us to investigate whether DNA synthesis inhibition by aphidicolin would affect drug-induced protein-associated DNA breaks and cytotoxicity. Aphidicolin (10 μM) treatments decreased the cytotoxicity of m-AMSA and VP-16 by approximately a factor of 2, but had no effect upon drug-induced DNA breaks. Similar experiments were performed with the topoisomerase I inhibitor, camptothecin. Aphidicolin did not affect camptothecin-induced DNA breaks, but protected completely against its cytotoxicity. An additional finding was that both inhibitors of topoisomerase II (m-AMSA and VP-16) and topoisomerase I (camptothecin) inhibit DNA synthesis rapidly and for several hours, even after 30 min drug exposures. Taken together these results suggest that drug-induced topoisomerase II or I complexes could exert their cytotoxic action by producing stable topoisomerase-DNA complexes which would destabilize DNA replication complexes and inhibit their normal activity.

3. Relationship Between Drug-Induced Topoisomerase II-mediated DNA Damage and Cytotoxicity. DNA strand breaks produced by VP-16 in sensitive and resistant Human breast cancer (MCF 7) cells

Several laboratories, including ours (Pommier et al., Cancer Res., 1987) have shown that resistant cells form markedly less drug-induced topoisomerase II-mediated DNA breaks than sensitive cells. Adriamycin is the most widely used topoisomerase II inhibitor in cancer chemotherapy. Adriamycin resistant cells belong to the group of pleiotropic drug-resistant cell lines. The present study was undertaken in order to investigate whether topoisomerase II alterations are also present in a pleiotropic resistant breast cancer cell line (MCF-7), selected for resistance to adriamycin. The resistant cells were found to be cross-resistant to VP-16 (125-200-fold). Alkaline elution studies showed that VP-16 produced much less DNA breaks in the resistant than in the sensitive cells (100-200-fold), indicating a good correlation between DNA breaks and cytotoxicity. Isolated nuclei from resistant cells formed only 2-fold less VP-16-induced DNA breaks than those from sensitive cells. In agreement with this result, nuclear extracts from resistant cells produced 2-3-fold less VP-16-induced DNA breaks than those from sensitive cells. VP-16 uptake measurements showed a 2-3-fold decreased drug cellular accumulation in resistant cells. These results indicate that this multidrug resistance of the MCF-7 cell line is multifactorial.

4. Topoisomerase Modifications in Drug Resistant Chinese Hamster Cells

We had previously reported the presence of an unusual topoisomerase activity with high DNA-linking properties in a line of Chinese hamster cells (DC3F/9-OHE) resistant to 9-hydroxyellipticine and other topoisomerase II inhibitors. We have now purified the enzyme to homogeneity by anion exchange chromatography. The purified enzyme protein migrated as a single band (silver stain, SDS-PAGE) with a molecular weight of 68 kDa (L1210 or HeLa topoisomerase I migrate at 95 kDa). In contrast to topoisomerase I, the new enzyme exhibited strong DNA-linking activity in filter binding assays. The

DNA-linking activity was stimulated by ATP and not by m-AMSA. The enzyme relaxed supercoiled SV40 DNA in the absence of ATP. ATP did not affect the rate of supercoil relaxation,but markedly enhanced the nicking of DNA circles even in the absence of drug (studied in chloroquine-containing electrophoretic gels). The optimum conditions for relaxation of supercoiled DNA were 250 mM KCl and 10 mM $MgCl_2$, similar to topoisomerase I. The enzyme decatenated kinetoplast DNA in the presence of 1mM ATP, under conditions in which topoisomerase I had little or no effect. Camptothecin, stimulated the new enzyme to cleave end-labeled SV40 DNA at sites identical to those seen with topoisomerase I; inhibitors of topoisomerase II (m-AMSA, etoposide, norfloxacin and nalidixic acid) produced no DNA cleavage. We conclude that the new enzyme is similar to topoisomerase I except for its ATP-stimulated DNA-linking and nicking activities, its decatenation activity and its lower molecular weight.

Proposed Course:

1. Study the interactions between topoisomerase II and topoisomerase I inhibitors with respect to cytotoxicity and protein-associated DNA breaks.

2. Design a new DNA cleavage assay for topoisomerase inhibitors; nuclear extracts and [^{32}P]-end labeled DNA will be used. Such an assay should allow the comparison of the topoisomerase-mediated DNA cleavage activities from sensitive and resistant cell lines and to determine the role of topoisomerase modifications in the mechanism of resistance of pleiotropic resistant cell lines.

3. Isolate DNA topoisomerases from sensitive and resistant cell lines by FPLC and determine their chemical, enzymic, and drug sensitivity characteristics. A long term project is to isolate the gene(s) of the modified topoisomerases for resistant cells.

4. Determine whether some genetic diseases are associated with topoisomerase alterations.

Publications:

Pommier Y, Kerrigan D, Kohn KW. Topoisomerase alterations associated with drug resistance in a line of Chinese hamster cells, Natl Cancer Inst Monogr 1987;4:83-7.

Kerrigan D, Pommier Y, Kohn KW. Protein-linked DNA strand breaks produced by etoposide (VP-16) and teniposide (VM-26) in mouse L1210, and human VA-13 and HT-29 cell lines. Relationship to cytotoxicity, Natl Cancer Inst Monogr 1987;4:117-21.

Kohn KW, Pommier Y, Kerrigan D, Markovits J, Covey J. Topoisomerase II as a target of anticancer drug action in mammalian cells, Natl Cancer Inst Monogr 1987;4:61-71.

Pommier Y, Kerrigan D, Covey JM, Kao-Shan C S, Whang-Peng J. Sister chromatid exchanges, chromosomal aberrations, and cytotoxicity produced by antitumor topoisomerase II inhibition in sensitive (DC3F) and resistant (DC3F/9-OHE) Chinese hamster cells, Cancer Res 1987;48:512-16.

Covey J., Kohn KW, Tilchen EJ, Pommier, Y. Topoisomerase II-mediated DNA damage produced by 4'-(9-acridinylamino)methanesulfon-m-anisidide (m-AMSA) and related acridines in L1210 cells and isolated nuclei: relation to cytotoxicity, Cancer Res 1987;48:860-65.

Objectives:

1. Determine the molecular interactions between topoisomerase II and DNA, and the sites and mechanism of action of anticancer drugs.

2. Study the inhibition of topoisomerase I by camptothecin and analogs, and determine whether this inhibition is responsible for the antitumor activity.

Methods:

1. Purification of DNA topoisomerases from mammalian cells in culture by FPLC chromatography (anion exchange) and glycerol gradient centrifugation.

2. [^{32}P]-end labeling of SV40 DNA fragments.

3. Agarose gel electrophoresis (+/- chloroquine) to separate supercoiled, relaxed, cleaved, nicked and catenated DNA molecules. Filter binding assays to detect topoisomerase DNA complexes.

4. DNA sequencing gels and autoradiography to determine the DNA sequence at topoisomerase-induced DNA cleavage sites.

Major Findings:

1. Topological Complexes Formed between L1210 Topoisomerase II and SV40 DNA and the Effects of Polyamines

The polyamines, spermine and spermidine, were found to enhance the formation of a stable non-covalent complex between mammalian topoisomerase II and DNA. This complex is not associated with DNA strand breaks, is associated with a stimulation of the enzymatic relaxation of DNA supercoils, and forms to a greater extent with supercoiled than with relaxed circular or with linear DNA. In these respects, the polyamine-enhanced complex differs from the covalent cleavable complexes stabilized by DNA intercalators such as amsacrine (m-AMSA) or epipodophyllotoxins such as teniposide (VM-26). In the polyamine enhanced complex, the topoisomerase II may be a bracelet-like structure topologically bound to the DNA and able to migrate and dissociate from the ends of linear DNA molecules. At relatively high concentrations, spermine (1 mM) enhances topoisomerase II-induced cleavage at certain sites on the SV40 genome that could have regulatory significance.

2. Topoisomerase II Inhibition by Anticancer Drugs is Antagonized by Distamycin

Antitumor topoisomerase II inhibitors, such as amsacrine, 5-iminodaunorubicin, and VM-26 have been shown, when reacted with purified enzyme, to produce sequence-specific DNA cleavage. By adding distamycin, a compound which binds to the DNA minor groove at A-T sequences specifically, to such reactions, we investigated the effects of DNA structural alterations upon drug-induced topoisomerase II-mediated DNA cleavage in [^{32}P]-end labeled SV40 DNA. Distamycin (1 μM) affected drug-induced DNA cleavage patterns, both by suppressing certain cleavage sites and enhancing others. The major enhancement sites were the same in the case of m-AMSA and VM-26. In the case of 5-Iminodaunorubicin, the only effect of distamycin was to suppress the major

topoisomerase II-induced cleavage site produced by this drug. Next, distamycin effects were examined at the nucleotide level in a region of the SV40 genome (nucleotides 4450-4600), where distamycin altered VM-26-induced topoisomerase II-mediated DNA cleavage markedly. Drug binding was determined by hydroxyl radical footprinting and topoisomerase II-mediated DNA cleavage by sequencing gel analysis. Distamycin binding suppressed VM-26-induced topoisomerase II-mediated DNA cleavage directly at drug binding sites and enhanced cleavage in the near proximity (within 20 bp) of distamycin binding sites by a propagated allosteric effect in DNA. In addition, distamycin enhanced topoisomerase II-mediated DNA relaxation and antagonized the inhibitory effect of VM-26. These results indicate that drug-induced DNA conformation alterations affect topoisomerase II-DNA interactions.

3. Structure-activity Study of the Relation Between Topoisomerase I Inhibition and Antitumor Activity of Camptothecin

Camptothecin is a potent antitumor agent which has been shown to be a specific inhibitor of mammalian topoisomerase I. In order to test the relationship between antitumor activity and topoisomerase I inhibition, we examined the effects of 21 camptothecin analogs on the ability of topoisomerase I (purified from mouse leukemia L1210 cells) to relax supercoiled SV40 DNA. Studies of substitutions on ring E of camptothecin showed that the 21-lactone and 20-OH are essential for topoisomerase I inhibition, and that the stereochemistry at position 20 is crucial (the natural S isomer being effective whereas the R isomer is not). Studies of ring A substitutions showed that the addition of NH_2 or NO_2 at position 12 abolished topoisomerase I inhibition, while similar substitutions at positions 9, 10, or 11, did not. 10,11-methylenedioxy-camptothecin was more effective than camptothecin, whereas 10,11-dimethoxy-camptothecin was ineffective. A similar structure dependence was observed for topoisomerase I-mediated nicking of SV40 DNA; hence the inhibition of supercoil relaxation is probably due to the stabilization of topoisomerase I-DNA complexes. The structure dependence of effectiveness in the topoisomerase I assays correlated well with the previously reported activity against experimental leukemias in mice. The sequence localization of camptothecin-induced DNA cleavage sites was determined in [^{32}P]-end-labeled SV40 DNA. All active derivatives generated similar DNA cleavage patterns. The results suggest that camptothecin binds to a specific receptor within topoisomerase I-DNA complexes, trapping the enzyme at crucial genomic regulatory sites. The structure-activity findings provide good evidence that effects on topoisomerase I are responsible for the antitumor action of the camptothecins.

4. Genomic Localization of Mouse Leukemia (L1210) Topoisomerase I-mediated DNA Strand Breaks Induced by Camptothecin in SV40 DNA

Topoisomerase I inhibition by camptothecin results in the formation of topoisomerase I-linked DNA strand breaks. The genomic and DNA sequence localization of these topoisomerase I-mediated DNA breaks were determined by analyzing autoradiographies of neutral, alkaline 1% agarose or DNA sequencing gels of the reaction products of purified L1210 topoisomerase I with [^{32}P]-3'-end-labeled SV40 DNA (restriction fragment Ban I-Hpa II). Densitometer scanning of the autoradiographies of agarose gels were analyzed by computer with respect to the migration positions of [^{32}P]-labeled standards. The standards. The general conclusions of the analysis are that: 1) camptothecin-induced topoisomerase I-mediated DNA cleavage is predominantly single-stranded, since it is best detected by using DNA denaturing gels; 2) camptothecin-induced DNA cleavage is

sequence selective, and most pronounced at a single site located near the origin of the early transcription region (T-antigens coding sequence); 3) camptothecin-induced DNA cleavage patterns were similar in supercoiled and linear DNAs; 4) The DNA sequence was determined at the major cleavage site (nucleotide position 4955); 5) Prominent cleavage sites also occur at a similar sequence in each of the two 72 bp-repeats in the major regulatory region of the SV40 genome; 6) The consensus sequence at the drug-induced cleavage sites was also found in pBR322 DNA.

5) Prominent cleavage sites also occur at a similar sequence in each of the two 72 bp-repeats in the major regulatory region of the SV40 genome; 6) The consensus sequence at the drug-induced cleavage sites was also found in pBR322 DNA.

Proposed Course:

1. Complete the genomic distribution mapping of drug-induced topoisomerase II cleavage sites in SV40. Determine the role of chromatin upon the cleavage sites distribution by studying SV40 minichromosomes.

2. Study topoisomerase binding to DNA in order to determine whether the DNA molecule wraps around the topoisomerase I (or II) molecules; carry out DNA footprinting of topoisomerase I with the SV40 DNA fragment where is located the major enzyme cutting site.

3. Investigate the molecular mechanisms of topoisomerase I inhibition by camptothecin. [^{3}H]-camptothecin will be used to determine whether drug-induced enzyme inhibition is due to drug binding to topoisomerase I or to topoisomerase I-DNA complexes.

4. Study topoisomerase I inhibition by other camptothecin analogs. This could lead to the discovery of more potent antitumor drugs and to a better definition of the topoisomerase I-DNA receptor for camptothecins.

Publications:

Pommier Y, Covey J, Kerrigan D, Pham R. DNA unwinding and inhibition of mouse leukemia L1210 DNA topoisomerase I by DNA intercalators, Nucleic Acids Res 1987;15: 6713-31.

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Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I, evidence for a specific receptor site and for a relation to antitumor activity, Proc Natl Acad Sci USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06170-04 LMPH

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of the Histone H2A Gene Family (modified title)

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

PI:	Christopher L. Hatch	Sr. Staff Fellow	LMPH NCI
Other:	William M. Bonner	Sr. Investigator	LMPH NCI
	Concepcion Muneses	Chemist	LMPH NCI

COOPERATING UNITS *(if any)*
Dr. Paul Swerdlow, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION NIH, NCI
Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1

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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 nucleotide sequences of cDNA's for the evolutionarily diverged but highly conserved basal H2A isoprotein, H2A.Z, have been determined for the rat, cow, and human. Each of the cDNA isolates encodes the entire H2A.Z polypeptide. The human isolate is about 1.0 kilobase long. It contains a coding region of 387 nucleotides flanked by 106 nucleotides of 5′ untranslated region (5′UTR) and 376 nucleotides of 3′ untranslated region (3′UTR), which contains a polyadenylation signal followed by a poly A tail. The bovine and rat cDNA's have 97 and 94% nucleotide positional identity to the human cDNA in the coding region and 98% in the proximal 376 nucleotides of the 3′UTR which includes a polyadenylation signal. A potential stem-forming sequence imbedded in a direct repeat is found centered at 261 nucleotides into the 3′UTR. Each of the cDNA clones could be transcribed and translated to yield H2A.Z protein.

A human genomic library in lambda has been screened with the human H2A.Z cDNA and the genomic form of the H2A.Z gene has been isolated. The 5′ half of the gene contains at least two introns. We are utilizing exon- and intron-specific H2A.Z DNA to probe restriction enzyme cleaved human genomic DNA in order to be able to determine the copy number of the functional H2A.Z gene as well as the presence or absence of processed pseudogenes. Sequences upstream and downstream from the H2A.Z gene are being determined and the regulatory elements will be studied.

The human genomic-lambda library has also been screened with the cDNA complement of a chicken major H2A variant gene and clones of the human H2A.1 gene have been isolated and partially sequenced. This gene has been used to isolate plasmid clones from a human cDNA library which are likely to be representative of the gene for another minor histone variant, H2A.X.

Project Description

Introduction:

The expression of different subsets of histone variant genes is very much related to the state of cell proliferation and cycling. This interrelationship suggests that changes in the chromatin composition, whether generalized or defined to particular locations, may influence chromatin structure and function, and, in turn, cell growth and homeostasis. Isolation and characterization of the particular histone variant genes will allow elucidation of how they themselves are differentially regulated.

Objectives:

Isolation and characterization of the human histone H2A gene family. We have cloned and sequenced the cDNA complements of the gene for the minor histone variant H2A.Z from three different mammalian species; human, cow, and rat. Although the synthesis of H2A.Z is not linked to DNA replication, it is still regulated with a 20 fold difference in expression between cycling and quiescent cells. In this regard, its regulation is perhaps more similar to that of proteins such as the myc oncogene product. We are at present analyzing the genomic organization of the gene for H2A.Z as well as the regulation of its expression during different states of cell growth and proliferation. The gene for the major histone H2A variant, H2A.1, has been isolated from a human genomic-lambda library and this gene has been utilized to isolate a number of cDNA clones potentially encoding the minor histone H2A variant, H2A.X. These clones will soon be analyzed by subcloning and sequencing. Several lines of evidence suggest that H2A.Z may be enriched in transcriptionally active chromatin. We have therefore arranged a collaboration with Dr. Paul Swerdlow of the Medical College of Virginia to study the effect on yeast phenotype when yeast mutant only in the expression of their own H2A variants are forced to express and utilize only mammalian major (H2A.1) variant H2A protein or mammalian minor (H2A.X) variant H2A protein.

Methods:

1. Recombinant DNA techniques.

2. Use of two dimensional gel electrophoresis for identification of histone variant proteins.

3. Rapid RNA methodology developed in this laboratory.

Major Findings and Accomplishments:

Isolation of Genes in the Histone H2A Isoprotein Family

As described in the Summary section we have isolated the cDNA and genomic forms of human histone H2A.Z and the genomic form of human histone H2A.1. We have also isolated cDNA clones which are likely to be representative of the gene for human histone H2A.X. The following paragraphs are presented as an elaboration of some of our findings and goals.

Analysis of Factors Which Affect Histone mRNA Utilization and Metabolism

A major component in the stability of mRNA's for the replication-linked histone isoproteins is inherent in the message structure. In general, mRNA's for replication-linked histone isoproteins contain only short 5' and 3' untranslated regions, are not polyadenylated, and contain a conserved stem-loop structure at the 3' end of the mRNA. In contrast, the mRNA's of the basal histone isoproteins appear to have longer 3' untranslated regions with a terminal poly A tail and do not contain the stem-loop structure at the 3' end of the mRNA. The mammalian H2A.Z 3' untranslated regions are more highly conserved than are their coding sequences, suggesting that these sequences have some sort of functional role. The mammalian H2A.Z cDNA 3'UTR is 64% AT and contains sequences which have been shown to be the kind of sequences that bind various non-histone proteins. We are at present studying the H2A.Z gene transcription and message stability in different states of cell cycling and proliferation (see WMB section). In addition, we will search for nuclear and cytoplasmic factors which might specifically bind the aforementioned sequences in the DNA and mRNA complements of this gene.

Yeast as a model system in which to study the roles of major and minor histone variant proteins in chromatin structure and function

While H2A.Z seems to be the H2A isoprotein that is least similar to its counterparts, its sequence also appears to have been highly conserved during evolution. The histone H2A.Z cDNA's from human, cow, and rat encode identical proteins. The cDNA's for the H2A.Z homologous gene has been identified in chicken, sea urchin, Tetrahymena, and Drosophila. The overall similarity between mammalian and urchin H2A.Z is 95%, but there is only a 59% homology between mammalian H2A.Z and H2A.1. Between H2A.Z residues 39 to 122, which are identical in mammal and urchin, there are 32 substitutions between mammalian H2A.Z and H2A.1. However, despite an overall similarity of only 59%, there are three regions of much higher similarity. Through evolution there appears to have been a conservation of certain polypeptide sequences to allow all H2A molecules to have a certain structural and functional commonality. By the same token, the allowed sequence differences may give each of the different H2A variants unique structural and functional capabilities. This is particularly relevant in light of evidence which suggests that H2A.Z may be enriched in transcriptionally active chromatin. It is precisely the aforementioned subtlety of difference between H2A.Z and its major variant counterparts that has made the isolation and analysis of chromatin showing both different levels of genetic activity and different histone variant compositions difficult. Tetrahymena presents an interesting natural case in that its transcriptionally active macronucleus contains the H2A.Z homologous protein, hv1, while its transcriptionally silent micronucleus does not. In order to address this question we have decided to utilize the yeast, Saccharomyces cerevisiae, as it is a lower eukaryote which has homologous core histone proteins but no homologue to the minor variant H2A.Z, and the organism is highly genetically manipulable. In order to expedite this approach we have arranged to collaborate with Dr. Paul Swerdlow, Medical college of Virginia, Virginia Commonwealth University, Richmond, Virginia. He had already conducted an investigation into the role of H2A-ubiquitin conjugate formation on the phenotype of this organism. In order to do so he utilized a strain of

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Yeast as a model system in which to study the roles of major and minor histone variant proteins in chromatin structure and function

While H2A.Z seems to be the H2A isoprotein that is least similar to its counterparts, its sequence also appears to have been highly conserved during evolution. The histone H2A.Z cDNA's from human, cow, and rat encode identical proteins. The cDNA's for the H2A.Z homologous gene has been identified in chicken, sea urchin, Tetrahymena, and Drosophila. The overall similarity between mammalian and urchin H2A.Z is 95%, but there is only a 59% homology between mammalian H2A.Z and H2A.1. Between H2A.Z residues 39 to 122, which are identical in mammal and urchin, there are 32 substitutions between mammalian H2A.Z and H2A.1. However, despite an overall similarity of only 59%, there are three regions of much higher similarity. Through evolution there appears to have been a conservation of certain polypeptide sequences to allow all H2A molecules to have a certain structural and functional commonality. By the same token, the allowed sequence differences may give each of the different H2A variants unique structural and functional capabilities. This is particularly relevant in light of evidence which suggests that H2A.Z may be enriched in transcriptionally active chromatin. It is precisely the aforementioned subtlety of difference between H2A.Z and its major variant counterparts that has made the isolation and analysis of chromatin showing both different levels of genetic activity and different histone variant compositions difficult. Tetrahymena presents an interesting natural case in that its transcriptionally active macronucleus contains the H2A.Z homologous protein, hvl, while its transcriptionally silent micronucleus does not. In order to address this question we have decided to utilize the yeast, Saccharomyces cerevisiae, as it is a lower eukaryote which has homologous core histone proteins but no homologue to the minor variant H2A.Z, and the organism is highly genetically manipulable. In order to expedite this approach we have arranged to collaborate with Dr. Paul Swerdlow, Medical college of Virginia, Virginia Commonwealth University, Richmond, Virginia. He had already conducted an investigation into the role of H2A-ubiquitin conjugate formation on the phenotype of this organism. In order to do so he utilized a strain of

Saccharomyces cerevisiae which had been made defective in the function of its two H2A genes and was dependent on the presence of H2A gene(s) maintained on extrachromosomal plasmids. He has sent us a yeast extrachromosomal plasmid which contains the normal yeast H2A promotor region and we have cloned into this plasmid the gene encoding mammalian H2A.Z (bovine nucleotide sequence) and the gene encoding human H2A.1. He is at present introducing these plasmids into the yeast H2A-mutant strain and will analyze the phenotypic characteristics of the resultant mutants. We will analyze the histone variants expressed on two-dimensional gels and we will analyze both histone messenger RNA levels as well as the levels of a variety of other mRNA's. Although it is difficult to anticipate the results, if yeast mutants containing either a major mammalian H2A variant (H2A.1) or a minor mammalian H2A variant (H2A.Z) are viable and grow normally but do show defined differences in phenotype and genetic expression then we will be able to construct hybrid major-minor variant H2A genes in order to localize the polypeptide sequences which afford the two classes of histone variant proteins different capabilities in genetic regulation.

<u>The Search for the Gene Encoding the Minor Histone Variant, H2A.X</u>

In order to extend our analysis of H2A minor variant gene structure we are presently working on the isolation and characterization of clones containing the cDNA complement of the gene for H2A.X. This gene has not been isolated from any source to date. A human cDNA library has been successfully made using the polyadenylated mRNA of HeLa cells treated with a combination of hydroxyurea and aphidicolin. The cDNA library has been constructed in a way that should strongly bias against the presence of non-polyadenylated major histone variant mRNA cDNA complements. The library has been probed with the human genomic major variant H2A.1 gene isolated in this lab. It is hoped that the homology within certain stretches of polypeptide sequences between H2A.1 and H2A.X will also extend at least in some degree to the nucleotide sequence and by carefully controlling the conditions of hybridization we will be able to find colonies containing the H2A.X-cDNA plasmid. Another interesting possibility is that if we isolate cDNA colonies positive for hybridization to the human H2A.1 gene is that we may identify a class of major H2A variant genes which produce polyadenylated mRNA's and are subject to a different mode of regulation than their counterparts which are DNA replication linked in their synthesis and stability. Approximately 20 cDNA clones positive for hybridization to the human H2A.1 gene probe have been isolated and are being analyzed.

<u>Proposed Course</u>:

1. Continue to study the genomic organization of the H2A.Z gene. Characterize the intron-exon structure of the gene, determine the copy number of the functional gene within the human genome and whether or not processed pseudogene copies are present. Identify regulatory elements within the DNA sequence and search for factors which will specifically bind to them.

2. Characterize the putative cDNA's for H2A.X already isolated. Compare message structure to those already known for H2A.1 and H2A.Z. Isolate the genomic form of this gene and compare to information known and to be gained

about the other H2A variant genes. Particular emphasis will be made to understand how minor histone variant genes, such as H2A.Z and H2A.X, are regulated similarly to each other but differently from the major histone variant genes, such as H2A.1.

3. Search for cytoplasmic factors which specifically bind minor histone variant mRNA's to affect their stability and translation in cycling and quiescent cells.

4. Continue a collaboration with Dr. Paul Swerdlow of the Medical College of Virginia in which we will utilize yeast as a model system to study the phenotypic characteristics and genetic activity of mutants which express and utilize only either a major histone variant H2A (human H2A.1) or a minor histone variant (mammalian H2A.Z).

<u>Publications</u>:

Hatch CL, Bonner WM. Sequence of cDNAs for mammalian H2A.Z, an evolutionarily diverged but highly conserved basal histone H2A isoprtein species. Nucleic Acids Res, 1988;16:1113-24.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06171-04 LMPH

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Chromatin Synthesis and the Control of Cell Proliferation

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

PI:	William Bonner	Sr. Investigator	LMPH NCI
Others:	Nancy Touchette	Sr. Staff Fellow	LMPH NCI
	Christopher Hatch	Sr. Staff Fellow	LMPH NCI
	Concepcion Muneses	Chemist	LMPH NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NCI, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

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 objective is to understand the relationship of various cell states and the transitions between them as reflected in the control of histone and chromatin biosynthesis. Currently we are investigating the transition of cells from the cycling state to the quiescent state, using hamster ovary (CHO) cells that can be synchronized. When S phase CHO cells are put into media that leads to the quiescent state, S phase continues at an undiminished rate although protein synthesis begins to significantly diminish.

We are using this system to investigate and characterize changes involved at the protein, mRNA, and gene level during this transition. One major question concerns the relationship of the cycling-quiescent transition to the reverse transition, in terms of the expression of oncogene as well as other genes. Present studies are focusing of the mRNA for H2A.Z, a basal histone the synthesis of which is not linked to DNA replication but is 20 fold lower in quiescent relative to cycling cells. One goal is to isolate factors that regulate transcription during this transition.

Differential scanning calorimetry is also being used to investigate apparent differences in chromatin topology found between cycling and quiescent cells and the effect of antitumor agents on this parameter.

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

Project Description

Introduction:

The biochemical mechanisms which regulate cell proliferation remain largely unknown. The rate of protein synthesis in quiescent cells in approximately 1/3 that of cycling cells. When S phase CHO cells are put into media that leads to the quiescent state, S phase continues at an undiminished rate although protein synthesis begins to significantly diminish. Currently we are investigating the transition of cells from the cycling state to the quiescent state, using hamster ovary (CHO) cells that can be synchronized through the cell cycle before quiescence.

Objectives:

1) The development of a greater understanding of the relationship of mechanisms regulating cell proliferation, with emphasis on the relationship of the chromosome cycle to the transition between the cycling and quiescent states.

2) A characterization of the responses of these regulatory mechanisms to the development of new regimens or compounds that might control inappropriate proliferation of transformed cells.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional electrophoresis. (Methods developed in this laboratory).

(2) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(3) Maintenance of cells and nuclei in viable non-dividing states using modified and defined media.

(4) Isolation and analysis of mRNA levels from different parts of the cell cycle or from quiescent cells, using methodology developed in this laboratory.

(5) Nuclear runoff assays to measure rates of mRNA transcription.

(6) FACS analysis of cell cycle distributions including the BrdU antibody technique for measuring S phase cells.

Major Findings and Accomplishments:

Relationship Between the Chromosome and Growth Cycles during the Transition from the Cycling to the Quiescent State

Previously, we have found that the level of histone mRNAs increases in response to moderate inhibition of protein synthesis to an extent sufficient to offset most of the inhibition, and have named this phenomenon chromosome cycle

compensation. Quiescent cells have rates of protein synthesis which are about 1/3 of that found in cycling cells. We have shown that when CHO cells, synchronized in or at the beginning of S phase, are transferred to serum-free growth media, the rate of protein synthesis starts decreasing almost immediately to the rate found in quiescent cells; however, the progression of the cells through S phase is not decreased. These results suggest that the cell is already changing to a quiescent state, even though the chromosome cycle is still in progress. Chromosome cycle progression could be maintained by the compensation mentioned above.

In order to test this hypothesis, the level and rate of transcription of certain mRNAs are being measured with respect to the rate and timing of their decrease. Present work is concentrating on the mRNA for H2A.Z, a basal histone, the synthesis of which is independent of DNA synthesis but which is decreased by 20 fold in quiescent relative to cycling cells.

Studies with Differential Scanning Calorimetry

Previous studies have suggested that there are differences in DNA topology between cycling and quiescent as assayed by differential scanning calorimetry. Cycling cells have a transition at about 105°C which quiescent cells lack. It is also known that supercoiled DNA has this high temperature transition while relaxed DNA has a transition at about 88°C. Because of the great sensitivity of supercoiling to DNA stand breaks, the effect of antitumor agents on this parameter may be a sensitive test of the interaction of these agents with DNA.

Significance:

Cancer cells grow and divide inappropriately. Understanding the mechanism regulating the transitions between the growth and quiescent states may lead to greater understanding of this inappropriate grow. While much attention has been focused of growth inducing substances, less has been focused on quiescence inducing substances. The inability to produce a quiescence inducing substance may transform a cell just as easily as the inappropriate production of a growth inducing substance. One aim of this study is to compare the two transitions, and thus gain insight into their similarites and differences.

Proposed Course:

1. To measure the level and rate of transcription of the mRNAs for H2A.Z and possibly H2A.X during the transitions from cycling to quiescebce and vice-versa.

2. To extend the above studies to include mRNAs of known oncogenes and other proteins of potential interest.

3. To extend studies of differences in DNA topology between cycling and quiescent as assayed by differential scanning calorimetry. To test the effect of antitumor agents on chromatin temperature transitions as a possible sensitive test of the interaction of these agents with DNA.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06172-04 LMPH

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

DNA Sequence-selective Alkylating Agents (Modified title)

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

PI:	Kurt W. Kohn	Lab Chief	LMPH NCI
Others:	Patrick O'Connor	Visiting Fellow	LMPH NCI
	Johann Hofmann	Guest Researcher	LMPH NCI
	Ann Orr	Microbiologist	LMPH NCI

COOPERATING UNITS *(if any)* Dr. Wayne Anderson, Department of Medicinal Chemistry, State University of New York at Buffalo. Drs. Maurizio D'Incalci and Massimo Broggini, Mario Negri Institute, Milan, Italy.

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION NIH, NCI

Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.9	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 ability of certain antineoplastic alkylating agents to react selectively with particular DNA sites, depending on DNA sequence and conformation, is being studied as a possible new avenue for the design of more specific drugs. The first objective is to understand at the chemical structure level the factors that govern the selectivity of DNA alkylation sites. Results so far show that the sequence selectivity of nitrogen mustards can be altered by changing the drug structure, and indicate that a structural understanding can be achieved. The second objective is to design new compounds optimized for selective reaction at certain DNA sequence sites. Synthesis and testing of the first new compounds has begun, and is being guided by computer aided molecular modelling based on specific structural hypotheses which are to be tested. The third objective is to investigate the possibility of enhancing the specificity of antitumor action. Studies have been initiated to determine the sequence selectivities in intact cells, and to determine whether there are significant hot-spots for DNA alkylation and crosslinking in the genomes of cells having different drug sensitivities.

PROJECT DESCRIPTION

Objectives:

1. Determine the structural basis for the nucleotide sequence selectivity of DNA alkylation reactions.

2. Design new alkylating agents having optimized DNA sequence selectivities.

3. Investigate the possibility of using this approach to enhance the specificity of antitumor action.

Methods:

1. DNA sequencing by gel electrophoresis, and application of this technique to the quantitation of extents of alkylation at specific sites in a sequence.

2. Densitometry and computer analysis of DNA sequence autoradiograms.

3. Molecular modelling and structure optimization by computer.

4. DNA separation, hybridization and standard molecular biology techniques to isolate and identify crosslinked DNA fragments derived from cells treated with alkylating agents.

5. DNA filter elution techniques to quantitate DNA lesions in cells.

Major Findings:

There is good evidence that the antitumor activity of most nitrogen mustards is due to the formation of DNA interstrand or intrastrand crosslinks. However other types of lesions are produced abundantly, including DNA-protein crosslinks, monoadducts at various DNA sites and alkylation of other molecules in the cell. Moreover, the extent of alkylation and crosslink formation can vary depending on DNA sequence and chromatin structure. A major premise of this project is that the antitumor activity depends mainly on a subset of these lesion types, and that compounds that predominantly produce those lesion types would be more effective and less toxic drugs when used alone or in combination.

To this end, we are investigating (a) the effects of DNA sequence and conformation on susceptibility to alkylation by various nitrogen mustards and other alkylating agents, and (b) the relationship between specific DNA lesions or genomic localization of lesions on cytotoxicity in various cell types.

Sequence Selectivity for Reactions with Purified DNA

We reported last year that nitrogen mustards generally tend to react with the more electronegative guanines in DNA sequences, but that certain nitrogen mustards, in particular uracil mustard and quinacrine mustard, have

characteristic reaction site preferences that deviate from this rule. The specific site preferences are of particular interest, because by understanding their chemical basis it may become possible to design alkylating agents that react more specifically at classes of DNA sites that are most selective for killing tumor cells. In addition, the relative reaction rate measurements may provide a new method for probing the conformation of DNA.

We have now confirmed the specific site preferences under a variety of conditions, and find that the reaction of uracil mustard is strongly enhanced at guanines located in sequences of the type, 5′-YGC-3′ (Y = C or T). This sequence preference is of particular interest because it contains the sequence, GC, which is the exclusive site where interstrand crosslinks can occur. Since the reactions of most other mustards tend to be suppressed in the sequence, GC, the question arises as to whether uracil mustard may be especially effective in producing interstrand crosslinks relative to other DNA lesions.

An important clue to understanding the peculiarities of uracil mustard at the structural level was our finding that the addition of a methyl group to the 6 position of the uracil moiety abolishes the specific reaction preferences and causes the molecule to revert to the reaction pattern common to most other mustards. We are now able to explain this observation on the basis of computer modelling studies that show that the 6-methyl group would prevent the molecule from adopting the configuration required for interaction with the amino group of the C.

A collaboration was initiated with Dr. Wayne Anderson (SUNY, Bufallo, NY) to design and synthesize structures that would optimize reaction preferences and test hypotheses on their structural basis.

In a collaboration with Drs. Maurizio D'Incalci and Massimo Broggini (Mario Negri Institute, Milan), we have utilized the DNA sequencing approach to investigate the mechanism of action of Pettit's mustard, which chemically should not itself undergo alkylation reactions, but which may be a pro-drug for an active alkylating species. The results indicate that the drug decomposes to the final alkylating species via an intermediate that exhibits a distinct sequence preference and suppression pattern.

<u>DNA Lesion Selectivity in Mammalian Cells</u>

Several nitrogen mustards were studied to determine the extent of variation that may exist among different mustards in the rates of formation and repair of interstrand and DNA-protein crosslinks and in the relative extent of production of these two types of crosslinks. The mustards studied: HN2, melphalan, uracil mustard and quinacrine mustard were found to have distinctive characteristics in L1210 cells. For example, HN2 showed distinctively rapid rates of formation and repair of both types of crosslinks, whereas with melphalan these processes were much slower. Uracil mustard formed crosslinks rapidly, but they showed little or no repair. Quinacrine mustard exhibited rapid formation and slow repair of DNA-protein crosslinks; however interstrand

crosslinks could not be detected, possibly because of the unusual production of large numbers of DNA strand breaks by this nitrogen mustard.

We will compare interstrand crosslink formation by uracil mustard and 6-methyluracil mustard, in order to test in cells the special reaction characteristics of uracil mustard predicted by the chemical studies of sequence-selective alkylation of purified DNA.

The unusual behavior of quinacrine mustard suggests special properties of intercalating mustards and possible effects on topoisomerases which will be investigated.

Significance:

The findings on alkylation site preferences and the dependence on DNA sequence show that reaction site selectivities can be altered by chemical modification of drug structure, and that the basis for the DNA sequence selectivity can be understood at the structural level. This provides a new avenue for drug design, based upon targeting of specific genomic regions so as to optimize the delivery of antitumor effective DNA lesions.

The findings on DNA crosslinking patterns in mammalian cells show that nitrogen mustards differ greatly with respect to the production of different types crosslinks, the rates of formation of crosslinks, and their susceptibility to repair. Hence nitrogen mustards are a diverse group of drugs with a broad potential for further investigation.

Proposed course:

1. Design new nitrogen mustards with the aid of computer modelling to test hypotheses regarding the structural origin of sequence selective alkylation of DNA, and use this information to obtain compounds having optimum selectivity for certain sequences.

2. Determine the effects of natural DNA binding molecules, including nuclear proteins, on DNA sequence selectivity of alkylation.

3. Determine DNA alkylation site selectivity in chromatin and intact cells.

4. Locate hot spots for alkylation or interstrand crosslinking in the genome of cells, and relate to the sensitivity of cells to alkylating agents.

Publications:

Kohn KW, Hartley JA, Mattes WB. Mechanisms of DNA sequence selective alkylation of guanine-N7-positions by nitrogen mustards, Nucleic Acids Res 1987;15:10531-549.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06186-02 LMPH

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

DNA Repair in Genes (modified title)

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

PI:	Vilhelm Bohr	Sr. Investigator	LMPH NCI
Others:	Diane S. Okumoto	Microbiologist	LMPH NCI
	Karsten Wasserman	Visiting Fellow	LMPH NCI
	Michele Evans	Medical Staff Fellow	LMPH NCI
	Katherine Ault	Summer Fellow	LMPH NCI

COOPERATING UNITS *(if any)*

Dr. David C. Thomas, University of North Carolina

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NIH, NCI
Bethesda, Maryland 20892

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.)*

Our objective is to study the DNA damage and repair in individual genes and in non-coding sequences within the genome. This is correlated to more traditional studies of DNA repair as an average over the genome. Findings reviewed in Bohr VA, Phillips DH and Hanawalt PC, Cancer Res. 47: 6426-6436, 1987, have indicated that active genes are preferentially repaired in mammalian cells and that determinations of DNA repair in specific genes are important for correlations to biological endpoints and risk assessments. Whereas our earlier studies were limited to UV as a damaging agent, we have now developed an approach which can be employed for a large variety of bulky agents that react with the DNA. We are currently further developing techniques that measure damage and repair of alkylating agents in specific genes including oncogenes.

We are studying preferential repair of genes in a number of human, cancer prone DNA repair deficient syndromes and in various human and rodent mutant cell lines, some of which are transfected with repair genes. We have found that transfection of a UV sensitive CHO cell line with the human repair gene, ERCC 1 restores preferential repair in the cells.

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

Objectives:

1) To study DNA damage and repair in specific genes using established methodology. To develop new methodology to examine damage and repair after alkylating agents in structural genes and oncogenes.

2) Examine preferential repair of genes in human and rodent mutant and transfected cell lines.

3) Develop assays to study DNA damage and repair in specific genomic sequences in human primary cell cultures and patient material.

4) Further explore the molecular rules that govern preferential repair of genes in normal and repair deficient mammalian cells.

Methods:

1) Cell culture techniques.

2) DNA damage and repair.

3) Drug pharmacology.

4) DNA molecular techniques, molecular hybridizations.

5) RNA techniques.

Brief Summary of previous results.

We have demonstrated that essential genes in rodent and human cells are preferentially repaired after UV damage. In rodent cells, some genes are repaired much more efficiently than the bulk of the genome. This may explain the long standing paradox that the overall genome repair capacity of rodent cells is low, whereas their UV survival is as high as for proficiently repairing human cells: Rodent cells appear to repair efficiently only genomic regions of vital importance. In normal human cells, we have found that genes are repaired faster than the bulk of the genome. We have demonstrated that determinations of DNA repair in specific genomic sequences may be more important than overall genome DNA repair measurements for correlations to other biological end points such as resistance to UV damage. Changes in preferential DNA repair could have profound effects on such parameters without noticeably altering overall genome repair levels since the vital regions only constitute a very small fraction of the genome. We have analyzed the genomic fine structure of DNA repair in and around the DHFR gene in CHO cells and find a region of preferential DNA repair of approximately 60-80 kb in length with maximal DNA repair efficiency at the 5′ end of the gene and in its 5′ flanking sequences.

We have found considerable differences in the repair efficiency of different genes within the same cell. The constitutively transcribed protooncogene c-abl is much more efficiently repaired than the transcriptionally silent proto-

oncogene c-mos. In experiments measuring the repair in genes which can be modulated with regard to activity, results have further suggested that when metallothionein genes which are normally inactive become activated, considerable corresponding increases in DNA repair efficiencies can be detected. These findings suggest a positive correlation between DNA repair efficiency and transcriptional activity in a gene.

We have studied the effect of the topoisomerase II inhibitor novobiocin on repair in the overall genome and in the DHFR gene. Whereas this compound inhibits overall genome repair, it had no effect on repair in the gene. This suggests that qualitative as well as quantitative differences exist between the "average" repair pathway in the cell and that responsible for the preferential repair seen in active genes.

MAJOR FINDINGS:

1) DNA repair in specific genes in particularly cancer prone DNA damage sensitive or repair deficient syndromes: Dysplastic naevus syndrome, Bloom syndrome, Cokaynes syndrome and Gardiner's syndrome.

A number of human disorders have been termed DNA damage sensitive or repair deficient syndromes. For none of these is the etiology of the disease known, nor is it known which aspect of the DNA repair mechanism is deficient. For the disorder Cockayne's syndrome, it has been demonstrated that there is no prefe rential DNA repair of active genes, a feature seen in normal mammalian cells. It seems possible that closer scrutiny of certain human syndromes by analysis of DNA repair in specific sequences could further our understanding of the pathomechanism of the disorders.

2) Preferential DNA repair in normal, mutant and transfected human and rodent cell lines.

The pattern of preferential DNA repair has been studied in Chinese hamster ovary (CHO) cells transfected with the bacterial repair gene, denV (which codes for the T4 endonuclease V) or with a human repair gene, ERCC-1. Repair efficiency after UV damage was studied in an active gene (dihydrofolate reductase, DHFR) and in non-transcribing sequences. Preferential DNA repair was examined in six cell lines: Two wild type CHO, two UV sensitive mutants which were the paren tal cell lines to the two transfectants, and the transfected CHO cell lines containing the denV gene or the ERCC-1 gene. The CHO cells transfected with the bacterial repair gene were capable of repairing transcribing and non-tran scribing sequences with equal and high efficiency, and it is likely that this is due to accessibility of mammalian chromatin to this small (16 kD) enzyme. In the UV sensitive cell lines, very little or no repair was found in the sequences studied. The CHO cells containing the ERCC-1 gene repair the active DHFR gene much more efficiently than the non-transcribing sequences. This pattern of repair is similar to the one in wild type cells, and different from that in the cells transfected with the bacterial gene. It suggests that the ERCC-1 gene product may be a normal mammalian repair enzyme involved in the early steps of the repair process.

3) ABC excision nuclease complex.

The complex of the E. Coli gene products UVR A, B and C recognizes a wide variety of damage in the DNA and cleaves the DNA at sites of damage. We have used this complex in our assay (in stead of the T4 endonuclease V enzyme) to nick the DNA at damaged sites. It is possible to measure the frequency of a variety of different adducts in specific genes using this approach, and so far we have measured the frequency of agents such as benzo(a)pyrimidines, acetylamino-fluorene, psoralens, 4 NQO and UV in some genes. As a measure of the reliability of this approach, we have found that determinations of the repair of UV damage in the DHFR gene in CHO cells are the same using the T4 endonuclease V approach and the ABC excinuclease approach. This approach has considerable general potential for the examination of damage and repair in specific genes including oncogenes after exposure of cell cultures to cytotoxic agents, alkylating agents and various forms of irradiation.

Significance to Biomedical Research and the program of the Institute:

A Further understanding of the molecular biology of preferential DNA repair in genes is of importance in basic cancer research. Furthermore, the lack of such repair appears to be associated with human disease. An understanding of these processes will have importance for diagnosis and therapy of cancer and DNA repair deficient disorders.

Proposed course:

1) We are currently investigating the preferential repair of genes including proto-oncogenes in cells from patients with various forms of xeroderma pigmentosum, Blooms syndrome, dysplastic naevus syndrome and other human syndromes suspect for DNA damage processing deficiencies. So far, we have demonstrated deficient repair of an essential gene and some oncogenes in Bloom Syndrome.

2) Preferential DNA repair is presently being examined in a newly established xeroderma pigmentosum cell line which is transfected with the bacterial repair gene, denV. This cell line represents the first transfectant of a xeroderma cell line with a repair gene, and thus an example of gene therapy. We have found that the DHFR gene is repaired very efficiently after UV light in this cell line. We are currently examining other genomic regions for repair after UV, but also plan to examine the repair in specific sequences after alkylation damage.

3) Preferential binding and removal of compounds from active genes may have therapeutical implications. Our techniques to measure damage and repair in specific sequences using the UVR A,B,C complex represent a direct assessment of the frequency of sites of damage within a certain genomic region or gene. Anti-cancer drugs are in most instances known to interact directly with the DNA, and the frequency of such sites can thus be directly measured. This would allow us to screen a number of compounds in order to find those that bind most strongly to active genomic regions including specific proto-oncogenes. Drugs with high affinity for genes may be candidates for anti cancer therapy since inactivation of certain genes are likely to be main targets of the therapy. And

since the active parts of the genome only constitute a very minor fraction of the genome (< 1%), drug affinity to those regions might increase the therapeutical efficiency dramatically.

4) Other methods to study DNA damage and repair in specific genes: The adducts formed by a number of bulky agents are not distributed homogeneously over the genome as is the case for pyrimidine dimers, but rather in a heterogenous fashion with a higher frequency in DNase I sensitive genomic regions. Aside from the ABC excision nuclease method mentioned above, we are developing two new approaches. One of these is based on the cleavage of DNA at alkaline labile sites after alkylation damage. It involves the probing for specific genes in a quantitative Southern approach similarly to the above mentioned technique, but is specific for alkylation damage. The other approach involves the probing for specific genes in eluted DNA from alkaline elution protocols. Our hope is to develop techniques that allow rapid detection of damage frequency in specific sequences and of preferential repair of genes.

Publications:

Bohr VA, Phillips DH, Hanawalt PC. Heterogeneity of DNA damage and repair in the mammalian genome, Cancer Res 1987;47:6426-36.

Bohr VA. Differential DNA repair within the genome, Cancer Rev 1987;7:28-55.

Bohr VA. Introduction (to issue on DNA repair), Cancer Rev 1987;7:1-3.

Okumoto DS, Bohr VA. DNA repair in the metallothionein gene increases with transcriptional activation, Nucleic Acids Res 1987;15:10021-31.

Bohr VA, Okumoto DS. Analysis of frequency of pyrimidine dimers in specific genomic sequences. In: Hanawalt PC, Friedberg EC, eds. DNA repair: a laboratory manual of research procedures. New York: Marcel Dekker, 1988;347-66.

Thomas DH, Morton AG, Bohr VA, Sancar A. General method for quantifying base adducts in specific mammalian genes, Proc Natl Acad Sci USA, press.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

DIVISION OF CANCER TREATMENT

October 1, 1987 - September 30, 1988

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 Response 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 and nutrition 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

During the past year seven Cancer Experts were converted to Medical Officer positions in the Civil Service. The seven are: Dr. Bruce Cheson; Dr. Andrew Dorr; Dr. Michael Hamilton; Dr. Michael Hawkins; Dr. Gisele Sarosy; Dr. Michaele Christian; and Dr. Jean Grem. All are employed in either the Clinical Investigations Branch or the Investigational Drug Branch. In addition, Dr. Maryellen Franko was converted from a Cancer Expert position to a Civil Service Microbiologist position.

Dr. Daniel Hoth, former Chief of the Investigational Drug Branch, joined the National Institute on Allergy and Infectious Disease (NIAID), as the Director of the AIDS Program.

Dr. Susan Ellenberg, Biometric Research Branch, joined the AIDS Program, NIAID, as the Chief of their Biometrics Branch.

Dr. Timothy Moore was recruited for a Senior Investigator position in the Clinical Investigations Branch following a three-year oncology fellowship at Ohio State University.

Dr. Paul Hiranaka was recruited from the FDA for the AIDS vacancy in the Regulatory Affairs Branch.

Mr. Alfred Fallavollita was recruited from the FDA for the AIDS vacancy in the Drug Management and Authorization Section (DMAS) of the Investigational Drug Branch.

HIGHLIGHTS IN PROGRAM DEVELOPMENT

1. INTRODUCTION OF NEW AGENTS INTO CLINICAL TRIALS

a. <u>IND Submissions</u>

For the FY '88, 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:

Drug	NSC Number
Dideoxyadenosine	NSC 98700
Buthionine Sulfoximine	NSC 326231
Hepsulfam	NSC 329680
Chloroquinoxaline Sulphonamide	NSC 339004
Fostriecin	NSC 339638
Ipomeanol	NSC 349438
Pyrazine Diazohydroxide	NSC 361456
Dideoxyinosine	NSC 612049

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

Drug	NSC Number
IL-2 + Tumor Infiltrating Lymphocytes	NSC 600664
Interleukin-1 Alpha	Not Assigned
Interleukin-1 Beta	Not Assigned
Interleukin-4	Not Assigned
Macrophage Colony Stimulating Factor (M-CSF)	Not Assigned
Monoclonal Antibody OKT-3	NSC 618843
Monoclonal Antibody 14.18	Not Assigned
Monoclonal Antibody 14G2A	Not Assigned
Monoclonal Antibody 11C64	Not Assigned
Monoclonal Antibody Lym-1	Not Assigned
Monoclonal Antibody 1F5	Not Assigned
Monoclonal Antibody B1	Not Assigned
Monoclonal Antibody NRCO-4	Not Assigned
Monoclonal Antibody 96.5	Not Assigned

Second Generation B72.3 Monoclonal Antibodies	Not Assigned
Chimeric Monoclonal Antibody B72.3	Not Assigned
Expanded LAK Cells	Not Assigned
Educated Lymphocytes	Not Assigned

b. INDs Discontinued

INDs for the following agents were discontinued:

Drug	NSC Number
THC	NSC 134454
Sodium Thiosulfate	NSC 45624
Prednisolone	NSC 9151

Biologic	NSC Number
Monoclonal Antibody Osteosarcoma (791 T/36)	NSC 377522

The Regulatory Affairs Branch currently maintains 160 active INDs for both cytotoxic and biologic anticancer and antiAIDS agents.

2. SELECTED STUDIES OF PARTICULAR INTEREST WITH INVESTIGATIONAL AGENTS

a. IL-2/LAK

Patients with malignant melanoma and renal cell carcinoma who obtained a complete remission following IL-2/LAK on the original extramural trials remain free of their disease 20 - 24 months after having started treatment. Screening for other malignancies sensitive to IL-2/LAK has begun. Serum free media is now being used to generate LAK cells, significantly reducing the risk of transmitting viral infections through the culture media. Semi-automated procedures are currently being developed which will significantly simplify the method of LAK cell generation. The availability of IL-2/LAK as treatment for renal cell carcinoma and malignant melanoma has been extended to 14 of the NCI Cancer Centers under the Modified Group C treatment program.

b. IL-2 without LAK cells

In addition to the studies of IL-2 with LAK cells, the NCI Extramural IL-2/LAK Working Group has also treated 25 patients with malignant melanoma with high doses of IL-2 alone and has observed 3 complete and 3 partial remissions. This IL-2 regimen is currently being tested in

combination with cis-platinum, a chemotherapeutic agent which also has antitumor activity against malignant melanoma. Another IL-2 alone treatment regimen using chronic administration of lower doses was found to be biologically active in NCI extramural studies and is currently in Phase II testing. Based on promising preclinical data, treatment regimens using IL-2 in combination with adriamycin, cyclophosphamide, alpha interferon, gamma interferon and tumor necrosis factor are currently in development.

c. Colony Stimulating Factors

As single agents, CSFs appear to be effective as treatment for some patients with myelodysplastic syndromes. Early studies also showed that G-CSF and GM-CSF can protect patients from the neutropenia associated with chemotherapy. To reduce toxicity or to permit administration of more treatment, the NCI is adding CSFs to standard chemotherapy and radiotherapy regimens used for the treatment of breast cancer, lung cancer, ovarian cancer, multiple myeloma, leukemia, lymphoma, testicular carcinoma, and sarcomas. CSFs are also being tested in combination with bone marrow transplantation to shorten the time to bone marrow recovery.

d. Monoclonal Antibodies (MoABs)

Studies to further define the activity of the R24 MoAB in malignant melanoma are currently in progress. Based on reports that the anti-colon MoAB 17-1A also has activity in pancreatic cancer, the ECOG is about to initiate a Phase II study to define response rate and duration. A protocol has been developed to permit rapid comparison of multiple MoABs directed against the same antigen. These data will be used to determine the optimal variable region for future MoAB constructs and will initially study MoABs directed against the TAG-72 antigen. An anti-CD 5 MoAB conjugated to ricin A chain, previously used to deplete T cell populations in patients with chronic graft versus host disease, will be tested in patients with CD 5 positive malignancies.

e. Gamma Interferon

Previous clinical trials conducted by the NCI identified a dose and schedule for gamma interferon administration which resulted in optimal biological activity. Large scale adjuvant trials which use this regimen of gamma interferon administration are currently in progress in malignant melanoma and small cell carcinoma of the lung. Monocyte function of patients treated on these studies is also being measured and will be correlated with antitumor activity.

f. Ifosfamide

Two ongoing trials seek to define the contribution of ifosfamide in the treatment of newly diagnosed adult patients with soft tissue sarcoma. In pediatric soft tissue sarcomas, the combination of ifosfamide and etoposide has shown exciting activity, particularly in refractory patients with Ewing's sarcoma and rhabdomyosarcoma. Trials are ongoing to define the role of this combination in newly diagnosed patients.

A treatment protocol for ifosfamide, mesna, cisplatin, and either etoposide or vinblastine for patients with refractory testicular cancer was recently approved by the FDA. Data from Indiana University suggest that this three drug combination is potentially curative in patients with refractory testicular cancer who have failed two prior cisplatin containing regimens; no other regimen has been reported to be potentially curative in this patient population. This treatment protocol (Group C) was initiated so that patients who are not eligible for research protocols can receive this investigational regimen with such promising activity.

g. SR 2508

A randomized trial in patients with head and neck cancer is ongoing to establish the efficacy of SR 2508 and radiotherapy versus radiotherapy alone. Several pilot studies are ongoing. A Phase I trial is seeking to define the maximally tolerated dose of SR 2508 when given with brachytherapy based on preclinical data which suggest that SR 2508 may be more effective when given with low dose rate radiotherapy. Patient accrual continues in a Phase II trial in prostate cancer which seeks to establish whether patients treated with SR 2508 and radiotherapy have a higher local control rate than one might anticipate.

Two Phase I trials are going in patients with refractory solid tumors to define the MTD of SR 2508 when given with cyclophosphamide. In addition, a recently initiated trial in patients with CLL will seek to determine the MTD in this patient population. Pharmacokinetic and pharmacodynamic studies are part of these proposed trials to establish the mechanism of the proposed chemosensitization.

h. WR 2721

Recent data from the University of Pennsylvania suggest that WR 2721 and cisplatin is an active regimen in the treatment of melanoma. A 53% objective response rate was observed in 36 patients with metastatic melanoma treated with this two drug combination, including 5 objective responses among 6 patients treated with WR 2721 and cisplatin 150 mg/m^2. Based on these promising data, a randomized Phase III trial has recently been initiated by ECOG. Because WR 2721 may increase the cytotoxicity of cisplatin, as well as ameliorate its toxicity, Phase II trials of this two drug combination are anticipated in patients with cancers of the breast and prostate.

i. Taxol

This unique natural product derived from the bark of *Taxus brevifolia* has shown promising antitumor activity. A 33% response rate has been observed in patients with refractory ovarian cancer. A Phase II trial will soon be initiated by the GOG to confirm these preliminary data. A Phase I trial of cisplatin and Taxol is anticipated to define the dose limiting toxicities of this two drug combination; if warranted, a Phase III comparison of cisplatin versus cisplatin and Taxol will be undertaken in newly diagnosed patients.

Some patients with malignant melanoma metastatic to soft tissue sites have experienced a brief response to therapy. Recently, one patient with non-small cell lung cancer was reported to experience an objective response during the ongoing Phase I study of the 6 hour schedule of administration.

j. L-Buthionine Sulfoximine

The drug inhibits glutathione biosynthesis and causes a depletion of cellular glutathione levels. The drug has been shown to reverse the induced resistance of human ovarian cell lines to melphalan. The initial proposed clinical study is a Phase I study of the combination of BSO and Melphalan. If the toxicity observed in this trial is acceptable, a randomized trial is anticipated in patients with ovarian cancer.

k. Flavone Acetic Acid

Based on the data from Wiltrout et al (J Immunology 140:3261, 1988) that natural killer cell activity is enhanced in mice treated with FAA, Phase I investigators looked at BRM parameters in patients at the higher dose levels. Stimulation of NK cell activity has been identified in several clinical trials. The lack of clinical activity in Phase II studies conducted on a weekly schedule of FAA (4.8 g/m^2 over 1 hr, and 8.6 g/m^2 over 6 hr) has created a need to develop alternative schedules. If FAA indeed works indirectly through host-mediated mechanisms, then chronic administration schedules might be necessary for optimal biological activity. FAA has demonstrated synergism with IL-2 in a preclinical model and a clinical trial of the combination has been initiated at the BRMP.

l. Teniposide

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol will be submitted shortly to the FDA for VM-26 in combination with Ara-C for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia. Two confirmatory trials in small cell lung cancer have been initiated based on the Finsen Institute data demonstrating extraordinary single agent activity of VM-26 (J Clin Oncol 4:524, 1986).

m. Liposomal Doxorubicin

Phase I trials with liposomal doxorubicin supplied by The Liposome Co. are being initiated by 3 contractors on weekly or every 3 week schedules. Preclinical studies with liposome-encapsulated doxorubicin have shown that the maximally tolerated dose of doxorubicin can be increased by approximately 2.5 fold. This has been accompanied by an alteration in the tissue distribution of doxorubicin, with less accumulation in cardiac tissue. Superior antitumor activity has been noted in some, but not all, preclinical models.

n. PALA/5-FU

The investigational agent, PALA, is being tested in Phase II trials at low dose (250 mg/m^2) with FUra (2600 mg/m^2/24 hr following PALA) in colon, gastric and pancreatic cancers to confirm the impressive results seen in a previous Phase I/II trial (2 CR, 11 PR in 28 patients).

o. Deoxycoformycin (dCF)

Phase III trials of dCF versus alpha-interferon are accruing well. In Phase II trials, dCF has produced remissions (CR + PR) in 85% of hairy cell leukemia patients (99 CR, 46 PR of 170 Phase II patients). A Group C protocol of pentostatin for Hairy Cell Leukemia patients refractory to alpha-interferon has been submitted to the FDA. In Phase II trials, dCF is a 26% agent (3CR, 48 PR of 196 patients) in CLL. Combination trials with FAMP and chlorambucil are planned in CLL. A Phase I trial in patients with impaired renal function has been initiated. A combination trial of dCF and alpha-interferon in mycosis fungoides and a trial of single agent dCF for the treatment of acute graft versus host disease after bone marrow transplant have also been initiated. An ex vivo purging trial to take advantage of the selective cytotoxicity of dCF with deoxyadenosine for T-lymphocytes is planned.

p. High Dose Methotrexate

For osteosarcoma Phase III trials have now proven a definitive advantage for an intensive adjuvant chemotherapy regimens which include high-dose methotrexate. Lederle's NDA submission, composed primarily of clinical data from NCI-supported trials, has been approved by the FDA in this past year.

q. Carboplatin

Phase III trials in ovary, testes, and head an neck cancer are in progress. Several interesting studies which exploit CBDCA's dose limiting toxicity, myelosuppression, are in progress or will be initiated soon. These include Phase II trials of single agent CBDCA in leukemia (significant activity was demonstrated in Phase I); CBDCA + VP-16 with autologous bone marrow transplant (which showed significant activity in testis cancer in Phase I); and CBDCA + GM-CSF. It is hoped that this latter trial will permit increased dose intensity which will then motivate a reevaluation of CBDCA in a number of diseases (i.e., melanoma where the response rate was 19% with conventional doses).

r. Suramin

This drug has generated considerable interest because of its novel mechanism of action and early evidence of activity, especially in adrenocortical carcinoma in intramural trials. Because of significant toxicity associated with this therapy, the dose and schedule are being further evaluated prior to a broader Phase II development.

s. HMBA

Three Phase II trials of this polar-planar differentiating agent in myelodysplastic syndrome and one in malignant melanoma are currently ongoing. The implementation of biologic endpoints (cytogenetics, measurement of early- or late- myeloid antigen levels, multilineage bone marrow progenitor cell assays, expression of proto-oncogenes (c-myc, c-fos, c-fms) are an intrinsic part of each of these trials. The Phase I oral study (between Walter Reed and the intramural program) using parenteral formulation administered via NG tube is being completed and the protocol has been amended to study oral bioavailability (bioequivalency) of the new tablet agent formulation.

t. Fludarabine Phosphate (FAMP)

The drug has demonstrated significant single agent activity in alkylator-refractory lymphoproliferative disorders, especially in chronic lymphocytic leukemia (CLL) and favorable histology non-Hodgkins lymphoma. Pilot trials of FAMP-containing combination regimens (FAMP + Prednisone, FAMP + chlorambucil, and FAMP + deoxycoformycin) in CLL are being activated. Application for Group C designation of the drug (for refractory CLL) is underway.

u. Provision of Investigational Agent for Treatment Purposes

During the past year the FDA approved CTEP's Group C application for ifosfamide for the combination treatment of relapsed/refractory testis cancer. An application for pentostatin for hairy cell leukemia has also been submitted. Before the end of FY '88, we anticipate the submission of the following additional Group C applications:

Drug	Disease
Teniposide (VM-26)	Refractory ALL of childhood
Methyl-CCNU	Adjuvant colorectal cancer
Fludarabine	Chronic lymphatic leukemia refractory to alkylators

v. Electronic Clinical Drug Request

The Drug Management and Authorization Section has developed an electronic clinical drug request system for the transmission of drug requests from investigators to NCI. After the system was designed, equipment was purchased and a User's Guide was written. A pilot project was initiated at two major cancer centers: Memorial-Sloan Kettering and M.D. Anderson. The pilot was later expanded to more diverse clinical practice settings, nation-wide, bridging several different time zones. These pilot programs have been successful in simplifying the drug ordering procedure and reducing overall drug distribution time from weeks to days. It is anticipated that the new system will minimize the need to maintain large drug inventories and thus reduce drug cost to NCI.

w. Drug Cost Containment

Drug cost containment has been successful during the past year. The total drug cost has decreased from $3.5 million in FY 86 to $2.9 million in FY 87. It is estimated that drug cost will increase in FY 88 to about $3.7 million to reflect the increase in drug distribution experienced in recent months.

3. CLINICAL TRIALS

In 1987 approximately 25,000 new patients were entered on therapeutic studies (Phase II and III) in CTEP sponsored trials conducted by the Cooperative Groups. Virtually every type of malignancy is being studied in this collaborative enterprise. Phase III definitive tests of efficacy are the central component of the effort to reduce cancer mortality.

a. High Priority Clinical Trials--The Need to Increase Accrual

A major impediment to progress in curing more cancer patients is that the necessary clinical investigation proceeds too slowly. For nearly every malignant disease, crucial studies accrue patients at an unacceptably slow rate, thus preventing the identification of new effective therapies in a timely and precise fashion. For the common adult malignancies only .5% to 3% of available patients are studied each year. Since definitive studies may require 1000 to 3000 patients, it has often taken a decade or more to complete the accrual phase of a study. With the dramatic expansion of basic and applied scientific research, there are an unprecedented number of research options.

Certain diseases have been selected in the attempt to achieve better clinical results more rapidly. Protocols for four potentially curable diseases--adjuvant colon, rectum, bladder and advanced lymphoma have been designated as "High Priority Clinical Trials" of national importance and are targeted for special attention.

These studies are likely to provide important new information and to have an impact on national mortality rates. In order to succeed, there must be greater awareness of and enthusiasm for clinical trials by the general public and health care deliverers.

Efforts to increase accrual to designated "High Priority Clinical Trials" are progressing along two parallel tracks. The Office of Cancer Communications (OCC) is coordinating assessment and information campaigns for the lay and professional communities. The general public is being educated about clinical trials via print and electronic media. 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.

The multidisease, adult Cooperative Groups are expanding their clinical bases. More than 4000 American Society of Clinical Oncology (ASCO) member physicians have been 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. Four Groups are making special efforts to incorporate these new participants and have submitted detailed proposals and budgets to CTEP.

Estimates of activity from these proposals may be summarized as:

Group	New Institutions/Practices	New Patients/Year For Priority Trials
CALGB	27	699
ECOG	27	1044
NSABP	54	930
SWOG	49	1902
Total	157	4575

The magnitude of impact of this accrual to adjuvant studies of patients with colon, rectum, and bladder cancer and for those with aggressive stage III-IV lymphomas will likely be substantial.

b. Breast Cancer

In the past year, three NCI-funded randomized clinical trials in node negative breast cancer were analyzed with each showing an advantage as measured by disease-free survival for the treated patients compared to those receiving no treatment. The results of these three studies were made public through the use of a Clinical Alert. In NSABP B-13 node-negative patients with estrogen receptor negative tumors were treated with methotrexate and 5-fluorouracil or were simply observed. At 4 years treated patients had an 80% chance of being disease free while untreated patients had only a 71% chance of being free of recurrence, a difference that was highly statistically significant ($p=0.003$). This therapeutic benefit was observed for patients ≤ 49 and ≥ 50 years of age. No difference in survival has yet been observed.

In NSABP B-14, estrogen receptor positive, node-negative patients were randomized to tamoxifen or to placebo. There was a statistically significant difference in disease-free survival favoring the tamoxifen treated patients ($p<0.00001$). This benefit also was found in patients ≤ 49 and ≥ 50 years of age. Again, no overall survival differences have been identified at this time.

In the Intergroup study conducted by ECOG, SWOG and CALGB, patients with node negative breast cancer were randomized to either CMFP or observation. Eligibility included all estrogen receptor negative tumors as well as estrogen receptor positive tumors greater than 3 cm. A disease-free survival advantage was identified for the treatment arm of this study which when analyzed by subsets was present for premenopausal and postmenopausal patients as well as for ER positive and ER negative patients. No survival advantage has been identified as of yet.

c. Urologic Cancer

1) During the past year, the prostate intergroup study has matured and has identified a modest but statistically significant survival advantage for combined leuprolide + flutamide compared to leuprolide alone. Further follow-up will better define the extent of this survival advantage.

2) A study to test the benefit of preoperative chemotherapy using the MVAC regimen in locally advanced bladder cancer has been started during the past year. The study is designed to give three courses of MVAC followed by radical cystectomy versus radical cystectomy alone. The study was initiated by SWOG with ECOG also involved in the study's development. In addition it has been designated a high priority trial by the NCI Board of Scientific Counselors.

3) Several studies in early stage prostate cancer have been developed during the past year including the role of adjuvant hormonal therapy for Stage D_1 disease, the role of adjuvant radiation in Stage C disease with positive margins following radical prostatectomy, and the relative merit of radical prostatectomy or radiation for Stages A_2 and B disease (not yet started).

4) The population of patients with poor risk germ cell tumors has been further defined by investigators at Memorial Sloan Kettering Institute. Additionally, the less toxic combination of cisplatin and VP-16 has produced equivalent results to VAB-6 in an early analysis by the same investigators.

5) An intergroup study of RT versus BEP chemotherapy in patients with advanced Stage II testicular seminoma has recently been started and will take three to five years to complete. This study follows important leads suggesting that chemotherapy reduces distant recurrence compared to the standard of treatment, radiotherapy.

d. Colorectal Cancer

1) NSABP Colon C01 Trial

The NSABP has published results of a trial of adjuvant therapy in colon cancer which accrued approximately 1200 patients between 1977-1984. It compared 5-FU/MeCCNU/VCR chemotherapy, BCG, and observation for patients with Dukes' B and C lesions. Average follow-up is 59 months. Approximately one-third of the patients have died. The data show improved disease-free and overall survival for chemotherapy compared with no postoperative therapy (JNCI 80:#1, 1988).

2) NSABP Rectal R01 Trial

In a companion publication for rectal cancer (JNCI 80:#1, 1988), the NSABP compared surgery plus combination chemotherapy or radiotherapy with surgery alone. More than 500 patients have been entered to

date, with results suggesting statistically significant benefit in survival for treatments with MOF chemotherapy.

3) NSABP Colon C02 Trial

A Phase III adjuvant trial of post-operative, seven day, 5-FU portal vein infusion versus surgery alone opened in March 1984. Nine hundred patients have been randomized.

4) NSABP CO_3 + RO_2 Trial

New adjuvant trials were activated by the NSABP in 1987 both in colon and rectal cancer. Both trials will employ the previous best chemotherapy arm of MOF (MeCCNU, Vincristine, 5-FU) randomized against chemotherapy with 5-FU/leucovorin. In addition a 2x2 factorial design will permit a further comparison in the rectal adjuvant trial of chemotherapy alone versus chemotherapy plus irradiation. Both the colon and rectal trials will also incorporate tumor sampling for in vitro determination of thymidylate synthetase activity and total folate pools to determine if some prediction of responsiveness can be made.

5) NCCTG Rectal Trial

The NCCTG reported the results of a trial of adjuvant treatment of rectal carcinoma in 200 stage B_2 and C patients (Proc. ASCO 5:318, 1986). Patients were randomized to radiation alone or radiation preceded and followed by chemotherapy. Median follow-up is 29 months. Preliminary analysis, confirmed now by longer follow-up, suggests a significant advantage in time to recurrence in the combined modality arm. The current NCCTG employs combined radiation + chemotherapy and uses a 2x2 factorial design. It 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.

6) NCCTG Colon Trial

The NCCTG reported the results of a trial of adjuvant treatment in 408 stage B_2 and C colon cancer patients (Proc. ASCO 5:316, 1986). Patients were randomized to levamisole with or without 5-FU versus observation. Both experimental arms show preliminary, significantly improved time to progression and survival compared to the surgery alone arm. Based on this experience, a confirmatory intergroup Phase III trial in adjuvant treatment of colon cancer was started in 1985 and recently completed. Accrual of 1200 patients was accomplished in 2.5 years.

e. Lung Cancer

1) At this year's ASCO meeting, the CALGB reported early closure of a positive trial showing the benefit of induction cisplatin and vinblastine before radiation for locally advanced NSCLC.

2) The Lung Cancer Study Group, in a series of trials examining the role of CAP chemotherapy, have shown that such treatment is superior to BCG/Levamisole in delaying recurrence and prolonging survival for patients with locally advanced (AJC regional stage III) tumors of non-squamous histology, who can first be rendered free of gross disease by surgery. These patients are at high risk of early relapse without such adjuvant therapy.

3) The LCSG plan to investigate a number of promising new approaches to early diagnosis, accurate staging, and treatment of early stage disease. These include the use of indium-labeled monoclonal antibodies and their correlation with pathological findings at the time of surgery; hematoporphyrin derivatives with argon lasers for the diagnosis and treatment of carcinoma-in-situ; evaluation of the use of IL-2 to alter the natural killer activity of tumor infiltrating lymphocytes; and clinical-pathological correlative studies of newer techniques of imaging the thoracic contents. In addition, a tumor repository has been established to determine whether oncogene and growth factor expression in patients is correlated with their clinical outcome. Other Groups, notably ECOG, have explored the possibility of participating in the tumor repository and lab clinical correlation.

f. Melanoma

1) The usefulness of high dose cisplatin, with the chemoprotector WR 2721 is under investigation by ECOG in a Phase III trial in advanced patients. This remains an important clinical test of the hypothesis that more effective drug doses may be tolerated if a protective agent is administered. In order to combine this potentially useful combination with other known active agents, a Phase I pilot of CDDP with WR 2721 and IL-2 plus DTIC will be opened in the near future.

2) The importance of large surgical margins and prophylactic lymph node dissection in clinical Stage I patients continue to be addressed in the large Intergroup Melanoma Study. Hyperthermic isolation perfusion with L-PAM is also being studied by U.S. surgeons, in cooperation with the EORTC and WHO investigators, to prospectively evaluate the advantage of prophylactic perfusion in patients with intermediate thickness extremity melanoma.

g. Ovarian

1) Phase III trials in advanced, suboptimal ovarian carcinoma continue to accrue patients. GOG is studying two dose-intensities of CDDP combined with CYT. SWOG is studying the role of carboplatin in a Phase III trial of CBDCA/CYT vs. CDDP/CYT.

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 up-front 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 examining the role of P^{32}.

h. Cervical

1) The Cooperative Groups have continued extensive Phase II screening of drugs in this disease. The GOG has demonstrated a 40% objective response rate with Dibromodulcitol in patients with advanced or metastatic disease.

2) This result will be the basis for a randomized Phase III trial, with CDDP versus DBD 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.

3) 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.

i. Leukemia

1) In a carefully conducted, prospective randomized trial, ECOG has demonstrated the need for post-remission therapy in adult ANLL in a 3-arm study which compares no further therapy (arm closed early) with maintenance vs intensification. The comparison of the latter two arms is ongoing.

2) CLL trials are either underway or nearly so, exploring various combinations of DCF and fludarabine with either conventional agents or with themselves. A national phase III trial will eventuate.

3) Both alpha- and gamma-IFN have been shown to be active in patients with chronic phase CML. CALGB and SWOG are currently exploring the tolerability of various schedules of combinations of these agents in previously untreated patients. An eventual phase III trial will be developed, although the "standard" therapy arm remains to be determined.

j. Adult Malignant Lymphoma

1) ECOG, with collaboration from CALGB, has successfully completed a direct comparison of CHOP vs m-BACOD in patients with intermediate grade NHL. Response rates in the two arms are comparable. Survival data remain to be analyzed.

2) CALGB has completed a three-arm, randomized comparison of MOPP vs ABVD vs MOPP/ABVD in patients with Hodgkin's Disease. CR rates, disease-free and projected overall survival were superior in the two ABVD-containing arms. A formal analysis of delivered dose with clinical correlations was an integral part of the study.

3) ECOG, CALGB, and SWOG are collaborating in an important comparison of MOPP/ABVD with the newly described MOPP/ABVD hybrid in previously untreated Hodgkin's Disease. Accrual is more rapid than projected.

k. <u>Bone Marrow Transplantation</u>

1) 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. Future directions include substitution of analogues to reduce toxicity and increase efficacy, and to develop disease-directed combinations in definitive studies.

2) 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 therapy for those high risk patients.

3) SWOG has recently activated an important prospective, randomized comparison of allogeneic BMT, ABMT, and conventional consolidation chemotherapy in patients with ANLL in first CR.

4) ECOG is conducting a pilot study of MACOP-B followed by autologous marrow transplantation as front-line therapy for patients with intermediate and high-grade NHL with poor prognostic features. Such risk-directed strategies are an important advance in our approach to therapy.

l. <u>Malignant Brain Tumors</u>

1) 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 high variable. The BTSG currently has a Phase III comparison of external beam versus external beam plus interstitial irradiation for malignant gliomas.

2) Efforts to improve the effectiveness of cranial irradiation through hyperfractionation or halopyrimidine radiosensitization are undergoing study in the RTOG. Other Cooperative Groups and Cancer Centers continue to explore phase II chemotherapeutic agents in malignant gliomas.

3) Intergroup Low-Grade Glioma Trial--An intergroup low grade glioma trial with the participation of the BTSG, RTOG, SWOG and University of California, San Francisco has been activated. The trial will evaluate the optimal timing of radiation therapy and test the role of chemotherapy for low grade tumors. With recent improvements in diagnostic technology (e.g. MRI), this disease entity is becoming increasingly important as are questions of optimal management. The expected accrual of approximately 400 patients will be achieved in three years with follow-up of up to 15 years anticipated.

m. Pediatrics

1) Final results of Intergroup Rhabdomyosarcoma Study IRS-II were reported. This study enrolled 1002 eligible patients and asked four randomized questions based on extent of disease at diagnosis (Group). Combining all patients, disease-free survival (65%) and survival (62%) in this study improved significantly over that obtained in the previous (IRS-I, 61% and 55%, p=.02 and p=<.001, respectively) study. The major improvement was in Group III (patients with bulky residual disease but no metastases, IRS II vs. I: 5 year survival 64% vs. 52%, p=.003), attributable to intensification of therapy and patients with cranial parameningeal primary sites (IRS II vs. I: 5 year survival 65% vs. 45%, p=<.001) associated with the introduction of CNS prophylaxis (Proc ASCO 7:255, 1988).

2) Investigators in the Pediatric Oncology Group reported promising results of therapy for patients with so-called "lower risk" acute lymphoblastic leukemia, who unfortunately still suffer a 20-40% relapse rate on current therapies. Utilizing early intensive 6-mercaptopurine and methotrexate in an effort to rapidly reduce leukemic cell numbers and thereby minimize mutation to a drug resistant state, and to eradicate leukemia cells in sanctuary sites, these investigators have observed 3 relapses among 62 previously untreated patients, with a median follow-up of 28 months. Fifty-nine patients, of whom 26 are off therapy, are in complete remission, for an actuarial disease-free survival of 94% at 3 years (Proc ASCO 7: 174, 1988).

3) The Children's Cancer Study Group has demonstrated that intrathecal methotrexate provides adequate central nervous system therapy in acute lymphoblastic leukemia in patients with intermediate risk features and an age of less than ten years, and in association with similar findings in patients with low risk features, permits the elimination of cranial irradiation, and the attendant risks of decrement in IQ and academic achievement, in over half of all patients without eliminating treatment success (Proc ASCO 7:178, 1988).

4) The Children's Cancer Study Group and the Pediatric Oncology Group have embarked on a collaborative randomized trial evaluating the benefit of ifosfamide and etoposide when added to the standard therapy of newly diagnosed Ewing's sarcoma. Preliminary evidence indicates that the former agents are extremely active in this disease, at least in the setting of recurrent disease. The

therapy of newly diagnosed Ewing's sarcoma. Preliminary evidence indicates that the former agents are extremely active in this disease, at least in the setting of recurrent disease. The establishment of benefit, or lack thereof, of these agents when combined with vincristine, adriamycin, dactinomycin and cyclophosphamide, is the leading question regarding the optimum treatment of this disease.

4. COORDINATION OF CLINICAL TRIALS STRATEGIES AND INTERGROUP STUDIES

In the past year CTEP has continued to emphasize to the oncology community the need for rigorous prioritization of research questions needing clinical trial. The need to concentrate on the key issues in developmental therapy and to address them with trials of adequate size is evident.

Strategy meetings help provide an overview and prioritize national efforts in selected disease sites. Expert oncologists from the Cooperative Group 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 and capitalizing of collaborative research.

During FY '88, strategy meetings were held on adjuvant colon and rectum cancer, melanoma, and adjuvant breast cancer. During the next year, sessions are planned in esophageal and gastric cancer, brain tumors, non-small cell lung cancer, the adult acute leukemias, and ovary cancer.

5. FACILITATING THE CONDUCT OF INTERGROUP STUDIES

a. With the input of the extramural community of cancer cooperative group investigators, CTEP has developed a set of guidelines for the conduct of studies involving two or more cooperative groups. In the past, intergroup studies have generally been developed and conducted in an informal manner. Many participants in intergroup studies have been frustrated by the lack of adequate quality control mechanisms, opportunities for input to study design, and regular monitoring reports. At the request of the cooperative group chairmen, we have drafted a set of guidelines for the design and conduct of intergroup studies. After further input from cooperative group personnel (including statisticians, data managers, operations office staff, etc.), a final set of guidelines has been developed.

b. CTEP is also working with group statisticians to determine, for several test intergroup studies, whether the amount of data collected can be substantially reduced without interfering with the quality or value of the research. Reduction in amount of data collection would both reduce costs and reduce the complexity of intergroup studies.

c. After many years of discussion and negotiation, CTEP staff and representatives of the cooperative groups have succeeded in developing a set of common toxicity criteria for use in all investigational drug studies sponsored by DCT. Use of these criteria will be phased in, as time is necessary for groups and centers to alter their computerized databases and re-educate their staffs.

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CLINICAL INVESTIGATIONS BRANCH

The Clinical Investigations Branch (CIB) is charged with the responsibility for fostering and supporting the best possible extramural clinical oncology research. In performing this task, the CIB integrates its activities with all other Cancer Therapy Evaluation Program Branches and with selected NCI and NIH components. Utilizing an integrated mixture of advisory, informational, and facilitative activities, CIB identifies promising scientific opportunities, stimulating specific trials conceived intra- or extramurally.

COMPREHENSIVE DISEASE/MODALITY INFORMATION

In order to promote optimal studies, the CIB attempts to assemble a comprehensive disease and modality perspective for clinical research activities. In order to identify and articulate the most demanding research questions, the 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
NUTRITION	NERENSTONE
PSYCHOSOCIAL	NERENSTONE
RADIATION	HAMILTON
SURGERY	FRIEDMAN (interim)

Specific disease responsibilities are divided as follows:

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

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 interest in 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, physician and patient resources. During the past year, particular administrative problems have included: the reallocation of resources to provide interim funding for the Brain Tumor Cooperative Group during the period preceding NCAB approval; providing interim funding for the Gynecologic Oncology Cooperative Group, and supervising that Group's reorganization and submission of an amended competing application; devising policy and guidelines for the submission of requests for administrative supplementation of Groups participating in the new high priority trials initiative; integrating per-case reimbursement within the Clinical Trials Cooperative Group Program, where appropriate; redefining the terms of award for cooperative agreements (currently in review); advising the Radiation Therapy Oncology Group concerning its upcoming competing reapplication; site visiting the headquarters of the Leukemia Intergroup study at the request of the CCIRC, to determine whether changes had been made as required by the summary statement; providing administrative supplementation to the Eastern Cooperative Oncology Group for continued supervision of the Intergroup Melanoma study; transfer of administrative responsibility for the Radiologic Physics Center to the Radiation Research Program, DCT; funding of the SWOG Urology Outreach application; and the finalization and publication of Guidelines for the Clinical Trials Cooperative Group Program to enable all participants (applicants, awardees, NCI staff and reviewers) to clearly understand his or her role in the conduct of the Program. 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 in the protocol generation process. Specifically, CIB staff regularly attend formal Group meetings promoting idea stimulation and information exchange. An effort is made to prevent duplicative protocols and to foster the very best science.

A second area of interaction is the Concept Review, which is an evaluation of the essence of a major Phase III study while still in an early stage of development. A brief document outlining the scientific background, objectives, eligibility, treatment schema and statistical section is sent to the CIB, which provides relevant criticism in return. Theoretically, it is more efficient and productive to evaluate a concept than to modify a protocol at the final stage of development. A total of 9 concepts were received from four Cooperative Groups;

one has become an active study seven are still in discussion or revision, and one is still being reviewed. This format invites fruitful dialogue between the investigators and NCI.

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. There is, consequently, a continuum of CIB interaction with the Cooperative Group system from the very earliest ideal formation to the review of the finished document.

The CIB works to promote 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 only a limited number of scientific questions of the highest priority. An intergroup study is deemed appropriate when a Cooperative Group study would require an inordinately long time for completion and/or might accrue too few patients to permit a powerful and complete statistical analysis.

Finally, the CIB has responsibility to promote relevant laboratory-clinical correlative interactions which would prove scientifically fruitful. Information concerning the best possible correlations comes not only from Group pilot activities, but also from information gained from the R01/P01 pool of grants which CIB manages.

The following is a list of the Cooperative Groups a funded intergroup organizations that were functioning with NCT support in 1987 and the CIB staff member who was responsible for scientific liaison with that organization.

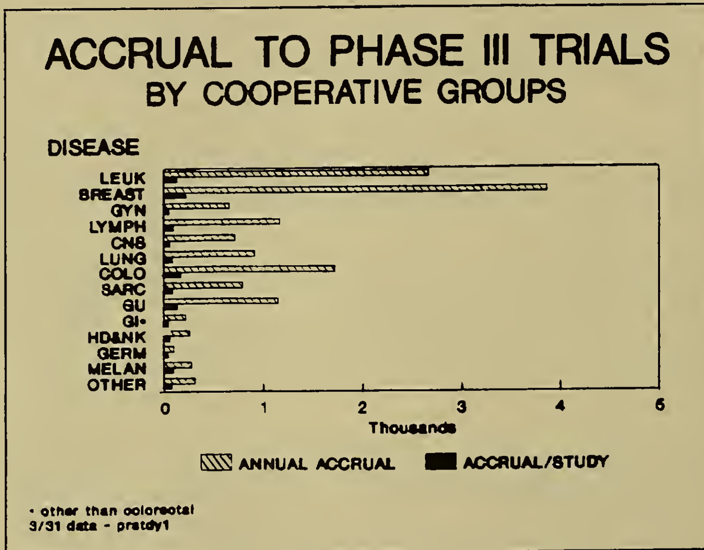
GROUP	CIB STAFF
Brain Tumor Study Group (BTSG)	Hamilton
Cancer and Acute Leukemia Group B (CALGB)	Cheson
Children's Cancer Study Group (CCSG)	Ungerleider
Eastern Cooperative Oncology Group (ECOG)	Dorr
European Organization for Research on Treatment for Cancer (EORTC)	Cheson
Gynecologic Oncology Group (GOG)	Nerenstone
Intergroup AML (IAML)	Cheson
Intergroup Melanoma Group (IMG)	Nerenstone
Intergroup Rhabdomyosarcoma Study (IRS)	Ungerleider
Intergroup Sarcoma Group (ISG)	Nerenstone
Lung Cancer Study Group (LCSG)	Hamilton
National Surgical Adjuvant Breast and Bowel Project (NSABP)	Dorr
National Wilms' Tumor Study Group (NWTSG)	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. Due to deficit reduction measures, the initial award was at 90% of FY87 levels for 3 months, but was followed by retroactive restoration to 100% of the amount recommended by peer review for a total of $4.5 million.

SCOPE OF ACTIVITIES

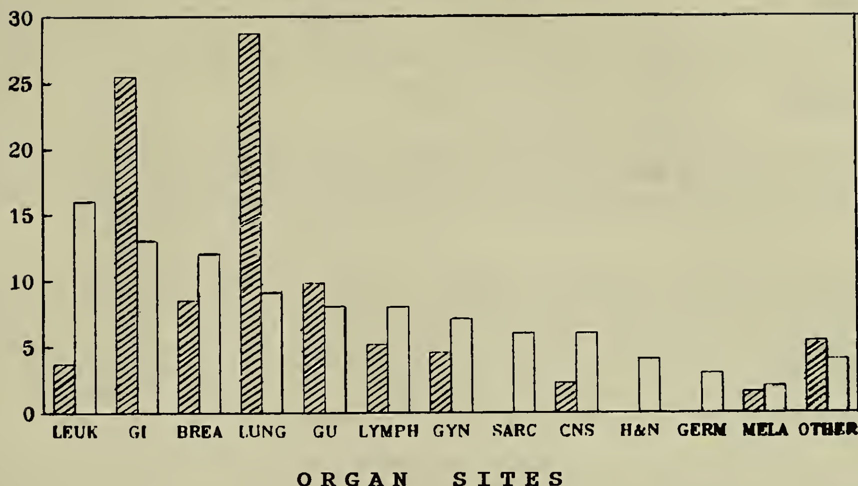
In 1987 approximately 25,000 new patients were entered on therapeutic studies (Phase II and III) in CTEP sponsored trials conducted by the Cooperative Groups. Virtually every type of malignancy is being studied in this collaborative enterprise. Phase III definitive tests of efficacy are the central component of the effort to reduce cancer mortality. Patient accession by disease is indicated below:



The following graph compares clinical research effort in relation to disease-specific causes of death. Lung and gastrointestinal malignancies, the major causes of cancer deaths in the U.S., are obvious targets for expanded clinical trials effort (see section on High Priority Trials and Strategy Sessions).

ACTIVE PHASE III CLINICAL TRIALS

ORGAN SITE VS FY86 DEATH RATE



|////| % OF CANCER DEATHS |____| % OF PHASE III TRIALS

<u>TERMS OF AWARD FOR GROUPS</u>

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 are being revised to reflect evolving expectations. The NCI will interact even more closely with each Group to implement clinical studies of the highest quality. New studies will be initiated only after much closer scrutiny to insure that all relevant scientific issues have been considered and that the study will be completed as quickly as possible. Intergroup collaborations will be required where appropriate, and capitation forms of reimbursement utilized to stimulate patient enrollment. The management of the large, complex and worthwhile U-10 mechanism will be tightened in order to maximize productivity.

<u>HIGH PRIORITY CLINICAL TRIALS--THE NEED TO INCREASE ACCRUAL</u>

A major impediment to progress in curing more cancer patients is that the necessary clinical investigation proceeds too slowly. For nearly every malignant disease, crucial studies accrue patients at an unacceptably slow rate, thus preventing the identification of new effective therapies in a timely and precise fashion. The Clinical Cooperative Groups are major contributors to clinical research, and currently enroll about 25,000 patients/year on therapeutic studies. However, this number is tiny when compared to the roughly 1,000,000 new cancer cases identified yearly in the USA. For the common adult malignancies only .5% to 3% of available patients are studied each year. Since definitive studies may require 1000 to 3000 patients, it has often taken a decade or more to complete

the accrual phase of a study. With the dramatic expansion of basic and applied scientific research, there are an unprecedented number of research options.

Certain diseases have been selected in the attempt to achieve better clinical results more rapidly. Protocols for four potentially curable diseases--adjuvant colon, rectum, bladder and advanced lymphoma have been designated as "High Priority Clinical Trials" of national importance and are targeted for special attention.

These studies are likely to provide important new information and to have an impact on national mortality rates. In order to succeed, there must be greater awareness of and enthusiasm for clinical trials by the general public and health care deliverers.

Efforts to increase accrual to designated "High Priority Clinical Trials" are progressing along two parallel tracks. The Office of Cancer Communications (OCC) is coordinating assessment and information campaigns for the lay and professional communities. The general public is being educated about clinical trials via print and electronic media. 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.

The multidisease, adult Cooperative Groups are expanding their clinical bases. More than 4000 American Society of Clinical Oncology (ASCO) member physicians have been 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. Four Groups are making special efforts to incorporate these new participants and have submitted detailed proposals and budgets to CTEP.

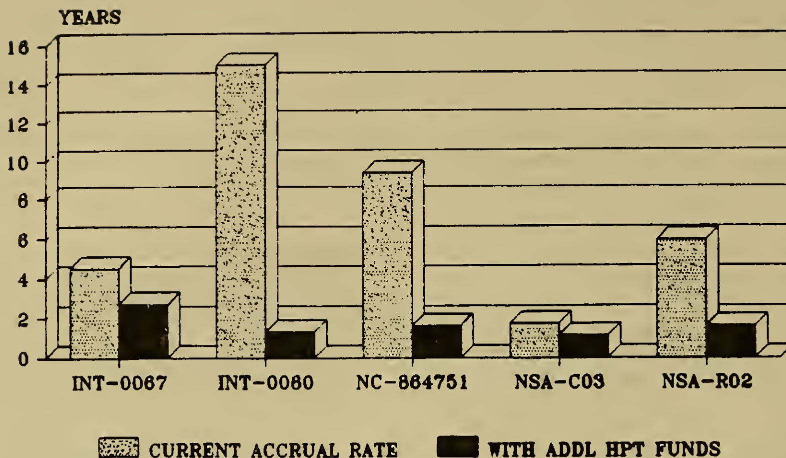
Estimates of activity from these proposals may be summarized as:

Group	New Institutions/Practices	New Patients/Year For Priority Trials
CALGB	27	699
ECOG	27	1044
NSABP	54	930
SWOG	49	1902
Total	157	4575

The magnitude of impact of this accrual to adjuvant studies of patients with colon, rectum, and bladder cancer and for those with aggressive stage III-IV lymphomas will likely be substantial. A description of these trials is as follows:

STATUS OF COOPERATIVE GROUP PHASE III STUDIES AS OF 3/31/88
HIGH PRIORITY TRIALS
CLOSURE DATE BASED ON ACCRUAL RATE FROM 1/1/88 TO 3/31/88

Protocol Number	Study	Target Accrual	Accrual as of 3/31/88	Current Annual Rate of Accrual	Activation Date
INT-0067	A Phase III comparison of CHOP vs. M-BACOD vs. PRO-MACE-CYTABOM vs. MACOP-B in patients with intermediate or high grade non-Hodgkin's lymphoma	824	274	220	04/04/86
INT-0080	A Phase III trial of cystectomy alone vs. neoadjuvant M-VAC + cystectomy in patients with locally advanced bladder cancer	298	10	20	08/01/87
NCCTG-86-47-51	Phase III protocol for surgical adjuvant therapy of rectal carcinoma: a controlled evaluation of protracted infusion 5-FU as a radiation enhancer and 5-FU plus methyl-CCNU chemotherapy	450	38	48	06/02/87
NSABP-C-03	A study to compare leucovorin and 5-FU with adjuvant MECCNU, vincristine and 5-FU (MOF) in patients with Dukes' B and C carcinoma of the colon	855	261	588	08/01/87
NSABP-R-02	A clinical trial to compare adjuvant methyl CCNU, vincristine, 5-FU (MOF) with and without radiation to adjuvant leucovorin and 5-FU (LV-5-FU) with and without radiation in patients with Dukes' B and C carcinoma of the rectum	750	60	132	08/01/87



Currently CTEP is analyzing the budget requests for these new participants and encouraging current group members to enroll their available patients. An orderly, meaningful expansion of patient accrual to studies addressing the most pressing scientific questions is underway.

CLINICAL TRIALS TRACKING SYSTEM

CIB has developed a clinical trials tracking system to focus attention on ongoing studies of importance 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. A major feature of this system is the ability to organize and identify studies by the scientific hypothesis being tested. Also CIB staff will analyse the clinical trials portfolio bared on hypothesis. It is also possible to accurately assess patient accrual and projected closure date. Thus the management of all Group studies can be improved.

STRATEGY MEETINGS

Strategy meetings help provide an overview and prioritize national efforts in selected disease sites. Expert oncologists from the Cooperative Group 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. The following topics have been discussed.

CIB STRATEGY MEETINGS

Date	Coordinator	Topic	Participants	Representing
9/21/87	Dorr	Testicular Cancer	George Bosl	MSKCC
			David Crawford	SWOG
			Michael Friedman	NCI
			Mark Garnick	DFCI
			David Johnson	ECOG
			Patrick Loehrer	Indiana
			Alison Martin	NCI
			Craig Nichols	Indiana
			Michael Sarosdy	SWOG
			Ronald Stephens	SWOG
9/28/87	Hamilton	Adjuvant Therapy of Colon Cancer	Susan Ellenberg	NCI
			Michael Friedman	NCI
			Daniel Haller	ECOG
			Bernard Levin	MDAH
			John Macdonald	SWOG
			Robert Mayer	CALGB
			Charles Moertel	NCCTG
			Anthony Russell	RTOG
			Robert Wittes	NCI
			Norman Wolmark	NSABP
12/18/87	Nerenstone	Melanoma	Dean Bajorin	MSKCC
			Robert Benjamin	MDAH
			Edward Creagan	Mayo
			Robert Dalton	
			Bruce Dana	
			Susan Ellenberg	NCI
			Lawrence Flaherty	Wayne St.
			Michael Friedman	NCI
			Donna Glover	U.PA CC
			Geoffrey Herzig	Wash. U.
			Alan Houghton	MSKCC
			Raymond Kempf	KNCC*
			John Kirkwood	PittCI*
			Sewa Legha	MDAH
			Michael Lotze	NCI
			Alison Martin	NCI
			Malcolm Mitchell	NCI
			Joyce O'Shaughnessy	NCI
			David Parkinson	NEMC*
			Carl Pinsky	NCI
			Ian Quirt	PMH*

Date	Coordinator	Topic	Participants	Representing
12/18/87 Continued	Nerenstone	Melanoma	Gisele Sarosy	NCI
			Tom Shea	DFCI
			Mario Sznol	NCI
			David Van Echo	UMCC
4/12/88	Dorr	Breast	Martin Abeloff	ECOG
			Judith Bader	NCI
			Kenneth Cowan	NCI
			Barbara Fowble	RTOG
			Richard Gelber	IBCSG*
			John Glick	ECOG
			Robert Gray	ECOG
			Stephanie Green	SWOG
			Thomas Hakes	MSKCC
			Craig Henderson	DFCC
			James Ingle	NCCTG
			Ann Korzun	CALGB
			Larry Norton	CALGB
			Kent Osborne	SWOG
			Kathleen Pritchard	NCIC
			Carole Redmond	NSABP
			Dan Schaid	NCCTG
			Thomas Smith	ECOG
			Sandra Swain	NCI
			Douglass Tormey	ECOG
			Lawrence Wickerman	NSABP
			Norman Wolmark	NSABP
			William Wood	CALGB

* KNCC = Kenneth Norris Cancer Center
NEMC = New England Medical Center
PittCI = Pittsburgh Cancer Institute
PMH = Princess Margaret Hospital, Toronto

STRATEGY SESSIONS PLANNED FOR FY89

1. Esophageal Cancer--A session to develop multimodality clinical questions for advanced and adjuvant squamous cell cancer will be held in the Summer of 1988 in preparation for a contract effort.

2. Gastric Adjuvant--A strategy meeting to develop an intergroup clinical trial in adjuvant therapy of gastric cancer will be held in the fall of 1988.

3. Radiation Therapy Quality Control--With the RRP, CTEP will sponsor a strategy session to review Group practices and develop intergroup

guidelines for external beam radiation therapy quality assurance. This meeting will be held in the Fall of 1988.

4. Brain Tumor Clinical Trials--A meeting to develop guidelines for eligibility data stratification, response measurement, and trial endpoints for brain tumor clinical trials will be held during 1988-89.

5. Non-small Cell Lung Cancer (NSCLC)--A strategy session to develop future direction for locally advanced and advanced NSCLC will be held in the Winter-Spring of 1989.

6. Adult Acute Lymphoblastic Leukemia--A session to discuss therapeutic options for this difficult disease.

7. Acute Non-Lymphocytic Leukemia (ANLL)--Currently there are discrepancies in the diagnostic criteria and criteria for complete response in acute non-lymphocytic leukemia among the cooperative groups and cancer centers. In addition, there are new technologies which accurately identify minimal residual disease. A meeting to standardize the diagnostic and response criteria for adult and pediatric ANLL will be held in August 1988.

8. Ovarian Cancer--A meeting to develop the next studies in advanced, bulky disease and to plan an Intergroup effort in patients with complete response to primary therapy.

9. Prostate Cancer--A meeting to develope the next generation of efforts for advanced stage patients.

LABORATORY-CLINICAL CORRELATIONS

Approximately 30 Phase III Group studies have formally described Lab-Clinical correlations. Some of the most interesting are:

1. Studies of the biologic significance of genetic rearrangements in the malignant cells of children with cancer offer increased reliability in correctly diagnosing patients and in predicting which patients are likely to relapse. Preliminary application of this methodology in the childhood neuroectodermal tumors has already demonstrated that (a) n-myc oncogene copy number is directly related to clinical outcome in neuroblastoma (CCSG), and (b) peripheral neuroepithelioma (PN), a tumor histopathologically indistinguishable from neuroblastoma, is in fact more closely related to Ewing's sarcoma; PN patients treated as Ewing's sarcoma do far better than those treated as neuroblastoma (M. Israel). These techniques will be applied to larger numbers of patients with solid tumors and leukemia. The cytogenetics data base being built by both pediatric cooperative groups will be available to identify patients whose tumor cells have rearrangements of specific gene regions of interest. Cells stored in group tissue banks or immunology reference laboratories will be used for studies of the relationship of immunophenotyping and cytogenetics in childhood ALL, and limited exploratory studies of the relationship between immunophenotyping and molecular genetics of childhood T-cell ALL. These efforts should provide a better understanding of the biology of ALL as it relates to overall response to the treatments offered.

2. Recent multivariate analyses of the CCSG data suggest that immunophenotype, using the cytoplasmic immunoglobulin marker and BA-1 (CD-24), adds to the prognostic importance of age and white blood cell count predicting outcome for ALL and displaces some of the prognostic and clinical markers thought to be quite important in the past. This and other important prognostic information supplied by cytogenetic analyses permits the tailoring of therapy to the particular risk that an individual child may have for a subsequent event or a successful remission induction. The conjecture that immunophenotype, early response data, FAB morphology and karyotype analysis will identify overlapping groups of patients with bad prognosis and that these patients may benefit from an early change in their therapy will be tested on the upcoming CCSG-164 study.

3. SWOG and, more recently, ECOG myeloma studies are incorporating labeling indices and $beta_2$-microglobulin determinations. $Beta_2$ microglobulin has been demonstrated in several successive SWOG studies to be the single best predictor of outcome.

4. SWOG and CALGB--a collaborative effort in ANLL and MDS to study phenotyping, cytogenetics, and sophisticated molecular biology studies with appropriate clinical correlations. Not only are all patients studied with currently available probes, but samples are being frozen which will be available to other scientists who propose a worthwhile scientific question.

5. Molecular studies and Deoxycoformycin (DCF) pharmacology are now being incorporated into the large-scale hairy cell leukemia trials.

6. Clinical pharmacology studies are planned for the phase I DCF/ fludarabine trials in CLL.

7. Based on the activity of both alpha- and gamma-IFN as single agents, trials are now ongoing in SWOG and CALGB which are evaluating the combination of these two agents in previously untreated CML patients. These studies also include state-of-the-art cytogenetics and molecular studies of the bcr gene using newly developed analytic techniques including polymerase chain reaction studies.

8. SWOG is currently evaluating patients with ANLL who are resistant to chemotherapy for MDR gene amplification.

9. SWOG is performing studies in myeloma which are exploiting the ability of verapamil to reverse MDR. In vitro correlates are an important part of the study. Future studies in lymphoma will be considered if supported by pilot data in myeloma.

10. Investigators in CALGB are evaluating the clinical importance of a number of newly identified phenotypic markers in CLL (e.g., CD5, shared idiotypes).

11. In CALGB's recently funded U10 application, support was provided for a clinical pharmacology program. One of the first drugs to undergo group-wide evaluation is amonafide.

12. CALGB is completing its study of ara-C pharmacology in patients with ANLL and attempts will be made to correlate blood levels with response.

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.

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.

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. Nerenstone coordinates these activities with CIB.

CONTRACTS

Extramural Clinical Trials Office (ECTO)--EMMES

This contract provides operations and administrative support for a number of CTEP supported extramural research efforts. The services provided include: assistance in protocol and forms design; patient randomization; quality control data; coordination of scientific activities of clinical investigators, statisticians and project officers; planning of meetings and preparation of agenda, minutes, reports, communications, and related administrative tasks. The contractor also provides analytical support to CTEP in evaluating data obtained from extramural clinical research resources (such as the LAK-IL2 trials).

Intergroup Testicular Study

This is a collaboration among seven Cooperative Groups and four large institutions having an interest in testicular cancer. The protocol is a randomized controlled study of adjuvant chemotherapy of Stage II resectable testicular cancer and monitoring of Stage I testicular cancer.

For Stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapse) versus surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to identify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Progress presentations have been made at various Cooperative Group meetings: CALGB, SEG, SWOG, AND NCOG. This study is nearing completion, and full analyses are forthcoming.

MANAGING INVESTIGATOR-INITIATED GRANTS AND CONTRACTS (R Series, P01's, U01's, SBIR's)

The purpose of the CIB 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 Coordi- nators 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 88, the CIB managed 151 investigator-initiated funded grants and contracts. These are broken down into the respective categories in the following table.

Code	Clinical Oncology	Cancer and Nutrition	Surgical Oncology	Total
R01	65	11	8	84
R13	7	0	0	7
R15	0	2	0	2
R21	0	0	2	2
R23	2	0	0	2
R29	4	1	0	5
R35	2	0	0	2
R37	2	0	1	3
R43	4	0	0	4
R44	3	0	1	4
Subtotal	89	14	12	115
P01	29	0	5	34
Subtotal	118	14	17	149
U01	1	0	0	1
Subtotal	119	14	17	150
SBIR Contracts	0	0	1	1
GRAND TOTAL	119	14	18	151

Most of the money in the grants/contracts pool was spent on P01 grants. CIB manages one of the largest portfolios of P01 grants both in terms of dollars and numbers of grants within the NCI. In FY 88, there was a net gain of 2 P01's, increasing the total to 34. During FY 88, the Grants Program Director attended 19 site visits for the review of P01's and performed 6 formal consultations on P01 submissions. During these formal consulting sessions in Bethesda, 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, numerous hours are spent on the telephone with both new and recompeting applicants. Approximately 40% of those P01 grants assigned to CIB 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 CIB and represents the "cutting edge" of both basic and clinical research.

The number of active R01 grants managed by CIB has declined to a total of 84, representing a net loss of 4 grants. There are two reasons for this decline: (1) the assignment of 3 grants into the R37 (Merit Award) category and (2) the tough standards of grading by the ET I and ET II Study Sections. The success rate of obtaining funding in the R01 pool is rather poor. Only <u>20%</u> of those grants assigned to CIB are being funded. With the advent of percentiling during the third round of the review/funding cycle in FY88, the success rate of funding for grants assigned to CIB has increased to approximately 28%. In collaboration with Dr. Dennis Cain, the Grants Program Director did a computer analysis of the impact of percentile scoring on both R01 and P01 grants. The projection is that percentile scoring will help fund more clinically oriented grants and should help those applicants whose grants will be reviewed by ET I and ET II.

The SBIR grants/contracts program has been a rousing success. Five SBIR grants/contracts have reached phase II. It is anticipated that useful software packages for clinical trials, two new types of laser surgical equipment and a data base on questionable cancer remedies will be available within two years.

One cooperative agreement (U01) was funded in FY88. This cooperative agreement was awarded to Dr. John Koch of the Mayo Foundation as the result of his response to a RFA (85-CA-13) issued by the Investigational Drug Branch. The laboratory and clinical investigations are directed toward the development and optimal clinical use of a combination of drugs which is synergistic in vitro. Using a rat animal model, Dr. Kovach will attempt to develop regimens of combinations of FdUrd and IdUrd to achieve a level of IdUrd replacement of thymidine in DNA necessary to enhance radiation effectiveness at least twofold. It is hoped that ultimately these regimens will be applied to cure human glioblastomas by enhancement of the radiosensitivity of the tumor through selective biochemical modulation of incorporating IdUrd into tumor DNA.

During FY88 there was a reorganization of the Organ Systems Program. All grants (R01/P01) previously assigned to the Organ Systems Program were distributed to the different Divisions of the NCI according to program relevance/scientific content. CIB gained one P01 grant, six R01 grants and one R43 grant as a result of this grant transfer. In the future, all new and competing renewals will be assigned by the Division of Research Grants (DRG) to the respective programs according to referral guidelines. Furthermore, all new initiatives (RFA's, PA's) in a scientific area will be the respon- sibility of the Program Director assigned to that particular scientific area.

Highlights of Investigator Initiated Grants/Contracts

Several significant discoveries/leads with potentially important clinical applications/implications were made in FY88 by PI's who were supported by grants managed by CIB.

Dr. James Speyer (2 R01 CA3654-04) reported a positive clinical study on the cardioprotective effect of ICRF 187 against adriamycin toxicity. The patients receiving ICRF 187 had less or little damage to the heart as compared to Adriamycin alone, determined by histopathological measurements from endomyocardial biopsies.

Dr. Emil Freireich (5 R35 CA-3909-04) and his colleagues have successfully used the polymerase chain reaction (PCR) to detect the 14;18 translocation in treated leukemia patients. This was the first clinical application of this important molecular biological technique (PCR) to malignant disease and was published in Science and will aid in the detection of minimal residual disease in treated patients.

Dr. Rowan Chlebowski (5 R01 CA-37320-03) reported in a small pilot study that treatment with hydrazine sulfate increased the survival of unresectable non-small cell lung cancer patients. Furthermore, the patients treated with hydrazine sulfate had an improvement in their appetite and a stable or increased weight. Overall, hydrazine sulfate seems to give a better quality of life to these incurable cancer patients.

Dr. Herbert Hoover, Jr. (5 R01 CA-45079-02) reported encouraging results in his randomized preoperative clinical trial assessing adjuvant active specific immunotherapy in patients with B_2-C_3 colorectal cancer. Patients treated with tumor cell-BCG vaccines showed an encouraging trend toward increased disease-free interval are survival in colon cancer patients but not in rectal cancer patients.

Dr. William J. Hrushesky (5 R01 CA-31635-06) has been performing studies to determin the efficacy of chronobiology in cancer therapy. He has reported that the respective toxicities of doxorubicin and cisplatin is dependent upon the day the drugs are given. He is currently expanding these chronoclinical studies to other drugs and biological response modifiers. He has accrued 200 colorectal and renal cell cancer patients and has used programmable pumps to regulate the delivery of the therapeutic agents in accordance with the established biological rhythm. Itg is clear from the preliminary data that infusion shape (in tissue) markedly affects all fluoropyrimidine toxicities and maximal dose intensities. His work with IL-2 and TNF indicates that circadian timing of biological agents will be even more important than the timing of chemotherapeutic agents.

Dr. Donald Kufe (5 R01 CA-42802-02) has developed a technique to distinguish leukemia cells from normal cells simultaneously with determination of cell differentiation. He is now able to track the maturation of leukemia myeloid cells. This technique will be useful for studies to determine effective differentiation-inducing agents in vivo. Several biologic agents including IL-3, GM-CSF and G-CSF have been tested for their differentiation-inducing capabilities on AML cells. IL-3 and GM-CSF were effective at inducing proliferation while G-CSF was not effective. These results suggest that G-CSF may not be a useful differentiating agent in human leukemia even though it is useful in murine leukemia.

Dr. Charles Moertel (2 P01 CA31224-04A1) piloted successful clinical trials of the regimen 5FU plus levamisole in Dukes' C colon cancer patients; and of the somatostatin analogue 201-995 for metastatic carcinoid and islet cell tumors.

Dr. William Evans has been conducting studies on hepatic drug clearance in children with ALL. He has published in the New England Journal of Medicine his finding that interpatient variability in the clearance of high-dose methotrexate can have a significant influence on the risk of relapse in children with ALL. This improved hepatic clearance of drugs may be related to the eradication of leukemic infiltration of the liver once complete remission was achieved.

Dr. Bayard Clarkson (5 P01 CA-05826-27) and his colleague, Dr. Alan Yagoda (Project Leader), reported a successful clinical study using M-VAC to treat patients with advanced bladder cancer. The estimated probability of surviving 2 and 3 years is 71% and 55% respectively in patients with metastatic disease.

In the same P01 grant, Dr. Nancy Kemeny (Project Leader) demonstrated that the intrahepatic round of chemotherapy is metastatic liver carcinoma. A 50% response rate was observed for the intrahepatic technique whereas only 15.6% response rate was observed in the systemic technique.

Dr. George Santos (5 P01 CA-15396-15) was named the recipient of the Bristol Meyers Award for his contributions to the field of bone marrow transplanta- tion. Durin the 15th year of his grant, Dr. Santos reported significant increases in disease-free survival for allogeneic transplanted patients with ALL in CR-1 and CR-2 and ANLL in CR-1. This improvement was primarily due to a decrease in several GVHD and lethal viral infections.

MERIT AWARD RECIPIENTS

Dr. William Evans (2 R37 CA36401-04), Dr. Robert Diasio (5 R37 CA40530-05) and Dr. Edmund Chao (2 R37 CA23751-10) were nominated by the Grants Program Director, were approved by the NCAB and became the recipients of MERIT awards. MERIT awards are given to those investigators who have demonstrated their research competence and productivity and who received a priority score on a competing R01 research application within the first two deciles.

Dr. William Evans demonstrated in children that maximally tolerated systemic exposure is a better measure of anticancer drug delivery than the average minimum toxic dose derived from traditional phase I studies.

Dr. Robert Diasio has attempted to improve the clinical effectiveness of fluoropyridmine (FP) drugs through a better understanding of the biochemical and pharmacologic factors that determine toxicity with a major focus on FP catabolism in humans.

Dr. Edmund Chao has centered his research activities around general orthopedic problems after limb sparing surgery in cancer patients. He is developing a new implant/prosthetic system.

Grants/Contracts Information Distribution System

Computer searches with their semiannual updates were provided to the drug monitors and disease coordinators. This information distribution system contributed toward the effort of improving intra-program communication among the branches. In FY88 the Grants Program Director also established a system to circulate relevant reprints received in the competing and non-competing renewal grant applications thereby providing program staff with access to unpublished/preprint material and information.

The challenge for the upcoming year clearly springs from our present activities in FY88. The first of these is the continuing refinement of the Cooperative Group system and ongoing potential remodeling and restructuring. In order to achieve the most efficient and productive Group system, considerable CIB energy will continue to be expended in this effort. Secondly, the establishment of national scientific priorities will continue to be an area of responsibility. Groups of extramural investigators will be called in to the Cancer Therapy Evaluation Program in order to help set goals which will be equitably determined, clearly communicated and efficiently achieved. This effort will not stifle innovation by the extramural community. Rather it is meant to provide a helpful priority framework for major Phase III investigations which consume such substantial effort and money. Obviously, pilot studies and Phase II studies will continue to be done as they are now, stimulated by Cancer Center or Cooperative Group activities and P01 grants. No attempt will be made to legislate scientific creativity. The only goal here is responsible management and disciplined science. Thirdly, new scientific initiatives must continue to be the challenge for the CIB. Important scientific hypotheses must be identified in a timely manner and properly addressed in a definitive way.

SPECIFIC PROGRAM ACCOMPLISHMENTS

PLANS FOR FY89

The Clinical Investigations Branch is oriented toward the clinical study of disease and/or modality issues. The following are selected highlights of the current program and specific plans for the future.

PEDIATRICS

Accomplishments

1. Final results of Intergroup Rhabdomyosarcoma Study IRS-II were reported. This study enrolled 1002 eligible patients and asked four randomized questions based on extent of disease at diagnosis (Group). Combining all patients, disease-free survival (65%) and survival (62%) in this study

improved significantly over that obtained in the previous (IRS-I, 61% and 55%, p=.02 and p=<.001, respectively) study. The major improvement was in Group III (patients with bulky residual disease but no metastases, IRS II vs. I: 5 year survival 64% vs. 52%, p=.003), attributable to intensification of therapy and patients with cranial parameningeal primary sites (IRS II vs. I: 5 year survival 65% vs. 45%, p=<.001) associated with the introduction of CNS prophylaxis (Proc ASCO 7:255, 1988).

2. Investigators in the Pediatric Oncology Group reported promising results of therapy for patients with so-called "lower risk" acute lymphoblastic leukemia, who unfortunately still suffer a 20-40% relapse rate on current therapies. Utilizing early intensive 6-mercaptopurine and methotrexate in an effort to rapidly reduce leukemic cell numbers and thereby minimize mutation to a drug resistant state, and to eradicate leukemia cells in sanctuary sites, these investigators have observed 3 relapses among 62 previously untreated patients, with a median follow-up of 28 months. Fifty-nine patients, of whom 26 are off therapy, are in complete remission, for an actuarial disease-free survival of 94% at 3 years (Proc ASCO 7: 174, 1988).

3. The Children's Cancer Study Group has demonstrated that intrathecal methotrexate provides adequate central nervous system therapy in acute lymphoblastic leukemia in patients with intermediate risk features and an age of less than ten years, and in association with similar findings in patients with low risk features, permits the elimination of cranial irradiation, and the attendant risks of decrement in IQ and academic achievement, in over half of all patients without eliminating treatment success (Proc ASCO 7:178, 1988).

4. The Children's Cancer Study Group and the Pediatric Oncology Group have embarked on a collaborative randomized trial evaluating the benefit of ifosfamide and etoposide when added to the standard therapy of newly diagnosed Ewing's sarcoma. Preliminary evidence indicates that the former agents are extremely active in this disease, at least in the setting of recurrent disease. The establishment of benefit, or lack thereof, of these agents when combined with vincristine, adriamycin, dactinomycin and cyclophosphamide, is the leading question regarding the optimum treatment of this disease.

<u>Future Plans</u>

1. The Intergroup Rhabdomyosarcoma Study has begun preparations for the successor to the current study. IRS-IV will use a staging system independent of the extent of surgery for the first time (Proc ASCO 7:255, 1988). Stage III patients will be entered in a randomized study to determine the benefit of ifosfamide and etoposide when added to standard VAC therapy, as well as the benefit of hyperfractionated vs. conventional irradiation; the experimental arms will enter the pilot (feasibility) phase shortly. Stage IV (metastatic) patients 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. The pilot phase will begin shortly.

2. A study of very high dose methotrexate as a therapeutic option that permits the elimination of cranial irradiation in high risk ALL patients has now been tested by the Children's Cancer Study Group and will be incorporated in a randomized study (vs. BFM therapy).

3. The next front-end CCSG protocol for acute non-lymphoblastic leukemia will include an induction question and a comparison of three arms for post-induction therapy. For induction, 5 drugs will be given simultaneously over 96 hours ("DCTER" regimen), and patients will be randomly assigned to repetition of the drugs on days 10-13 irrespective of marrow cellularity <u>vs.</u> administration using cellularity on day 14 as the basis to decide when to repeat the cycle (the standard approach). The post-induction therapy will consist of allogeneic transplant <u>vs.</u> autologous transplant after in vitro treatment with hydroxycyclophosphamide <u>vs.</u> standard consolidation therapy.

4. Preliminary evidence indicates that transplantation of autologous bone marrow purged of malignant cells with magnetic immunobeads is an effective therapy for newly diagnosed advanced stage neuroblastoma in remission. The relative benefit of this toxic, complicated and costly procedure will be established by the Pediatric Oncology Group through a concurrently controlled clinical trial comparing transplant with conventional therapy.

<u>MELANOMA</u>

<u>Accomplishments</u>

1. Immune manipulation remains an area of active research in melanoma patients. Various cytokine combinations, either with cytotoxics (such as IL-2 plus cyclophosphamide or DTIC), or with other biologicals (such as IL-2 plus monoclonal antibodies) have continued to be tested in Phase II trials in advanced disease, attempting to optimize response while minimizing the severe toxicity seen with high doses of agents such as IL-ECOG has also started a randomized Phase II study of gamma interferon in advanced disease patients in an attempt to find the most active dose of an agent which, in preclinical studies, may exhibit a bell-shaped dose-response curve. SWOG is continuing its adjuvant gamma- interferon study, while ECOG and NCCTG continues their alpha adjuvant trial.

2. Dose-intensity questions are being asked in patients with advanced melanoma using cytotoxic agents. High dose alkylating agents with autologous bone marrow transplantation has demonstrated significant activity in patients with metastatic disease, with metastatic CNS disease responding in some patients. However, the response durations have been short. Researchers are continuing to investigate combinations of drugs in order to select agents that may have synergistic antitumor efficacy.

3. The usefulness of high dose cisplatin, with the chemoprotector WR 2721 is under investigation by ECOG in a Phase III trial in advanced patients. This remains an important clinical test of the hypothesis that more effective drug doses may be tolerated if a protective agent is administered. In order to combine this potentially useful combination with other known active agents, a Phase I pilot of CDDP with WR 2721 and IL-2 plus DTIC will be opened in the near future.

4. The importance of large surgical margins and prophylactic lymph node dissection in clinical Stage I patients continue to be addressed in the large Intergroup Melanoma Study. Hyperthermic isolation perfusion with L-PAM is also being studied by U.S. surgeons, in cooperation with the EORTC and WHO investigators, to prospectively evaluate the advantage of prophylactic perfusion in patients with intermediate thickness extremity melanoma.

5. IL-2 and LAK continue to be administered through the contract and Modified Group C mechanisms.

Future Plans

1. Exploration of combinations of biologicals will continue, with such combinations as IL-2 and alpha interferon, tumor infiltrating lymphocytes and IL-2, and monoclonals being tested in Phase I/II trials.

2. Consolidation of ABMT with several courses of IL-2 is under consideration by several cancer centers in carefully selected patients.

3. The use of CSF's will be evaluated using repeated courses of high dose therapy, in an attempt to duplicate the high response rates seen with the ABMT trials, but with the ability to repeat the cycles of therapy.

4. Phase II drug screening will continue in order to identify new, potentially active agents. A Phase I/II trial of L-PAM for isolated limb perfusion will be started later this year, in an attempt to clearly define the perfusion MTD of this drug. It is hoped that the involved investigators will be able to continue Phase I and II drug testing of perfused drugs after the successful completion of this pilot study.

GYNECOLOGIC MALIGNANCIES

Ovarian

Accomplishments

1. Phase III trials in advanced, suboptimal ovarian carcinoma continue to accrue patients. GOG is looking at two dose-intensities of CDDP combined with CYT. SWOG is studying the role of carboplatin in a Phase III trial of CBDCA/CYT vs. CDDP/CYT.

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. Follow-up is now available for the GOG studies of early ovarian cancer. Patients with well and moderately differentiated St IAi and IBi tumors did well without any therapy. Patients with IC, II(A,B,C), and selected IAii and IBii tumors did equally well with L-PAM as with P^{32} therapy. Thus, standard treatment would be no further therapy for early patients, and P^{32} for patients with more advanced (but still localized) tumors outside a clinical trial.

5. Patients who fail standard treatment or recur after response to CDDP-containing regimens, require salvage therapy. Preliminary data from Johns Hopkins indicate taxol, a mitotic spindle poison, may have activity in this setting. Intraperitoneal single agents (such as mitoxantrone) and combinations (such as alpha interferon with CDDP) are actively being studied in the GOG, SWOG, and single institutions. Promising agents will be taken forward by the cooperative groups for definitive Phase III testing. Biological agents, such as tumor necrosis factor, and new cytotoxics, are also being studied.

Future Plans

1. For the treatment of patients who are NED after therapy CTEP will encourage the collaboration of SWOG and GOG in exploring useful therapies in this group of patients.

2. The positive study of taxol will be verified in a Phase II study by the GOG. If their results confirm the Hopkins' data, then combining taxol with CDDP would be the next logical step. Investigators at Memorial Sloan Kettering plan to study the drug when given intraperitoneally.

3. Pilot studies of ABMT in patients with relapsed ovarian cancer are being piloted in several cancer centers. This approach will be expanded to several GOG member institutions. In the future, CSF's may be added to decrease myelosuppression, thereby avoiding the need for bone marrow infusion.

4. Intraperitoneal ^{131}I labeled B72.3 monoclonal antibody will be evaluated in Phase I studies in patients with refractory ovarian cancer, in collaboration with NCI investigators. Several other monoclonals will be ready for toxicity pilots in the near future.

Cervical

1. The Cooperative Groups have continued extensive Phase II screening of drugs in this disease. The GOG has demonstrated a 40% 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 DBD 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 5FU + bolus CDDP after radiation therapy for selected early patients (St IA_2, 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. Novel radiation fractionation schemas will be tested by RTOG and GOG in an attempt to increase local control in advanced cervical cancer patients.

SARCOMAS

Accomplishments

1. With a median follow-up of 32 months, the Intergroup Sarcoma Trial of adjuvant doxorubicin after optimal local treatment in patients with localized large (>5 cm) high or intermediate grade sarcomas has failed to show a survival advantage in the treated arm. This finding has important implications on the design of future trials.

2. Accrual to an intergroup osteosarcoma trial comparing adjuvant post-operative therapy with 3 (HDMTX-DOX-CDDP) versus 6 (same + BLEO-ACTD-CTX) drugs has been slow. In addition, unexpected toxicity has been seen on the 3 drug arm, possibly related to the short interval between the administration of CDDP and high dose MTX, which may be dropped from the regimen.

3. The Phase III trial of doxorubicin with DTIC ± Ifosfamide in advanced soft tissue sarcomas continues to rapidly accrue patients.

4. ECOG continues accruing on its advanced sarcoma protocol of 3 regimens: single agent doxorubicin, doxorubicin + Ifosphamide, and doxorubicin + mitomycin C + cisplatin.

Future Plans

1. The Intergroup Sarcoma Committee will use the results of material collected for pathologic review in subsequent analysis of data, in an attempt to advance the clinico-pathologic understanding of these tumors. Basic biological questions will also be addressed, such as oncogene expression and multidrug resistance, using fresh tumor specimens.

2. Confirmatory studies of IUdR as a radiosensitizer will be undertaken by cooperative group investigators, based on the preliminary work of radiation oncologists at the NCI. A Phase III study will follow if the initially positive results are confirmed.

3. An Intergroup Sarcoma Trial of adjuvant therapy of soft tissue sarcomas will start accruing patients shortly, using doxorubicin, DTIC, and Ifosfamide.

BREAST CANCER

Accomplishments

1. In the past year, three NCI-funded randomized clinical trials in node negative breast cancer were analyzed with each showing an advantage as measured by disease-free survival for the treated patients compared to those receiving no treatment. The results of these three studies were made public through the use of a Clinical Alert. In NSABP B-13 node-negative patients with estrogen receptor negative tumors were treated with methotrexate and 5-fluorouracil or were simply observed. At 4 years treated patients had an 80% chance of being disease free while untreated patients had only a 71% chance of being free of recurrence, a difference that was highly statistically significant ($p=0.003$). This therapeutic benefit was observed for patients ≤ 49 and ≥ 50 years of age. No difference in survival has yet been observed.

   In NSABP B-14, estrogen receptor positive, node-negative patients were randomized to tamoxifen or to placebo. There was a statistically significant difference in disease-free survival favoring the tamoxifen treated patients ($p<0.00001$). This benefit also was found in patients ≤ 49 and ≥ 50 years of age. Again, no overall survival differences have been identified at this time.

   In the Intergroup study conducted by ECOG, SWOG and CALGB, patients with node negative breast cancer were randomized to either CMFP or observation. Eligibility included all estrogen receptor negative tumors as well as estrogen receptor positive tumors greater than 3 cm. A disease-free survival advantage was identified for the treatment arm of this study which when analyzed by subsets was present for premenopausal and postmenopausal patients as well as for ER positive and ER negative patients. No survival advantage has been identified as of yet.

Future Plans

1. A study of preoperative versus postoperative chemotherapy has recently been started by the NSABP for Stages I and II disease.

2. Several pilot studies attempting to intensify breast cancer chemotherapy by employing colony stimulating factors are being developed to identify a regimen which can be safely studied in the adjuvant setting in order to rigorously test the concept of dose-intensity.

3. In node negative breast cancer, the role of sequential chemotherapy and hormonal therapy is to be tested in estrogen receptor positive patients. In estrogen receptor negative patients, duration of chemotherapy as well as the relative benefit of doxorubicin versus non-doxorubicin containing regimens will be tested.

4. Several different high-dose chemotherapy regimens with autologous bone marrow support are being developed with and without colony stimulating factors.

UROLOGIC CANCER

Accomplishments

1. During the past year, the prostate intergroup study has matured and has identified a modest but statistically significant survival advantage for combined leuprolide + flutamide compared to leuprolide alone. Further follow-up will better define the extent of this survival advantage.

2. A study to test the benefit of preoperative chemotherapy using the MVAC regimen in locally advanced bladder cancer has been started during the past year. The study is designed to give three courses of MVAC followed by radical cystectomy versus radical cystectomy alone. The study was initiated by SWOG with ECOG also involved in the study's development. In addition it has been designated a high priority trial by the NCI Board of Scientific Counselors.

3. Several studies in early stage prostate cancer have been developed during the past year including the role of adjuvant hormonal therapy for Stage D_1 disease, the role of adjuvant radiation in Stage C disease with positive margins following radical prostatectomy, and the relative merit of radical prostatectomy or radiation for Stages A_2 and B disease (not yet started).

4. The population of patients with poor risk germ cell tumors has been further defined by investigators at Memorial Sloan Kettering Institute. Additionally, the less toxic combination of cisplatin and VP-16 has produced equivalent results to VAB-6 in an early analysis by the same investigators.

5. An intergroup study of RT versus BEP chemotherapy in patients with advanced Stage II testicular seminoma has recently been started and will take three to five years to complete. This study follows important leads suggesting that chemotherapy reduces distant recurrence compared to the standard of treatment, radiotherapy.

Future Directions

1. Biologic therapies in renal cell cancer are being further developed including tumor infiltrating lymphocytes ± IL-2, tumor vaccines, and LAK + IL-2.

2. A direct comparison of BCG with mitomycin-C in superficial bladder cancer will be conducted by SWOG.

3. Efforts to limit the toxicity of therapy for good risk germ cell cancer are being conducted in separate studies by MSKCC (with SWOG) and by ECOG. The MSKCC trial compares VP-16 + cisplatin with VP-16 + carboplatin. The ECOG study will compare VP-16 + cisplatin with or without bleomycin.

NEUROENDOCRINE TUMORS

1. Pilot studies continue in advanced patients, including a randomized Phase II study of alpha interferon and doxorubicin versus VP-16 and cisplatin.

2. A Phase III intergroup study will soon be opened, randomizing advanced islet cell patients between chlorozotocin + doxorubicin versus streptozotocin + doxorubicin versus a phase II drug. On relapse, refractory patients would receive one of the doublets. This would allow the testing of new agents in unpretreated patients, in order to maximize the likelihood of finding antitumor effects. The initial phase II drug may be pibenzimol, a drug found to induce pancreatic necrosis and cause diabetes mellitus during phase I studies.

ADULT HEMATOLOGIC MALIGNANCIES (LEUKEMIA AND LYMPHOMA)

Adult Leukemia

Accomplishments

1. A number of clinically important subsets of adult acute lymphoblastic leukemia (ALL) patients have been identified by the CALGB using newly developed monoclonal antibodies. In approximately 30% of morphologically and histochemically diagnosed ALL, the immunologic phenotype is ambiguous. Patients whose blasts carry myeloid antigens have a particularly poor prognosis. This may provide the basis for phenotype directed clinical trials.

2. In a carefully conducted, prospective randomized trial, ECOG has demonstrated the need for post-remission therapy in adult ANLL in a 3-arm study which compares no further therapy (arm closed early) with maintenance vs intensification. The comparison of the latter two arms is ongoing.

3. CLL trials are either underway or nearly so, exploring various combinations of DCF and fludarabine with either conventional agents or with themselves. A national phase III trial will eventuate.

4. Both alpha- and gamma-IFN have been shown to be active in patients with chronic phase CML. CALGB and SWOG are currently exploring the tolerability of various schedules of combinations of these agents in previously untreated patients. An eventual phase III trial will be developed, although the "standard" therapy arm remains to be determined.

Adult Malignant Lymphoma

Accomplishments

1. ECOG, with collaboration from CALGB, has successfully completed a direct comparison of CHOP vs m-BACOD in patients with intermediate grade NHL. Response rates in the two arms are comparable. Survival data remain to be analyzed.

2. CALGB has completed a three-arm, randomized comparison of MOPP vs ABVD vs MOPP/ABVD in patients with Hodgkin's Disease. CR rates, disease-free and projected overall survival were superior in the two ABVD-containing arms. A formal analysis of delivered dose with clinical correlations was an integral part of the study.

3. ECOG, CALGB, and SWOG are collaborating in an important comparison of MOPP/ABVD with the newly described MOPP/ABVD hybrid in previously untreated Hodgkin's Disease. Accrual is more rapid than projected.

Future Plans

CALGB is developing an intensive CHOPE regimen which will require G-CSF with the eventual comparison of CHOP at its MTD vs CHOP/CSF at that MTD.

Bone Marrow Transplantation

Accomplishments and Future Plans

1. 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. Future directions include substitution of analogues to reduce toxicity and increase efficacy, and to develop disease-directed combinations in definitive studies.

2. 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 therapy for those high risk patients.

3. SWOG has recently activated an important prospective, randomized comparison of allogeneic BMT, ABMT, and conventional consolidation chemotherapy in patients with ANLL in first CR.

4. ECOG is conducting a pilot study of MACOP-B followed by autologous marrow transplantation as front-line therapy for patients with intermediate and high-grade NHL with poor prognostic features. Such risk-directed strategies are an important advance in our approach to therapy.

Adult Malignant Lymphoma

1. Patients with advanced stage, low-grade, or indolent NHL have been considered incurable with conventional chemotherapy, with or without radiation. Unfortunately, these patients have rarely been treated aggressively with one of the third generation multi-agent regimens. SWOG is proposing to treat such patients with the intensive ProMACE/MOPP program, randomizing responders to alpha-interferon or observation alone.

   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 studies on larger numbers of patients, correlating these findings with response to treatment and survival.

MALIGNANT BRAIN TUMORS

Accomplishments

1. Interstitial Versus External Beam Radiation Therapy--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 high variable. The BTSG currently has a Phase III comparison of external beam versus external beam plus interstitial irradiation for malignant gliomas.

   Efforts to improve the effectiveness of cranial irradiation through hyperfractionation or halopyrimidine radiosensitization are undergoing study in the RTOG. Other Cooperative Groups and Cancer Centers continue to explore phase II chemotherapeutic agents in malignant gliomas.

2. Intergroup Low-Grade Glioma Trial--An intergroup low grade glioma trial with the participation of the BTSG, RTOG, SWOG and University of California, San Francisco has been activated. The trial will evaluate the optimal timing of radiation therapy and test the role of chemotherapy for low grade tumors. With recent improvements in diagnostic technology (e.g. MRI), this disease entity is becoming increasingly important as are questions of optimal management. The expected accrual of approximately 400 patients will be achieved in three years with follow-up of up to 15 years anticipated.

Future Plans

1. The current standard treatment of primary CNS lymphoma is surgery and/or radiation therapy. A study involving the BTSG and the RTOG will determine the roles of chemotherapy and radiation. Patients with AIDS will be ineligible for this trial due to the altered natural history of lymphoma in the setting of HIV infection.

2. A close liaison has been maintained with the Organ Systems Coordinating Center's (OSCC) CNS working group in an effort to coordinate their epidemiologic and basic biology proposals with the interest and resources of investigators in the CIB supported clinical trials apparatus. A concept to evaluate prospectively the immediate and long term clinical and pathologic consequences of cranial irradiation is under development between OSCC and CIB.

GASTROINTESTINAL CANCERS

Accomplishments

Esophageal Cancer

The RTOG has undertaken a Phase III comparison in localized esophageal cancer of radiation alone or with cisplatin plus 5FU. The study tests whether four courses of chemotherapy plus radiation controls distant metastatic disease following potentially curative local therapy that avoids the morbidity of surgery (standard therapy). As noted previously, a multicenter contract group is being established for the conduct of adjuvant and advanced esophageal trials.

Colorectal Cancer

1. NSABP Colon C01 Trial

   The NSABP has completed a trial of adjuvant therapy in colon cancer which accrued approximately 1200 patients between 1977-1984. It compared 5-FU/MeCCNU/VCR chemotherapy, BCG, and observation for patients with Dukes' B and C lesions. Average follow-up is 59 months. Approximately one-third of the patients have died. Preliminary data suggest benefit for chemotherapy.

2. NSABP Rectal R01 Trial

   In a companion trial for rectal cancer, the NSABP compared surgery plus combination chemotherapy or radiotherapy with surgery alone. More than 500 patients have been entered to date, with results suggesting statistically significant benefit in survival for treatments with MOF chemotherapy.

3. NSABP Colon C02 Trial

   A Phase III adjuvant trial of post-operative, seven day, 5-FU portal vein infusion versus surgery alone opened in March 1984. Nine hundred patients have been randomized.

4. NSABP CO_3 + RO_2 Trial

   New adjuvant trials were activated by the NSABP in 1987 both in colon and rectal cancer. Both trials will employ the previous best chemotherapy arm of MOF (MeCCNU, Vincristine, 5-FU) randomized against chemotherapy with 5-FU/leucovorin. In addition a 2x2 factorial design will permit a further comparison in the rectal adjuvant trial of chemotherapy alone versus chemotherapy plus irradiation. Both the colon and rectal trials will also incorporate tumor sampling for in vitro determination of thymidylate synthetase activity and total folate pools to determine if some prediction of responsiveness can be made.

5. NCCTG Rectal Trial

   The NCCTG reported the results of a trial of adjuvant treatment of rectal carcinoma in 200 stage B_2 and C patients (Proc. ASCO 5:318, 1986). Patients were randomized to radiation alone or radiation preceded and followed by chemotherapy. Median follow-up is 29 months. Preliminary results suggest a

significant advantage in time to recurrence in the combined modality arm. The newly launched NCCTG employs combined radiation + chemotherapy and uses a 2x2 factorial design. It 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.

6. NCCTG Colon Trial

   The NCCTG reported the results of a trial of adjuvant treatment in 408 stage B_2 and C colon cancer patients (Proc. ASCO 5:316, 1986). Patients were randomized to levamisole with or without 5-FU versus observation. Both experimental arms show preliminary, significantly improved time to progression and survival compared to the surgery alone arm.

7. IG Colon Adjuvant Trial

   Based on the NCCTG experience (above) with the role of 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.

Future Plans

Colon Cancer

1. Intergroup Colon Adjuvant Trial

   A trial targeted to accrual 800 patients in 1.5 years will compare 2 FU/Leucovorin schedules.

LUNG CANCER

Non-Small Cell

Accomplishments

1. The CALGB reported early closure of a positive trial showing the benefit of induction cisplatin and vinblastine before radiation for locally advanced NSCLC.

2. The Lung Cancer Study Group, in a series of trials examining the role of CAP chemotherapy, have shown that such treatment is superin to BCG/Levamisole in delaying recurrence and prolonging survival for patients with locally advanced (AJC regional stage III) tumors of non-squamous histology, who can first be rendered free of gross disease by surgery. These patients are at high risk of early relapse without such adjuvant therapy.

3. The Eastern Cooperative Oncology Group have examined their data from several Phase III comparative trials of four separate chemotherapy regimens for NSCLC, and have shown that initially promising response rates have not led to improved survival for many patients with advanced disease. Indeed, their regimen which resulted in the highest response

rate was associated with the shortest survival, perhaps due to toxicities of the drugs used. They have concluded that no regimen can currently be recommended as "standard" therapy, particularly for patients with advanced tumors and poor performance status.

4. The Southwest Oncology Group have evaluated the effectiveness of combination chemotherapy with respect to initial performance status in patients with advanced (metastatic) malignancy at the time of diagnosis, and shown that beneficial effects from drug treatment are seen only in patients with good (grade 0-1) performance status before treatment. Hence, future combination regimens may be more accurately assessed if the study population is restricted to patients who are potential responders.

5. As a result of their comparative trials of different types of adjuvant therapy, the LCSG are opening a study comparing immediate post-surgical adjuvant CAP chemotherapy to observation for patients with completely resected NSCLC. A similar comparative trial is being performed by the North Central Cancer Treatment Group.

6. Based on results showing the effectiveness of cisplatin against NSCLC, and early evidence of enhanced activity in combination with 5-fluorouracil, the LCSG will conduct a trial using this "neo-adjuvant" chemotherapy in an effort to reduce tumor bulk in locally advanced disease patients, converting those who are currently inoperable to an operable, potentially curable, state.

7. Improvements in radiotherapy are an active area of investigation, including alterations in fractionation (high doses once weekly, low doses several times daily), combinations with radiosensitizing drugs (SR-2508, WR-2721, misonidazole), intraoperative brachytherapy, and neutrons rather than conventionally used photons. The RTOG, CALGB, and ECOG will be exploring these questions in several clinical trials.

8. The LCSG plan to investigate a number of promising new approaches to early diagnosis, accurate staging, and treatment of early stage disease. These include the use of indium-labeled monoclonal antibodies and their correlation with pathological findings at the time of surgery; hematoporphyrin derivatives with argon lasers for the diagnosis and treatment of carcinoma-in-situ; evaluation of the use of IL-2 to alter the natural killer activity of tumor infiltrating lymphocytes; and clinical-pathological correlative studies of newer techniques of imaging the thoracic contents. In addition, a tumor repository has been established to determine whether oncogene and growth factor expression in patients is correlated with their clinical outcome. Other Groups, notably ECOG, have explored the possibility of participating in the tumor repository and lab clinical correlation.

9. An enhanced interrelationship between the NCI epidemiologic program and CIB has been developed. An epidemiology developed protocol administering dibrisoquine to assess drug (and possibly carcinogen) metabolic rates has been adopted by the LCSG. CIB is helping to develop an epidemiologic survey of patients with bronchioalveolar carcinoma.

HEAD AND NECK CANCER

Accomplishment

1. The head and neck intergroup trial compares post-operative radiation with post-operative radiation plus cisplatin-5-FU in the adjuvant setting (pathologically negative margins). The trial has encountered difficulties with patient compliance on the combined modality arm, reflecting difficulties encountered in treating this population. Accrual and data acquisition continues.

Future Plans

1. Nasopharyngeal cancer has been identified as a potential area for an intergroup clinical trial testing radiation with or without chemotherapy. The trial is expected to be activated in 1988.

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INVESTIGATIONAL DRUG BRANCH

BIOLOGICS EVALUATION SECTION

IL-2/LAK

Patients with malignant melanoma and renal cell carcinoma who obtained a complete remission following IL-2/LAK on the original extramural trials remain free of their disease 20 - 24 months after having started treatment. Screening for other malignancies sensitive to IL-2/LAK has begun. Serum free media is now being used to generate LAK cells, significantly reducing the risk of transmitting viral infections through the culture media. Semi-automated procedures are currently being developed which will significantly simplify the method of LAK cell generation. The availability of IL-2/LAK as treatment for renal cell carcinoma and malignant melanoma has been extended to 14 of the NCI Cancer Centers under the Modified Group C treatment program.

IL-2 without LAK cells

In addition to the studies of IL-2 with LAK cells, the NCI Extramural IL-2/LAK Working Group has also treated 25 patients with malignant melanoma with high doses of IL-2 alone and has observed 3 complete and 3 partial remissions. This IL-2 regimen is currently being tested in combination with cis Platinum, a chemotherapeutic agent which also has antitumor activity against malignant melanoma. Another IL-2 alone treatment regimen using chronic administration of lower doses was found to be biologically active in NCI extramural studies and is currently in Phase II testing. Based on promising preclinical data, treatment regimens using IL-2 in combination with adriamycin, cyclophosphamide, alpha interferon, gamma interferon and tumor necrosis factor are currently in development.

Colony Stimulating Factors

As single agents, CSFs appear to be effective as treatment for some patients with myelodysplastic syndromes. Early studies also showed that G-CSF and GM-CSF can protect patients from the neutropenia associated with chemotherapy. To reduce toxicity or to permit administration of more treatment, the NCI is adding CSFs to standard chemotherapy and radiotherapy regimens used for the treatment of breast cancer, lung cancer, ovarian cancer, multiple myeloma, leukemia, lymphoma, testicular carcinoma, and sarcomas. CSFs are also being tested in combination with bone marrow transplantation to shorten the time to bone marrow recovery.

Tumor Necrosis Factor

Broad Phase II testing is currently in progress to define the antitumor activity of TNF when given as a single agent.

Gamma Interferon

Previous clinical trials conducted by the NCI identified a dose and schedule for gamma interferon administration which resulted in optimal biological activity. Large scale adjuvant trials which use this regimen of gamma interferon administration are currently in progress in malignant melanoma and small cell carcinoma of the lung. Monocyte function of patients treated on these studies is also being measured and will be correlated with antitumor activity.

Monoclonal Antibodies (MoABs)

Studies to further define the activity of the R24 MoAB in malignant melanoma are currently in progress. Based on reports that the anti-colon MoAB 17-1A also has activity in pancreatic cancer, the ECOG is about to initiate a Phase II study to define response rate and duration. A protocol has been developed to permit rapid comparison of multiple MoABs directed against the same antigen. These data will be used to determine the optimal variable region for future MoAB constructs and will initially study MoABs directed against the TAG-72 antigen. An anti-CD 5 MoAB conjugated to ricin A chain, previously used to deplete T cell populations in patients with chronic graft versus host disease, will be tested in patients with CD 5 positive malignancies.

Alpha Interferon

Although licensed in this country only for Hairy Cell Leukemia, alpha IFN has anti-tumor activity and continues to be studied in non-Hodgkin's lymphoma, multiple myeloma, chronic myelogenous leukemia, carcinoid, mycosis fungoides, and CNS malignancies. Alpha Interferon is also being tested as adjuvant therapy for malignant melanoma, renal cell carcinoma, and ovarian cancer.

DRUG MANAGEMENT AND AUTHORIZATION SECTION

Drug Accountability

The drug accountability system, implemented in January 1983, has continued to function well. All investigational drugs must be ordered and dispensed to patients by protocol and be documented on an investigational drug accountability form. This has proven to be an essential addition to the site visit monitoring as conducted by the Quality Assurance and Compliance Section. This form has been accepted by several drug companies for use in many of the cancer centers across the country. During the past year we have added a drug transfer form to the NCI Investigational Drug Accountability procedure. We have applied to OMB for renewal of the Drug Accountability Form and have begun working with the NCI Information Resources Branch to reprint the Drug Accountability procedures book. The Drug Accountability form and the drug transfer form should reduce the amount of drug returned to NCI.

Electronic Clinical Drug Request

The section has developed an electronic clinical drug request system for the transmission of drug requests from investigators to NCI. After the system was designed, equipment was purchased and a User's Guide was written. A pilot project was initiated at two major cancer centers: Memorial-Sloan Kettering and M.D. Anderson. The pilot was later expanded to more diverse clinical practice settings, nation-wide, bridging several different time zones. These pilot programs have been successful in simplifying the drug ordering procedure and reducing overall drug distribution time from weeks to days. It is anticipated that the new system will minimize the need to maintain large drug inventories and thus reduce drug cost to NCI.

Computer Cost Containment

All contract work activities, distribution reports and special queries continue to be scrutinized closely for need. As a result, the section's computer account at DCRT, NIH continues to remain within budget.

Investigator Registration

The administration of the annual reregistration of investigators continues to be an important function of DMAS. The investigator compliance is presently 100%. Also, during the past year we have implemented the new FDA-1572 forms. Because of the changed format and content of the form and the requirement of more detail, greater interaction with investigators has been required to explain the form changes.

Drug Cost Containment

Drug cost containment has been successful during the past year. The total drug cost has decreased from $3.5 million in FY 86 to $2.9 million in FY 87. It is estimated that drug cost will increase in FY 88 to about $3.7 million to reflect the increase in drug distribution experienced in recent months.

Drug Supply Management

The following steps have been taken to ensure improved drug supply management:

1. DMAS is meeting with each monitor every 3 to 4 months to review all drugs and plans for that drug.

2. An alert system was established for drugs that are in short supply or potentially in short supply.

3. A drug expiration date system was developed for monthly review.

4. A DMAS staff person has been assigned to the Biologic Agents so information, supply and authorization activities can be focused in one individual. It is hoped that this concentration will improve communications for biologics.

Drug Distribution Data for the Past Year

Number of Drug Orders (Line Items)	Number New Special Exception Protocols (Reorders)	New Group C Orders	Total Containers (Vials/ampules bottles) Distributed
14,389 (27,670)	593 (605)	520	1,092,520

DEVELOPMENTAL CHEMOTHERAPY SECTION

Ifosfamide

Two ongoing trials seek to define the contribution of ifosfamide in the treatment of newly diagnosed adult patients with soft tissue sarcoma. In pediatric soft tissue sarcomas, the combination of ifosfamide and etoposide has shown exciting activity, particularly in refractory patients with Ewing's sarcoma and rhabdomyosarcoma. Trials are ongoing to define the role of this combination in newly diagnosed patients.

A treatment protocol for ifosfamide, mesna, cisplatin, and either etoposide or vinblastine for patients with refractory testicular cancer was recently approved by the FDA. Data from Indiana University suggest that this three drug combination is potentially curative in patients with refractory testicular cancer who have failed two prior cisplatin containing regimens; no other regimen has been reported to be potentially curative in this patient population. This treatment protocol (Group C) was initiated so that patients who are not eligible for research protocols can receive this investigational regimen with such promising activity.

In addition, clinical trials are ongoing to establish the role of ifosfamide containing combinations in patients who are less extensively pretreated.

SR 2508

A randomized trial in patients with head and neck cancer is ongoing to establish the efficacy of SR 2508 and radiotherapy versus radiotherapy alone. Several pilot studies are ongoing. A Phase I trial is seeking to define the maximally tolerated dose of SR 2508 when given with brachytherapy based on preclinical data which suggest that SR 2508 may be more effective when given with low dose rate radiotherapy. Patient accrual continues in a Phase II trial in prostate cancer which seeks to establish whether patients treated with SR 2508 and radiotherapy have a higher local control rate than one might anticipate.

Two Phase I trials are going in patients with refractory solid tumors to define the MTD of SR 2508 when given with cyclophosphamide. In addition, a recently initiated trial in patients with CLL will seek to determine the MTD in this patient population. Pharmacokinetic and pharmacodynamic studies are part of these proposed trials and seek to establish the mechanism of the proposed chemosensitization.

Amonafide

Broad Phase II evaluation of this agent was initiated during the past year. Although the data concerning activity are not yet available, early toxicity data suggest that this drug is well tolerated with reversible myelosuppression as its dose limiting toxicity.

WR 2721

Recent data from the Unversity of Pennsylvania suggest that WR 2721 and cisplatin is an active regimen in the treatment of melanoma. A 53% objective response rate was observed in 36 patients with metastatic melanoma treated with this two drug combination, including 5 objective responses among 6 patients treated with WR 2721 and cisplatin 150 mg/m^2. Based on these promising data, a randomized Phase III trial has recently been initiated by ECOG. Because WR 2721 may increase the cytotoxicity of cisplatin, as well as ameliorate its toxicity, Phase II trials of this two drug combination are anticipated in patients with cancers of the breast and prostate.

Taxol

This unique natural product derived from the bark of *Taxus brevifolia* has shown promising antitumor activity. A 33% response rate has been observed in patients with refractory ovarian cancer. A Phase II trial will soon be initiated by the GOG to confirm these preliminary data. A Phase I trial of cisplatin and Taxol is anticipated to define the dose limiting toxicities of this two drug combination; if warranted, a Phase III comparison of cisplatin versus cisplatin and Taxol will be undertaken in newly diagnosed patients.

Some patients with malignant melanoma metastatic to soft tissue sites have experienced a brief response to therapy. Recently, one patient with non-small cell lung cancer was reported to experience an objective response during the ongoing Phase I study of the 6 hour schedule of administration.

L-Buthionine Sulfoximine

The drug inhibits glutathione biosynthesis and causes a depletion of cellular glutathione levels. The drug has been shown to reverse the induced resistance of human ovarian cell lines to melphalan. The initial proposed clinical study is a Phase I study of the combination of BSO and Melphalan. If the toxicity observed in this trial is acceptable, a randomized trial is anticipated in patients with ovarian cancer.

Fostriecin

This novel compound, produced by *Streptomyces pulvaraecus*, inhibits macromolecular synthesis and is thought to inhibit DNA topoisomerase. 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 are anticipated to begin in the near future.

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, and the DCT closed the IND for porfiromycin in the early 1970's. Recent preclinical data by Sartorelli et al. suggest that porfiromycin is preferentially toxic to hypoxic cells compared to oxic cells. Based on these data, investigators at Yale University wish to undertake a clinical trial of porfiromycin in patients with head and neck carcinoma who are undergoing radiotherapy. The Decision Network voted to reopen the IND for porfiromycin in May, 1988 for limited clinical evaluation for this indication.

Trimetrexate (TMTX)

A randomized clinical trial to test the value of murine data in predicting schedule dependency was performed. Based on murine data demonstrating superior antileukemic activity when TMTX was administered on an every 3 hr x 8 doses days 1, 5, 9 schedule, the daily x 5 schedule was chosen for the broad Phase II screening of TMTX. A head-on clinical comparison of the dx5 schedule versus the every other week schedule in colon cancer produced responses only on the every other week arm. Broad Phase II screening of TMTX has revealed activity on a dx5 schedule (4 PR in 24 patients) in soft tissue sarcoma patients who had failed a prior adriamycin-containing regimen). Phase II investigation of the every other week schedule in sarcoma is now being pursued. A Phase I pharmacokinetic trial in patients with hepatic/renal dysfunction is planned, and a Phase I trial of TMTX/cisplatin is underway which will evaluate the effect of cisplatin on the renal excretion of TMTX and its active metabolites. TMTX continues to produce excellent responses against Pneumocystis carinii pneumonia in trials performed by Masur and Allegra.

Flavone Acetic Acid

Based on the data from Wiltrout et al (J Immunology 140:3261, 1988) that natural killer cell activity is enhanced in mice treated with FAA, Phase I investigators looked at BRM parameters in patients at the higher dose levels. Stimulation of NK cell activity has been identified in several clinical trials. The lack of clinical activity in Phase II studies conducted on a weekly schedule of FAA (4.8 g/m^2 over 1 hr, and 8.6 g/m^2 over 6 hr) has created a need to develop alternative schedules. If FAA indeed works indirectly through host-mediated mechanisms, then chronic administration schedules might be necessary for optimal biological activity. FAA has demonstrated synergism with IL-2 in a preclinical model and a clinical trial of the combination has been initiated at the BRMP.

Teniposide

VM-26 has become an important component of therapy for acute lymphoblastic leukemia/lymphoma and for neuroblastoma. A Group C protocol will be submitted shortly to the FDA for VM-26 in combination with Ara-C for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia. Two confirmatory trials in small cell lung cancer have been initiated based on the Finsen Institute data demonstrating extraordinary single agent activity of VM-26 (J Clin Oncol 4:524, 1986).

Liposomal Doxorubicin

Phase I trials with liposomal doxorubicin supplied by The Liposome Co. are being initiated by 3 contractors on weekly or every 3 week schedules. Preclinical studies with liposome-encapsulated doxorubicin have shown that the maximally tolerated dose of doxorubicin can be increased by approximately 2.5 fold. This has been accompanied by an alteration in the tissue distribution of doxorubicin, with less accumulation in cardiac tissue. Superior antitumor activity has been noted in some, but not all, preclinical models.

Fazarabine

Phase I trials (4 trials on 3 schedules) of this analog of both Ara-C and 5-azacytidine have been initiated. Toxicity at the first dose level was observed on the dx5 schedule (@ 72 $mg/m^2/d$ over 1 hr) and the 24 hr continuous infusion (@ 20 $mg/m^2/hr$) despite initiating therapy at 1/50th of the $MELD_{10}$. Dose escalations continue on the two trials utilizing a 72 hr continuous infusion schedules. Toxicity has been limited to myelosuppression, with leukopenia greater than thrombocytopenia. Phase I trials in leukemia are planned.

PALA/5-FU

The investigational agent, PALA, is being tested in Phase II trials at low dose (250 mg/m^2) with FUra (2600 mg/m^2/24 hr following PALA) in colon, gastric and pancreatic cancers to confirm the impressive results seen in a previous Phase I/II trial (2 CR, 11 PR in 28 patients).

Deoxycoformycin (dCF)

Phase III trials of dCF versus alpha-interferon are accruing well. In Phase II trials, dCF has produced remissions (CR + PR) in 85% of hairy cell leukemia patients (99 CR, 46 PR of 170 Phase II patients). A Group C protocol of pentostatin for Hairy Cell Leukemia patients refractory to alpha-interferon has been submitted to the FDA. In Phase II trials, dCF is a 26% agent (3CR, 48 PR of 196 patients) in CLL. Combination trials with FAMP and chlorambucil are planned in CLL. A Phase I trial in patients with impaired renal function has been initiated. A combination trial of dCF and alpha-interferon in mycosis fungoides and a trial of single agent dCF for the treatment of acute graft versus host disease after bone marrow transplant have also been initiated. An ex vivo purging trial to take advantage of the selective cytotoxicity of dCF with deoxyadenosine for T-lymphocytes is planned.

High Dose Methotrexate

After over a decade of supplying and supporting trials of high-dose methotrexate in osteosarcoma, Phase III trials have now proven a definitive advantage for an intensive adjuvant chemotherapy regimens which include high-dose methotrexate. Lederle's NDA submission, composed primarily of clinical data from NCI-supported trials, has been approved by the FDA in this past year.

Oxantrazole

This is in Phase I trials and has been escalated from 7.5 mg/m^2 to 150 mg/m^2. The initial dose escalations were done by factors of 2 according to the blocd level working group method. It is estimated that this reduced the number of patients required for this trial by 9-12.

Deoxyspergualin

Continues in Phase I currently at a dose level of 1700 $mg/m^2/d$ x 5.

Ipomeanol

Is currently beginning Phase I evaluation on a single dose schedule to be followed shortly by repetitive daily dosing and then tolerance schedules.

Carboplatin

Phase III trials in ovary, testes, and head an neck cancer are in progress. Several interesting studies which exploit CBDCA's dose limiting toxicity, myelosuppression, are in progress or will be initiated soon. These include Phase II trials of single agent CBDCA in leukemia (significant activity was demonstrated in Phase I); CBDCA + VP-16 with autologous bone marrow transplant (which showed significant activity in testis cancer in Phase I); and CBDCA + GM-CSF. It is hoped that this latter trial will permit increased dose intensity which will then motivate a reevaluation of CBDCA in a number of diseases (i.e., melanoma where the response rate was 19% with conventional doses).

Antifols

NCI development plans for antifols were reviewed within DCT. It was felt that new novel agents with significant differences from the parent compound Methotrexate would be pursued in combination with industry. Acquisition efforts in this regard are underway. Of the current antifols in development, the following actions are planned: Dichloromethotrexate IND will be closed; Triazinate will remain open only to allow completion of ongoing commitments to define its role in gastric cancer; Trimetrexate development will continue.

Suramin

This drug has generated considerable interest because of its novel mechanism of action and early evidence of activity, especially in adrenocortical carcinoma in intramural trials. Because of significant toxicity associated with this therapy, the dose and schedule are being further evaluated prior to a broader Phase II development.

HMBA

Three Phase II trials of this polar-planar differentiating agent in myelodysplastic syndrome and one in malignant melanoma are currently ongoing. The implementation of biologic endpoints (cytogenetics, measurement of early- or late- myeloid antigen levels, multilineage bone marrow progenitor cell assays, expression of proto-oncogenes (c-myc, c-fos, c-fms) are an intrinsic part of each of these trials. The Phase I oral study (between Walter Reed and the intramural program) using parenteral formulation administered via NG tube is being completed and the protocol has been amended to study oral bioavailability (bioequivalency) of the new tablet agent formulation.

Fludarabine Phosphate (FAMP)

The drug has demonstrated significant single agent activity in alkylator-refractory lymphoproliferative disorders, especially in chronic lymphocytic leukemia (CLL) and favorable histology non-Hodgkins lymphoma. Pilot trials of FAMP-containing combination regimens (FAMP + Prednisone, FAMP + chlorambucil, and FAMP + deoxycoformycin) in CLL are being activated. Application for Group C designation of the drug (for refractory CLL) is underway.

Merbarone

Phase I trials of Merbarone using either 5-day continuous infusion or 2-hour infusion daily for 5 days are being completed. A number of Phase II trials in a panel of solid tumors are now being planned.

Dihydrolenperone

This is the first compound chosen for clinical trials based on its activity in the human tumor colony-forming assay; the highest response rate (27%) in this assay was seen in lung cancers. A Phase I trial in lung cancer patients (disease-oriented) is nearly completed at the Navy, which will be followed by disease-specific Phase II trials.

Methyl CCNU

Based on the results of trials by GITSG, NSABP and NCCTG, applications for Group C designation of Methyl CCNU for adjuvant therapy of high-risk colon and rectal carcinoma are in preparation.

Chloroquinoxaline Sulfonamide

This is the second compound with outstanding activity in the human tumor cloning assay. The compound is especially active preclinically against melanoma, ovary, breast, and lung tumors. Phase I trials are ready to be started using 1-hour infusion schedule. Based on drug's pharmacokinetic characteristics with the initial schedule of administration, other schedules would be explored.

Administrative Highlights

1. A symposium on the preclinical and clinical development of radiosensitizers and chemosensitizers (nitroimidazoles and halogenated pyrimidines) was held in conjunction with the Phase I meeting in November 1987.

2. Phase II/III Contract - Clinical Development of Anticancer Agents - Has seen an improvement in productivity, with a significant increase in the initiation of a number of high priority trials including:
   - WR 2721 + CDDP — breast, prostate
   - 5-FU + leucovorin + RT — colorectal, esophagus
   - Ifn + 5-FU — pilot and colon
   - CBDCA + CDDP — head and neck
   - 6TG — unfavorable NHL
   - Active agents in patients with abnormal end organ function (renal and hepatic) doxorubicin, etoposide, ifosfamide, and 5-FU.

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REGULATORY AFFAIRS BRANCH

The Regulatory Affairs Branch is responsible for preparing and submitting investigational new drug (IND) applications to the Food and Drug Administration (FDA) for initiating clinical trials with anticancer and antiAIDS agents and complying with all FDA regulatory requirements pertaining to these agents. In addition, the Regulatory Affairs Branch implements, coordinates and administers 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 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 and Diagnosis;

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/Treatment 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. Serving as an educational resource to the cancer community for site visit monitoring and regulatory requirements for clinical trials; and

9. Review of informed consent documents.

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.
Jane Cassidy, R.N., B.S.
Gary Smith, B.S.

A summary of the activities for FY '88 includes:

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

2. The INDs for 4 agents were discontinued.

3. The data from all adverse drug reactions reported to the FDA since August 1, 1985 have been entered into a data base on a personal computer. Reports were prepared and distributed to the Drug Monitors and other staff in CTEP to help in their evaluation of agent toxicities. During CY '87 397 adverse drug reactions were reported to FDA.

4. On-site audits were made to 17 Cancer Centers or other single institutions which are conducting trials with DCT-sponsored investigational agents.

5. 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.

6. Procedures were implemented for the monitoring of limited multi-institutional Phase I studies carried out by the Cooperative Groups.

DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY '88, 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:

Drug	NSC Number
Dideoxyadenosine	NSC 98700
Buthionine Sulfoximine	NSC 326231
Hepsulfam	NSC 329680
Chloroquinoxaline Sulphonamide	NSC 339004
Fostriecin	NSC 339638
Ipomeanol	NSC 349438
Pyrazine Diazohydroxide	NSC 361456
Dideoxyinosine	NSC 612049

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

Drug	NSC Number
IL-2 + Tumor Infiltrating Lymphocytes	NSC 600664
Interleukin-1 Alpha	Not Assigned
Interleukin-1 Beta	Not Assigned
Interleukin-4	Not Assigned
Macrophage Colony Stimulating Factor (M-CSF)	Not Assigned
Monoclonal Antibody OKT-3	NSC 618843
Monoclonal Antibody 14.18	Not Assigned
Monoclonal Antibody 14G2A	Not Assigned
Monoclonal Antibody 11C64	Not Assigned

Monoclonal Antibody Lym-1	Not Assigned
Monoclonal Antibody 1F5	Not Assigned
Monoclonal Antibody B1	Not Assigned
Monoclonal Antibody NRCO-4	Not Assigned
Monoclonal Antibody 96.5	Not Assigned
Second Generation B72.3 Monoclonal Antibodies	Not Assigned
Chimeric Monoclonal Antibody B72.3	Not Assigned
Expanded LAK Cells	Not Assigned
Educated Lymphocytes	Not Assigned

INDs Discontinued.

INDs for the following agents were discontinued:

Drug	NSC Number
THC	NSC 134454
Sodium Thiosulfate	NSC 45624
Prednisolone	NSC 9151

Biologic	NSC Number
Monoclonal Antibody Osteosarcoma (791 T/36)	NSC 377522

The Regulatory Affairs Branch currently maintains 160 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 '87 397 adverse drug reactions were reported to FDA. An additional 434 ADRs were received and processed and held for the Annual Reports to FDA. A package outlining the reporting of adverse drug reactions was mailed to all DCT investigators. The data from these forms 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 to systematically update Clinical Brochures continue to be implemented, 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 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's office in Brussels.

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/LAK cell trials and validate the IL-2/LAK cell process at each new institution.

The Sections's staff continues to disseminate information and guidelines for the process validation and monitoring of LAK cell generation to all NCI investigators performing human studies with IL-2/LAK.

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

1. Guidelines for the process validation of effector cells for adoptive immunotherapy (TIL, Expanded LAK, Educated Lymphocytes, etc.);

2. Guidelines on requirements for IND submissions for IL-2 in combination with adoptive transfer of cytotoxic cells; and

3. Guidelines for the manufacture and testing of monoclonal antibodies.

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

The Sections's staff developed a computer tracking system for all new INDs and subsequent IND amendments. The staff also developed a computer tracking system for all FDA correspondence.

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 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 is being developed.

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-sponsored investigational agents.

Cooperative Group Site Visits.

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 160 member institutions, 166 affiliates and 43 CCOPs (or CCOP components).

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

New guidelines were implemented for the monitoring of limited multi-institutional Phase I trials conducted by Cooperative Groups.

Phase I and Single Institution Site Visits.

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, 17 visits to Cancer Centers or other single institutions conducting trials with DCT-sponsored investigational agents were accomplished.

Additional Activities.

Five special audits were carried out to examine the data and verify response determinations in promising Phase II trials. These included: taxol in melanoma and ovarian cancer; WR-2721 and Cisplatin in melanoma and fludarabine in CLL.

The adverse drug reaction (ADR) reporting from Cooperative Groups and other investigators using DCT-sponsored investigational agents is monitored closely. A standard ADR reporting section is required in each protocol.

A series of followup letters for obtaining delinquent data from the Phase I investigators has been implemented. As a result of the closer monitoring of timeliness, the Phase I database has been brought up-to-date. The requirements for monitoring by the Clinical Trials Monitoring Service are now included in either the Phase I Letter of Intent (LOI) approval letters or the consensus review for the protocol.

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.

All protocols submitted to CTEP were reviewed to determine whether they would be conducted as single institution or multicenter trials, and to assure that the conduct of multicenter trials would meet quality assurance guidelines.

The Sections's professional staff participated in national meetings to provide updates on ADR monitoring and quality assurance issues.

The contract for the Clinical Trials Monitoring Service (CTMS) was recompeted and awarded to the incumbent, Theradex Systems, Inc.

The audit results database for Cooperative Groups has been transferred from CTMS to a personal computer. The database has been updated to include all audits conducted since January, 1985.

1. STATISTICAL PLANNNING AND REVIEW OF CTEP SPONSORED CLINICAL TRIALS

The Biometric Research Branch collaborates with CTEP physicians in the development of clinical trials to evaluate new chemotherapeutic and biological agents. The BRB reviews all 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. BRB staff perform interim analyses of contract supported clinical trials and evaluate reports of promising therapeutic regimens for the planning of possible future clinical trials.

The BRB serves as liaison to extramural statistical centers. BRB staff visits centers and organizes national meetings in order to improve statistical and data management procedures.

2. PRECLINICAL DRUG DISCOVERY

a. Methods for the evaluation of differential cytotoxicity (histologically related or otherwise) have been developed for the in-vitro colorimetric human tumor cell line assay in collaboration with Drs. Ken Paull, Lou Hodes and Robert Shoemaker of the Developmental Therapeutics Program.
b. Cluster analysis of the preliminary data from the in-vitro colorimetric human tumor cell line assay has been performed. This analysis explores the extent to which the in-vitro sensitivities of the cell lines demonstrate patterns associated with histology or other known characteristics.

3. DISCOVERY OF IN-VIVO SYNERGISM

a. Methods for the design and analysis of in-vivo murine tumor studies of the efficacy of drug combinations, based on response surface methodology, have been developed in collaboration with Drs. Grem, Christian and Hawkins. A large number of experiments have been analyzed in order to identify therapeutically synergistic combinations of cytotoxic and biological agents.

4. COMPARATIVE STUDIES TO EVALUATE MAGNETIC RESONANCE IMAGING

The BRB is collaborating with the Diagnostic Imaging Branch (Dr. Matti Al-Aish, Acting Chief) of the Radiation Research Program in the conduct of prospective multi-institution evaluations of MRI relative to CT scanning and other modalities in the diagnosis of brain neoplasms, liver metastases, musculoskeletal tumors, cervical myelopathies, lung cancer,

uterine neoplasms, and congenital heart disease. The BRB participates in the following ways:

a. Primary statistician in the design, supervision and analysis of the following protocols:
   - Brain neoplasms (Dr. Meredith Weinstein)
   - Cervical myelopathies (Dr. Scott Rosenbloom)
   - Congenital heart disease (Dr. Charles Higgins)
   - Uterine neoplasms (Dr. Hedvig Hricak)

b. Chairman of the statistical advisory group for the MRI studies: Developed the design for the centralized multi-institutional comparative reading of MRI vs. CT in which semi-annual 3-day meetings are held to read approximately 1200 images per meeting. The design balances potentially biasing effects between the two modalities.

c. Supervision of the data management contract.

d. Preliminary analyses were prepared for the NIH Consensus Conference on MRI and final analyses are in progress.

5. NATIONAL CLINICAL TRIALS OF EARLY OVARIAN CANCER

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 submitted for publication. 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). Analyses of prognostic determinants will be performed.

6. COLLABORATIVE RESEARCH WITH THE LUNG CANCER STUDY GROUP

a. Analysis of a protocol comparing CAP + RT vs. RT in patients with residual non-small cell lung cancer has been completed and two papers are in press (Dr. Thomas Lad).

b. Development of a protocol to evaluate the role of magnetic resonance imaging in determining the presence of mediastinal and hilar nodal disease and renal and hepatic metastases in patients with Stages II and III non-small cell lung cancer (Dr. Frederick Grover).

c. Analysis of the prognostic value of routine screening for brain, bone and liver metastases in non-small cell lung cancer patients has been completed and reported at the annual ASCO conference, and the results are being prepared for publication (Dr. Thomas Lad).

d. Analysis of toxicity and compliance associated with CAP in non-small cell lung cancer patients has been completed and the results are being prepared for publication (Dr. Thomas Lad).

e. Supervision and analysis of the following are ongoing:
   - Protocol comparing CAP vs. no treatment in Stage I (T1N1 and T2N0) NSCLC patients (Dr. Ronald Feld)
   - Protocol comparing lobectomy vs. limited resection in Stage I (T1N0) NSCLC patients (Dr. Robert Ginsberg)

7. TRIMETREXATE TOXICITY

In collaboration with Dr. Jean Grem of the Investigational Drug Branch, toxicity experience with trimetrexate in the Phase I studies supported by CTEP was investigated. This review was motivated by the surprisingly varied patterns of toxicity observed in the initial Phase II studies. Multivariate analysis indicated that schedule, dose, tumor type (leukemia vs. other) and pre-treatment albumin level were independent significant predictive factors for occurrence of grade 3 or worse toxicity. These results were presented at the annual meeting of the American Society of Clinical Oncology in May, 1988; a manuscript has been submitted for publication.

8. PROGNOSTIC DETERMINANTS 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 written and prepared on long-term survival results within the Working Formulation histologic subtypes. A second manuscript has been submitted 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.

9. EVALUATION OF CRITERIA WHICH MAY PREDICT RESPONSE TO IL2/LAK TREATMENT

Preliminary data suggest that tumor cells that express Class II antigens (HLA-DR) may be more sensitive to LAK/IL2. Tumor specimens analyzed in the surgical pathology department of five institutions participating in LAK/IL2 trials are being evaluated for expression of HLA-DR. The reliability of the technique (agreement within and between observers) will be studied. The project will also provide an estimate of the frequency of HLA-DR for different histologies, and will determine whether expression of HLA-DR can be predicted by other morphologic features. Relation of HLA-DR expression to the response to LAK/IL2 treatment is the final goal of this study.

10. MONOCLONAL ANTIBODY TRIALS

The BRB is collaborating with Drs. O'Shaughnessy, Hawkins and Schlom in the design and conduct of clinical trials to evaluate monocolonal antibodies for diagnosis, staging and treatment of patients with cancers. The BRB has primary statistical responsibility for these trials.

11. MAGNETIC RESONANCE SPECTROSCOPY

The BRB is participating in the development, monitoring and analysis of an extramural clinical trial to evaluate magnetic resonance spectroscopy of blood samples as an adjunct in the diagnosis of breast cancer. The trial will be conducted in patients with a suspicious mammography

result. The predictiveness of spectroscopy will be evaluated relative to the biopsy result. This study is being performed in collaboration with Drs. Sheila Taube, Eric Fossel and Michael Friedman.

12. ADVERSE DRUG REACTIONS

In collaboration with Dr. Leyland-Jones we are evaluating the incidence of adverse drug reactions resulting from special exception drug access compared to use of the same drugs in research protocols.

13. 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 submitted. A manuscript with Dr. Foster has also been prepared based on a review of in-vitro data on the modulation of adriamycin resistance. A review of the clinical trials conducted in the development of AMSA has been performed with Dr. Leyland-Jones. A manuscript has been prepared on this case-study in the development of an anti-leukemia agent.

14. 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. Three protocols have been developed to date:

R88-0001: Treatment of patients with refractory germ cell carcinoma with Cisplatin, Etoposide (or Vinblastine), Ifosfamide and Mesna.

R88-0002: Pentostatin in patients with active hairy cell leukemia previously treated with alpha-Interferon.

R88-0003: VM-26 in combination with ARA-C for the treatment of patients with relapsed acute lymphoblastic leukemia.

15. INTERGROUP STUDIES

a. With the input of CTEP staff and the extramural community of cancer cooperative group investigators, the BRB has developed a set of guidelines for the conduct of studies involving two or more cooperative groups. For scientific as well as financial reasons, it is expected that the number

of intergroup studies will increase. In the past, intergroup studies have generally been developed and conducted in an informal manner. Many participants in intergroup studies have been frustrated by the lack of adequate quality control mechanisms, opportunities for input to study design, and regular monitoring reports. At the request of the cooperative group chairmen, and with input from the Clinical Investigations Branch, we have drafted a set of guidelines for the design and conduct of intergroup studies.

b. Facilitating the conduct of intergroup studies is an important priority of CTEP. The intergroup guidelines should contribute to this. The BRB is also working with group statisticians to determine, for several test intergroup studies, whether the amount of data collected can be substantially reduced without interfering with the quality or value of the research. Reduction in amount of data collection would both reduce costs and reduce the complexity of intergroup studies.

c. BRB staff continues to attend meetings of the Intergroup Rhabdomyosarcoma Study and to provide advice concerning statistical issues arising in the group, particularly with regard to the ongoing investigation of pathology classification systems.

d. BRB worked with cooperative group data managers to develop a national workshop on intergroup studies which was held in Denver, Colorado on June 20-21, 1987. More than 150 representatives of the cooperative groups participated in this workshop. Discussion topics included general issues in intergroup studies as well as specific problems in ongoing studies. The proceedings of a workshop on intergroup study issues held in Denver, Colorado in June, 1987, were prepared and circulated to the cooperative groups by BRB staff with the assistance of CTEP contractors. Special issues for intergroup pediatric studies were considered by a CTEP "working group" which included a BRB representative. A policy letter was prepared and sent to the chairs of the pediatric cooperative groups.

16. A BAYESIAN MODEL FOR EVALUATING WHETHER TREATMENT DIFFERENCES VARY AMONG PATIENT 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 using the Bayesian notion of a-priori exchangability of interactions and using 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 decompositon methods. We have re-analyzed the rectal cancer adjuvant clinical trial (R01) of the National Surgical Adjuvant Breast and Bowel Cancer Project using this method. A manuscript has been submitted for publication.

17. SUBSET ANALYSIS

BRB staff have investigated the problem of misleading results arising from analysis of patient subsets in clinical trials, have reviewed statisticial tools for more reliable subset analyses and have prepared a set of recommendations to clinical trials investigators concerning the conduct and interpretation of such analyses. Parts of this work have been presented at the Third Heidelberg Conference on Cancer Clinical Trials and at the International Biometric Conference in Namur, Belgium. Results will appear in Recent Advances in Cancer Research and a second manuscript is in preparation.

18. SAMPLE SIZE CONSIDERATIONS FOR STUDIES COMPARING SURVIVAL CURVES USING HISTORICAL CONTROLS

It is generally impractical to test all new regimens in randomized comparative trials; preliminary screening based on non-randomized pilot investigations is necessary. In addition, for some rare diseases historically controlled comparisons are the only possibility. Although there is an extensive literature on specification of sample size for randomized clinical trials, this is not the case for studies involving historical controls. The required sample size depends on the extent and follow-up maturity of the historical data base. We have developed methods of sample size planning for historically controlled studies when the primary endpoint is survival or disease-free-survival. A manuscript describing these results has been accepted for publication.

19. CUBIC SPLINE REGRESSION

Continuous variables are problematic in the development of predictive models. Most often a simple linear or quadratic effect is assumed. In some cases, however, there may be a more complex relationship between response and the continuous covariate and it is desirable not to impose a standard form but rather to discover the nature of the relationship. We have investigated the use of restricted cubic splines in such models, described how to use standard statistical software for fitting such models and compared this approach to the standard approaches and to other nonparametric methods. A manuscript has been submitted describing these results.

20. WHEN TO RANDOMIZE?

It is often the case that a study is designed for comparing two therapeutic approaches which are similar during a certain time, and subsequently differ. A typical example is chemotherapy alone being 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 develop a model comparing adequate

sample sizes with each design in order to provide guidelines in these situations.

21. DESIGN OF PHASE II CLINICAL TRIALS

We have reviewed the statistical approaches to the design of single agent phase II trials, and have reviewed the response rates found in such trials for drugs tested since 1975. Recommendations concerning the size, design and number of such trials for a drug have been developed. A new two-stage design for phase II trials has been developed which is optimal in the sense that the expected number of patients accrued is minimized. Two manuscripts describing this research have been accepted for publication.

22. SELECTION OF THE MOST EFFECTIVE TREATMENT

The BRB has conducted research on two-stage clinical trial designs. During the first stage several experimental treatments are examined. The most promising treatment is selected and then during the second stage that experimental treatment is compared to a standard therapy. Provision for termination after the first stage if no experimental treatment is sufficiently promising and for possibly including the standard treatment in the first stage have also been evaluated. The operating characteristics of these designs have been studied and their parameters optimized for most efficient utilization of patients. These designs provide a more reliable basis for selecting the experimental regimen to be evaluated in a large phase III trial than is available from uncontrolled pilot studies. These designs are appropriate where there are several experimental treatments of interest but insufficient patients to evaluate them all in a phase III trial. Three manuscripts have been accepted for publication and we are working to extend these ideas to provide innovative approaches to the clinical screening of new agents.

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.

24. OVERVIEWS OF RANDOMIZED CLINICAL TRIALS

This major symposium was organized and sponsored jointly by the Biometric Research Branch and by the Clinical Trials Branch of the National Heart, Lung and Blood Institute. It brought together statisticians and clinical

investigators from the fields of oncology and cardiovascular disease to discuss the controversial issues involved in "pooling" or coming to an overall assessment of the effectiveness of a therapeutic intervention based on multiple randomized clinical trials. It was a very successful and stimulating symposium. The proceedings, including discussion, were published as the May 1987 issue of Statistics in Medicine. Subsequently, the branch has evaluated the role that overviews should play in cancer therapeutics. The BRB has organized sessions and given presentations on this topic at meetings of the American Society for Clinical Oncology, American Public Health Association, International Biometrics Society, Eastern North American Region of the Biometrics Society and the Health Protection Agency of Canada. An editorial has been published in Cancer Treatment Reports and an invited paper has been accepted by Seminars in Oncology.

25. SURROGATE ENDPOINTS

Surrogate endpoints are frequently used in clinical research when the end-point of primary interest is difficult or expensive to measure. We have described some of the uses of surrogate endpoints in cancer studies and have considered the problem of determining when a surrogate endpoint may be appropriately used. An invited paper on this subject was presented at the Biometric Society Meeting in March 1987 and a manuscript has been accepted for publication in Statistics in Medicine.

26. UICC PROJECT ON CONTROLLED THERAPEUTIC TRIALS

Dr. Simon is a participant in the UICC project on controlled clinical trials. This project will conduct an international survey of oncologists to determine their enthusiasm for the questions addressed by ongoing clinical trials in selected disease areas.

27. IMPROVING THE EFFICIENCY OF CLINICAL TRIALS

Much of the research being conducted by the BRB is directed toward improving the reliability and efficiency of clinical trials. An invited paper providing a critical review of approaches to improving efficiency was presented at the Third Heidelberg Conference on Cancer Clinical Trials and a manuscript has been accepted for publication.

28. SUPPORT OF SURGICAL ONCOLOGY RESEARCH

In collaboration with the Clinical Investigations Branch, we investigated trends in the submission and award of research grants to surgical oncologists. Variables studied included type of academic department, degree of principal investigator, type of grant and year of submission. A manuscript has been published.

29. SEQUENTIAL ANALYSIS OF CLINICAL TRIALS

The BRB has been conducting research on several aspects of the use of sequential analysis to enhance the efficiency of clinical trials.

a. Ellenberg and Eisenberger developed a two-stage design for comparison of a new experimental treatment to a standard regimen. The trial can be terminated at the end of the first stage if results are not promising for the new treatment. Within the past year we have optimized the parameters of this design to enable it to make more efficient use of patient resources in screening experimental regimens. One manuscript has been published in a statistical journal. A second manuscript has also been submitted that illustrates the value of this design for the evaluation of treatments in advanced head and neck cancer and the results were presented at ASCO.

b. As treatments for various diseases improves, studies are undertaken in order to develop new therapeutic approaches that would be as efficacious, and less toxic. When the new treatment is more conservative than the standard, proper design of the study should insure that adoption of the new treatment will not result in an unacceptable loss in efficacy. Such trials require large numbers of patients. We have developed a group-sequential approach for planning and monitoring these trials and show that the reduction in sample size can be substantial. A manuscript has been submitted for publication. A paper describing approach was presented at the 9th meeting of the Society for Clinical Trials, San Diego, May 1988.

30. OTHER NIH CONSULTING

The BRB provides short-term statistical consulting to the NCI and other institutes on a variety of issues. For example, during the past year, we have advised the National Institute of Child Health and Human Development against proceeding with a pertussis vaccine trial in Massachusetts, advised the National Heart, Lung and Blood Institute on the design of a diet, exercise and anti-smoking intervention trial for children in grades 3-7, and advised the National Institute of Allergy and Infectious Diseases on the organization of statistics and data management for their extramural AIDS trials.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06308-17 BRB

PERIOD COVERED

October 1, 1987 through September 30, 1988

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, Chief, Biometric Research Branch, CTEP, DCT, NCI
Others:
Susan S. Ellenberg, Statistician, BRB, CTEP, DCT, NCI
Lawrence V. Rubinstein, Statistician, BRB, CTEP, DCT, NCI
Peter Thall, Visiting Researcher
Sylvain Durrleman, Guest Researcher

COOPERATING UNITS (if any)

Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Biological Response Modifiers Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Environmental Epidemiology Branch, DCE, NCI.

LAB/BRANCH

Biometric Research Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.25	4.75	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 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 methods and biomathematical approaches for the development and efficient evaluation of improved cancer treatments.

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

SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
OCTOBER 1, 1987 - SEPTEMBER 30, 1988

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). It is an extramural program that has two Branches: the Diagnostic Imaging Research Branch (DIRB) and the Radiotherapy Development Branch (RDB). 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 management 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. It requires the coordination of research activities with related programs at NCI and NIH, with other Federal agencies, and with 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
Ethel L. Meyer, Secretary to Associate Director
Wendy R. Fredericks, Biologist
Collette Patrice Thorne, Clerk-Typist

2. Administrative Office

Ann Duane, Administrative Officer
Sigrid Blassingame, Administrative Technician

3. Diagnostic Imaging Research Branch

Matti Al-Aish, Ph.D., Acting Chief
Roger Powell, Program Director
Arlyce Peterson, Branch Secretary

4. Radiotherapy Development Branch

Francis Mahoney, Ph.D., Acting Chief
Robert Morton, M.S., Program Director
Thomas Strike, Ph.D., Program Director
Sandra Zink, Ph.D., Cancer Expert
Vacant, Secretary

B. Recruitments
Chief, Diagnostic Imaging Research Branch
Chief, Radiotherapy Development Branch
Radiation Oncologist, Radiotherapy Development Branch

III. MAJOR ACTIVITIES

The Radiation Research Program continues to stimulate, develop, administer and evaluate basic science and clinical research areas in diagnostic imaging, radiation therapy, and their related subspecialty areas. At the October 1-2, 1987, Division of Cancer Treatment Board of Scientific Counselors meeting, a thorough radiation research program review was carried out.

The following areas were highlighted:

(1) A high priority project of the Radiotherapy Development Branch, the neutron clinical trials program, was carefully reviewed. After 10 years of planning and development it is rewarding to note that Phase III trials have been carefully designed and were successfully being carried out. The institutions participating in these trials are the University of Washington, Seattle, Washington; UCLA, Los Angeles, California; and the University of Texas Cancer Center, M.D. Anderson Hospital, Houston, Texas. The advantage of neutron beam therapy for the treatment of salivary gland tumors has been demonstrated. Data continues to be obtained in the treatment of non-small cell carcinoma of the lung, prostate cancer, and head and neck tumors. Unfortunately, the cyclotron at M.D. Anderson Hospital, Houston, Texas, experienced a breakdown; however, this is being corrected, and neutron clinical trials will resume at that institution as soon as possible.

(2) Continued excellent results in the control of clival chordomas, base of skull chondrosarcomas and uveal melanomas are reported by the research team of the Massachusetts General Hospital, Radiation Medicine Department, at the Harvard Cyclotron Laboratory, Cambridge, Massachusetts. Because of these encouraging results there is increasing interest in the use of proton beams in the treatment of malignant disease. A dedicated clinical proton treatment unit is in the development phase at Loma Linda Medical School in Riverside, California.

Data from the Heavy Ion Project at the Lawrence Berkeley Laboratory in Berkeley, California, is supportive of the data being obtained at the Harvard Cyclotron. This beam, along with the proton beam, demonstates that there is a definite place for this form of therapy in the treatment of well-defined and localized cancers.

(3) Intraoperative radiation therapy continues to be clinically investigated and appears to be effective in the treatment of advanced local gynecological and rectal tumors. Research continues in the treatment of these tumors, retroperitoneal sarcomas and gastric cancers.

(4) Radiation modifiers: The radiation sensitizer contracts of RRP continue to identify substances with radiosensitizing properties. Encouraging early results with SR-2508 have been followed by clinical trials being carried out by the Radiation Therapy Oncology Group (RTOG), and other cooperative groups. The sensitizer program is being reassessed and there is hope that a large scale automated modifier screening program can be developed. The expertise and advice of the Developmental Therapeutics Program is being utilized for the development for this new screening program. Interestingly, the radioprotector, WR-2721, has shown a significant 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. Larger doses of chemotherapy can be given with the normal tissues being protected.

Although greater tumor effects are being reported this finding requires substantiation by further clinical trials.

(5) Hyperthermia continues to have promise in the management of malignant disease. In addition to being used with radiation for the improved control of local tumors it is now undergoing investigation as an adjunct to systemic chemotherapy for the treatment of systemic disease.

(6) The exciting field of photodynamic therapy is a promising research area in which systemically administered tumor seeking light sensitizing compounds are used with activating wave lengths of light, usually generated by a laser. Improvements in the light sensitizing compounds are being made and new compounds are now entering the Decision Network of DCT, NCI. Potential for the treatment of closed space neoplasms such as carcinoma of the ovary and bladder cancer are being explored. The effectiveness of this therapy in the re-establishment of airway in totally occluded bronchi from lung cancer has been established. Hopefully, this research area will mature into a clinical tool which will give improved results for tumors which commonly recur following conventional therapy, e.g., ovarian cancer.

(7) Dosimetry studies: Research into determining optimal radiation treatment planning is ongoing. These activities include the dosimetry of electron beams, interstitial radiation therapy, and neutron beams. The need for research in radionuclide conjugate dosimetry is extremely important as this therapeutic area is experiencing rapid expansion and is being supported by NCI.

8) The contract for a new "Patterns of Care" study to evaluate the effects of radiation therapy in the United States will be funded in 1988. This retrospective analysis will focus on data from patients with breast, cervix, prostate cancer, Hodgkin's lymphoma, and recto-sigmoid cancer. Early patterns of care studies have proven helpful in identifying methods to improve patient survival and reduce complications. Improvement in the treatment of prostate and cervical cancers have resulted from this retrospective data analysis.

9) The rapidly emerging area of medical informatics, also known as "expert systems", is being exploited in the optimization of radiation treatment and planning. An RFP has been approved and will be funded in 1988 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 is expected to be great, and an even greater impact on the field of medicine in the near future should be anticipated.

In diagnostic imaging, several research areas were reviewed.

(1) Diagnostic ultrasound has the potential for tissue characterization which has not been totally exploited. Program emphasis will be placed on attempts to make specific tissue diagnosis in a non-invasive manner. Hopefully, applications of ultrasound will be helpful in accomplishing this goal. This potential does exist in breast disease where ultrasonographic techniques can be readily applied.

(2) Of the rapidly evolving imaging modalities, none is more dynamic than that of magnetic resonance imaging (MRI) and spectroscopy (MRS). Contracts supported by the Radiation Research Program in the comparison of magnetic resonance imaging and computerized tomography resulted in data that were used in a NIH Consensus Conference on MRI which was

held on October 26-28, 1987. Results of these contracts will be presented to the DCT Board of Scientific Counselors in October 1988.

(3) Another goal of the Diagnostic Imaging Research Branch of the Radiation Research Program is to develop anatomic and functional diagnosis of neoplasms employing singular and multiple modality imaging related technology. An RFA dedicated to this purpose was funded in 1988. Most likely, this RFA will deal with 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.

(4) PET scans have been extremely helpful in obtaining functional 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 has made possible the differentiation of normal brain from brain tumors and the determination of tumor viability following therapy such as radiation therapy.

(5) 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 for treatment planning.

(6) Perhaps the most exciting of the rapidly evolving radionuclide related research areas is that of radiolabeled ligands to be used for tumor identification and treatment. The possibility of tumor specific radiodiagnosis is made possible by this technique. An RFA titled "Development, Evaluation and Biodistribution of Chelated Conjugated Radiolabeled Monoclonal Antibodies Specifically for Diagnostic Imaging" was released during this fiscal year.

At the February 18-19, 1988, DCT BSC, Dr. Antoine once again reviewed the neutron therapy clinical trials including its history and development. The fact that the technical problems of developing units for neutron generation were overcome and that Phase III clinical trials had been developed by the Neutron Working Group were again emphasized. Also, it was noted that there is increasing interest in neutron therapy clinical trials in the international radiation research community among investigators from as far away as Seoul, Korea, and Capetown, South Africa. These investigators would not require additional funds but wished to become involved in the clinical trials. It was the feeling of the Board of Scientific Counselors that due to difficulties with monitoring data and quality control these activities should be limited to information exchange at this point in time. Also mentioned was the fact that a dosimetry center had been started at Johns Hopkins University which would be funded and administered through the Radiation Therapy Oncology Group. This center would develop expertise in radionucide dosimetry which would be made available to members of the cooperative groups in which radionuclide clinical trials were to be carried out.

Two areas of technical difficulty persist in local regional hyperthermia. These are the need for non-invasive thermometry and the difficulty occurring with most heating units in the heating of deep-seated tumors. A workshop addressing these problems would be sponsored by the Radiation Research Program.

Boron Neutron Capture Therapy (BNCT) is a therapeutic method which could potentially achieve tissue and cell specific radiation therapy. This is a major goal of the Radiation Research Program. If a boron compound can be 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 radiation. Early BNCT trials in the treatment of malignant gliomas were carried

out in the United States in the 1950s and 1960s but were discontinued because of unacceptable normal tissue side effects. However, Dr. Hatanaka, a neurosurgeon in Japan, has been treating patients with boron containing compounds for malignant brain tumors and continues to do so. Another Japanese investigator, Dr. Mishima, has used 10 borono-phenylalanine to treat melanoma in an animal model (pig) and is now treating lesions in humans. At the request of Congress, a proposal for the treatment of malignant gliomas using BNCT from the Idaho National Engineering Laboratory (INEL) was reviewed by a scientific committee established by the Radiation Research Program. A summary of the recommendations of that site committee was presented at the June DCT BSC meeting.

At the June 6-7, 1988, DCT BSC, the subcommittee chaired by Dr. William Hendee for the evaluation of the neutron clinical trials made its report. The subcommittee felt that scientific questions asked in the Phase III protocols should be answered. This should be done in as expeditious and financially responsible manner as possible. Although it was realized that some of the clinical trials would extend beyond the 1989 maturation date of the neutron contracts, it was felt that mechanisms should be developed to complete the high prioritiy Phase III trials in prostate cancer, head and neck, and lung cancer. This is consistent with the position taken by the Radiation Research Program over the past several years.

Dr. Albert Soloway presented the results of a RRP workshop on compound development in Boron Neutron Capture Therapy held in Annapolis, Maryland, on May 3-4, 1988. This exciting area of radiation research makes possible tissue specific radiation therapy. As discussed previously boron activated by thermal neutrons becomes unstable and disintegrates giving off local radiation including alpha particles. If these boron compounds can be selectively localized in tumors then focal radiation for neoplastic disease with sparing of normal tissues becomes possible. At this workshop, compounds were discussed that could be used for tumors including glioblastoma multiforme, other primary lesions such as the epithelial tumors, and metastatic tumors including metastatic melanoma. The results of this workshop will be published and recommendations made by the members of the workshop will result in scientific projects which will be announced by the appropriate RFAs and RFPs. The results of a site visit to INEL in Idaho Falls, Idaho, where a BNCT project for the treatment of glioblastoma multiforme was being proposed, were discussed in closed session. The substance of that discussion cannot be reported here; however, it was acknowledged that the Power Burst Facility Reactor at INEL was capable of generating the proper energy neutrons for the activation of the BNCT process.

The exciting area of radionuclide conjugate/ligand diagnosis and therapy was again discussed. An RFA was passed by the Board of Scientific Counselors for the establishment of a research team to develop the optimal dosimetry of this rapidly emerging diagnostic and therapeutic technique.

The Radiation Research Program being a completely extramural program uses the scientific members of its extramural community for the development of research projects. This advice is obtained at scientific meetings, from representative members of the Board of Scientific Counselors, and from the utilization of workshops in promising research areas. The results of these workshops are discussed in the reports from the branches of the Radiation Research Program.

IV. RADIATION RESEARCH PROGRAM RESEARCH GRANT AND CONTRACT SUPPORT

FY88 BUDGET
(Dollars in Thousands)

	Regular Contracts Number/Amount		SBIR Contracts Number/Amount		Grants Number/Amount	
Diagnostic Imaging Res. Branch	-	-	2/	165	214/	39,738
Radiotherapy Dev. Branch	12/	5,652	6/	1,244	219/	50,569
Total RRP	12	5,652	8/	1,409	433/	90,307

V. SCIENTIFIC OVERVIEW

A. DIAGNOSTIC IMAGING RESEARCH BRANCH

Since its inception in 1982, The Diagnostic Imaging Research Branch (DIRB) continues to develop and administer basic practical and clinical diagnostic imaging research using both ionizing and nonionizing radiations. Areas of research include nuclear medicine with emphasis on the use of monoclonal antibodies in diagnosis, light scanning, nuclear magnetic resonance imaging and spectroscopy, as well as NMR microscopy, ultrasound, digital radiography, or x-ray diagnosis and instrumentation, and image perception. 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 $40 million in 1988.

Nuclear medicine and magnetic resonance imaging/spectroscopy are the 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 DIRB actual budget FY87 and estimated budget FY88.

FY87 AND 88 DIRB BUDGETS

			$ (thousands)	
GRANTS	FY87	FY88	FY87	FY88
Coop. Agree. (RDOG-I) (U01, RFA 86-CA-10)	7	7	923	676
Coop. Agree. (RDOG II) (U01, RFA 88-CA-02)	-0-	6	-0-	600
Traditional (R01)	124	131	22,963	24,448
Program Projects (P01)	9	11	9,537	8,641
Conf. & New Investigator (R13 & R23)	9	7	313	219
SBIR	26	30	1,095	2,352
First Awards	2	5	474	474
RFA, 87-CA-33	-0-	5	-0-	500
RFA, 87-CA-36	-0-	7	-0-	800
OSP (Transferred R01)	-0-	5	-0-	937
TOTAL GRANTS	178	214	35,264	39,738
CONTRACTS				
NMR (Extension Funds)	5	-0-	137	-0-
SBIR*	2	2	757	165
Data Management	1	-0-	118	-0-
TOTAL CONTRACTS	8	2	1,012	165
TOTAL DIRB BUDGET	186	216	36,264	39,903

*Number of grants to be funded FY88 is estimated.

DIRB ACCOMPLISHMENTS

CLINICAL DIAGNOSTIC IMAGING RESEARCH TRIALS

A major accomplishment in FY88 is the establishment of the National Collaborative Diagnostic Imaging Group. Seven grant awards including an operations control center and a statistical center have been made for determining the most effective imaging procedures required to stage and monitor carcinoma of the lung and prostate. The result desired is the development of specific algorithms for the appropriate sequential use of state of the art imaging procedures in the diagnostic staging and timely follow-up of these tumors.

Several imaging technologies of recent development (Magnetic Resonance, Computed Tomography, Ultrasound, Digital Radiography, Positron Emission Tomography, Single Photon Emission Tomography) have reached a stage of progress which justifies investigation into the capacity of each of these modalities to detect cancers and to determine its extent, that is, to stage the disease employing a single modality or a combination of modalities, and to monitor therapy. Early assessment of these technologies is important for evaluating their impact on the management of cancer. Based on this rationale, the clinical research group for the staging of cancer of the prostate and lung was established in 1987 and patient accrual by six institutions started last January.

Patient accrual in most participating institutions is ahead of schedule. When enough data are accumulated and analyzed, specific decision trees or algorithms for the appropriate sequential use of state-of-the-art imaging procedures in the diagnosis, staging, and follow-up of these specific tumors will be established. Two additional clinical imaging trials for staging carcinomas of the colon and pancreas have been recently announced and applications are being received and reviewed. We anticipate about five new institutions will be funded and added to the RDOG by September 1988.

MAGNETIC RESONANCE IMAGING/SPECTROSCOPY

The T1, T2 RFA has been in operation for over 2 years. Four institutions are involved. This study of tissue relaxation times in magnetic resonance imaging and spectroscopy shows promising results in understanding the mechanisms of relaxation times of tissues and the ability to differentiate various normal and abnormal tissues.

The five contracts for Comparative Clinical Magnetic Resonance Imaging Studies have been completed and the investigators are continuing the final analysis of data and preparation of scientific publications. The data management contract has also been completed and the data are stored in the NIH computer system. Preliminary results, presented at the MRI Consensus Conference (NIH), October 26-28, 1987, indicated that CT and MRI are approximately equivalent for detection and delineation of tumors in the brain and liver, and of metastatic disease in the mediastinum. Compared with CT, MRI provides improved first look diagnosis at certain regions like the pelvis and the joints because of multiplanar views, but is slower and thus less comprehensive in overall viewing of the abdominal region. MRI is superior to myelography for imaging of the cervical spine in the majority of cases.

NEW PROJECTS

Four new initiatives have been concept approved, funded and announced in FY88.

1. RFA 87-CA-33, entitled "Development, Evaluation and Biodistribution of Chelate Conjugated Radiolabeled Monoclonal Antibodies Specifically for Diagnostic Imaging", has been announced and applications are being reviewed for funding in FY88. This initiative is intended for improvement of existing and development of new chelated monoclonal antibodies which appear to be the most promising for diagnostic purposes. In addition, Phase I Clinical Trials are included in this effort. There exists the possibility of cell-specific diagnosis and the capability of detecting tumor beyond the range of other currently employed imaging modalities.

2. RFA 87-CA-36, entitled "Anatomic and Functional Diagnosis Before, During and Following the Treatment of Neoplasm Employing Imaging Related Technology (MRI/MRS, PET, SPECT, Monoclonal Antibodies) for the Purpose of Planning of Treatment and Monitoring of Tumor Response" has been announced and is being reviewed for funding in FY88. The relative applicability of the various technologies (often used in combination), will be assessed to determine what modality, or what combination of modalities is most suitable, in a given situation for both anatomic and functional diagnosis. Functional information obtainable by imaging and imaging related methods includes the evaluation of various metabolites and determination of such parameters as PH, the tissue redox state and regional tissue perfusion by magnetic resonance spectroscopy. Positron Emission tomography (PET) studies also offer opportunities for obtaining similar information. The proper combination and sequence of modalities that provide functional information associated with specific anatomic sites is extremely important.

3. RFA 88-CA-02 to supplement the existing Cooperative Agreement (RDOG) studying lung and prostate cancer was announced. The new awards will be used for staging and monitoring, by imaging methodology, cancer of the colon/rectum and the pancreas tumors. It is expected that five or more new institutions will be funded in FY88 and added to the on-going collaborative group, RDOG.

4. RFA-CA-10, "Investigation of Tissue Composition and Function by MRI Using Paramagnetic and/or Superparamagnetic Contrast Agents" was issued to solicit grant applications on this subject. The objective of this study is to promote MRI studies employing paramagnetic and supermagnetic substances in an effort to determine tissue composition, function, localization of tumors, and quantitative measurement of physiological and pathological processes. It is anticipated that four grants will be funded in FY89.

5. Radiopharmaceutical development for single photon emission computerized tomography which received prior concept approval in 1985 has been issued in FY88 as an RFP, (NCI-CM-57744-26) entitled "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization". Successful proposals will be funded in FY89. Single photon emission tomography is a promising technology for non-invasive anatomic and functional diagnosis and is much less expensive than PET. The major aim of this RFP is designing, synthesizing, labeling and initial testing of radiopharmaceuticals (radiotracers) which may be biomedically useful as probes of physiologic processes suitable for imaging with the gamma-camera, and single photon emission computed tomography (SPECT). the number of radioisotope combinations now available is limited.

FY88 Annual Report Summary
Diagnostic Imaging Research Branch

The Diagnostic Imaging Research Branch (DIRB), Radiation Research Program (RRP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), supports and administers research leading to the development of radiologic instrumentation and methodology utilizing ionizing and nonionizing radiations to improve the diagnosis of cancer and other diseases. The ultimate goal is non-invasive specific anatomical and functional diagnosis.

Several modalities, both ionizing and non-ionizing, are employed in the field of diagnostic imaging which include diagnostic radiology and nuclear medicine. In the "Ionizing Section", equipment developments, especially digital radiography have resulted in improved x-ray technology. Nuclear medicine remains the most active field in the DIRB research effort. In the non-ionizing area of research such as magnetic resonance imaging/spectroscopy, light scanning and ultrasound increased activity is evident in FY88.

GRANTS AND CONTRACTS

There are three mechanisms of research support at DIRB; (1) grants, (2) contracts, and (3) collaborative agreements. Grants constitute most of DIRB's portfolio. Traditional grants (R01) leading the way in 1988 with 136, program projects (P01) with 11, collaborative agreements with 13, and others with 42. A new contract is being added in FY89, "Single Photon Radiopharmaceuticals for Function, Metabolism and Tissue Localization". The budget for this contract is $600,000. The estimated total expended for grants for FY88 is $39,903,000 as compared to the 1987 figure of $36,264,000.

Five MRI contracts (5 centers) have been extended to November 1987 to complete data review and analysis using FY87 funds. The total number of contracts in DIRB in FY88 is 2 small business contracts (SBIR) at a total budget of $165,000.

RADIOLOGIC DIAGNOSTIC ONCOLOGIC GROUP (RDOG)

The group is commonly known as the Radiologic Diagnostic Oncology Group (RDOG) and 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. The findings of each study should lead to an improved patient management and considerable cost savings resulting from the elimination of inappropriate or unnecessary diagnostic studies. Furthermore, the development of clinical trial groups allow for the rapid accrual of patients into a study within a short period of time. This assures rapid evaluation of imaging modalities for imaging procedures in diagnostic staging in cancer patients and timely follow-up of their tumors.

The clinical trials group is currently evaluating carcinomas of the lung and prostate. Semi-annual reports indicate satisfactory progress, especially patient accrual. Patients accrual with prostate carcinoma is ahead of schedule and that of the lung is slightly lower. A second RFA for clinical trials involving carcinoma of the pancreas and colorectal carcinoma was announced, and applications were received and are being reviewed. It is anticipated that six more institutions will be added to the RDOG.

Six institutions are currently participating in the clinical trials. This includes 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. An operations control center managed by the American College of Radiology and a Statistical center operated by Harvard University constitute the remaining members of the RDOG.

The clinical trials will contribute significantly to the development of a comprehensive comparative teaching file by site. This should prove to be an excellent resource for training radiologists in the selection of the optimal imaging modality and for performing additional studies on the impact of external factors on radiology interpretations. Moreover, the 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 characteristics, predictive factors for sites of disease involvement, impact of workup bias on test performance, and many other important areas in clinical diagnostic radiology.

DIGITAL RADIOGRAPHY

The advent of high speed digital electronics and communication technology changes the methods of acquiring, sorting, viewing, and communicating diagnostic images in radiology departments in this country. Our program took a leading role in the important area of imaging research. Presently, we support research in the area of electronic development to enhance image contrast, compress images into a more compact form before storage, and improve speed of image acquisition and transmission.

We recently added two new program projects. One is at the University of Pittsburgh. The major objective of this research is to significantly improve picture archiving and communication systems (PACS). The unique expertise of this team in high resolution storage phosphor imaging and fast digital acquisition brings a vital addition to digital radiography research in our program.

Another new program project was funded in FY88 at the University of North Carolina, the first university in the United States to establish a Computer Science Department. The primary objective of this program project grant is to develop improved means of medical image presentation utilizing data obtained from tomography (SPECT) imaging systems as well as radiation therapy port films. Efforts will be made: a) to produce better grey-scale 2D images by utilizing regionally adaptive contrast enhancement techniques and targeting context-sensitive-human vision; b) to develop dynamic presentation of 3D images both for anatomical and functional information; and c) to define anatomic objects for fast 2D/3D object definition.

A highly successful development has been under way for some years in a pioneering effort to a means for handling, examining, and storing the vast number of radiological images now being generated on film and to provide the means for all-electronic storage of images by means of digital radiology. This project at UCLA has resulted in a new image viewing console which has six television monitor displays on which images can be recalled, selected, magnified, examined, and compared in a rapid and flexible mode of operation. The monitors presently display 1000 X 1000 pixels in each image with a spatial resolution on the tube displays exceeding conventional radiographic films (except for mammography) and 2000 X 2000 pixel displays will be installed in the coming year. Laser-printed hard copy is available. Although primary diagnoses are made by film, all subsequent interpretation and storage has been converted to digital (or photoelectronic) operation in the Pediatric Radiology Department.

NUCLEAR MEDICINE

Nuclear medicine research comprises the majority of traditional research grants (R01). Development of new radiolabeled compounds, their biodistribution, toxicity, use in various modalities, i.e., PET and SPECT, and Chelate conjugated antibody research are just a few examples to illustrate the wide range of this program. Program project grants deal with various aspects of nuclear medical research. One deals with the development and evaluation of promising compounds which when radiolabeled, have the potential to scintigraphically diagnose and at higher doses treat cancer. A second program project will explore the improvement of image information by the administration of a novel contrast agent; improvement of the qualitative and quantitative aspects of imagery by SPECT and utilization of pharmacoangiography to improve information content and understanding of functions of imaged organs. A major development by another program project is to design and develop a scintillation probe for intraoperative tumor detection. By using two detectors, the newly constructed probe effectively discriminates against background radiation that might otherwise be mistaken for tumor, improving detection of small metastases when compared to external imaging or other surgical probes. It is expected that a commercial probe will be constructed based on this prototype and used routinely in surgery.

In another investigation, the effects of different chemical bonds between antibody and DTPA-In-111 on target to non-target ratios were evaluated. The primary initial screening test indicates that the linkages with the different chemical bonds gives rise to different target to non-target ratios and it appears possible that linkage of the antibody, the chemical spacer and the radionuclide conjugate may be optimized to improve target localization for efficacious external detection and therapy.

At the University of Cincinnati two major accomplishments were reported. First, they demonstrated that neutral complexes of Tc(II) could pass the blood-brain barrier in rats. This demonstration shows that Tc-99m brain perfusion agents do not have to be based on Tc(V) (as are all of those that have been evaluated to date), but can be based on any technetium center that affords a neutral, lipophilic complex. Second, they have developed a synthetic route to a new class of thiolato Tc complexes; the chemical and biological properties of the members of this class can be readily and systematically varied by altering the nature of the coordinated thiolato ligand. This new class of thiolato Tc-99m complexes is likely to contain neutral Tc(II) species which pass the blood brain barrier and thus can be used as the basis for the development of Tc-99m brain perfusion imaging agents.

Recent studies of the biochemistry of thymidine using PET indicates that de-iodination of I-125-IUDR was occurring in vivo and that there is little local thymidine reutilization. While the degradation pathway appears active in slowly growing tissues, it appears to be less of a concern in rapidly growing tissues and tumors, where these studies found between 75 and 95% of retained thymidine is incorporated into DNA. This information along with previous work on the effects of blood flow and endogenous synthesis are being used to produce models of thymidine metabolism for PET.

At the City of Hope, a study to determine the factors that affect the tumor uptake of monoclonal antibodies directed against tumor-associated antigens in humans was undertaken. By examining tumors for general characteristics, specific antigen properties and uptake of radiolabeled monoclonal antibody; criteria for matching individual tumors with the most appropriate antibody, for radioimmunodetection and/or radioimmunotherapy, will be developed.

The anti-CEA monoclonal antibody has been labeled with In-111 using DTPA to form "Indacea". Indacea has been used to image patients with cancer who were scheduled for a major

operative procedure and/or tumor resection. By comparison of imaging with actual human operative findings, the true specificity and sensitivity of Indacea imaging is calculated. Human tumor imaging (Indacea imaging) is compared with resected tissue analysis for tumor size, tumor content and histological distribution of CEA (CEA characteristics), and tumor and normal tissue uptake of In-111 (biodistribution) to identify those factors associated with effective tumor imaging.

Eighty-six (86) patients have been studied using Indacea. They have observed that approximately 70% of primary tumors are imaged, 30% of extrahepatic metastases are imaged and that 40% of liver metastases are identified as negative defects by scan. A significant correlation has been documented between tumor imaging, tumor uptake of In-111 and total CEA content of tumors (by EIA). Tumor CEA content was high for primary tumors that were not imaged or extra hepatic intra-abdominal metastases. Primary tumors that were large, polypoid (well vascularized) and had CEA located on the surface of the tumor cells by immunohistology (apical or intraluminal) were imaged best.

Single photon emission computed tomography (SPECT) use as a clinical modality in diagnostic imaging is well established. Over two thousand SPECT systems are installed worldwide. In addition to diagnostic application, SPECT also has the potential to provide quantitative information on the effectiveness of cancer therapy e.g, change in size of primary or metastatic lesions following therapy. The Diagnostic Imaging Program is supporting research leading to the construction of new and improved SPECT systems, improvement and development of SPECT, and development of radiolabeled compounds, especially using Tc-99m for use in SPECT. The recent announcement of SPECT RFP, (described elsewhere in this report) is an example of our interest in this area of research. A specially designed cone beam collimator by one of our grantees at Duke University is an illustration of the research we fund in this field. The measurements of point source sensitivity and spatial resolution of the newly designed collimator is three times as sensitive as other collimators. Another grantee developed a ring tomograph capable to image contiguous slices of entire brain at 8mm resolution with sensitivity more than six times that of a conventional camera with parallel hole collimator.

MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY (MRI/MRS)

Basic and developmental research studies of many kinds have continued to make rapid and revolutionary advances in improving the equipment, materials, and techniques for clinical diagnostic imaging, noninvasive tissue characterization, and studies of normal and pathologic tissues by MRI and MRS.

In the improvement of instrumentation and techniques highly successful developments have been realized in the design of surface coils and other special electronic coils for imaging all parts of the body, for localization of tissues, and for spectroscopic determinations. Optimal magnetic shielding designs have been developed as well as phantoms for quality control measurements.

Major interest has been centered on the development and evaluation of new MRI contrast agents, such as paramagnetic iron and gadolinium complexes, to be used as hepatobiliary contrast agents. Stable liposomes of manganese cations and nitroxide free radicals alter the NMR relaxation rates of liver tissue and intracellular water and enhance MR image contrast. Brain tumors and edema can be differentiated using gadolinium (DTPA); magnetic fluorine nuclei are being used to study 5-Fluorouracil chemotherapy and to detect tumor sensitivity or

resistance; and special immunodirective agents, including monoclonal antibodies, can be incorporated with contrast agents to improve the specific imaging of tumors.

Basic research studies continue to measure the magnetic resonance relaxation properties and rates (T1 and T2) of normal and pathological tissues as well as differences in the electrochemical characteristics of physiological water in normal and cancer cells, the transformation from preneoplastic to metastatic hepotocellular carcinomas, and the metastatic potential of prostate cancers.

New improvements developed by physicists and electrical engineers in applied NMR techniques have permitted high speed real time dynamic imaging of the beating human heart and of vascular flow patterns. High magnetic field spectral imaging and chemical shift imaging techniques now can display and measure intracellular and extracellular sodium in the assessment of cancers, stroke, and trauma and can display lesions not seen without these techniques. It is now possible to use MRI to assess both perfusion of organs and diffusion within cells, to image only the aerated portions of lungs, and to study the actions of macrophages in lung tissues. New developments in magnetic resonance microscopy promise to provide magnified, noninvasive imaging of tissues at a spatial resolution of ten microns in the near future.

Final analyses of data from a three year clinical imaging study are being carried out to compare the diagnostic accuracy of MRI vs. X-ray CT (computed tomography). More than 2300 patients were examined for a variety of cancers and disorders. An NIH Consensus Meeting on Magnetic Resonance Imaging was held in October 1987 which provided current state of the art assessments of the use of MRI in a large number of clinical applications. Preliminary results of the NCI comparative clinical imaging study provided an important contribution to the presentations and discussions of the Consensus Meeting and the findings of the comparative study. The experience of a large number of practicing radiologists and MRI specialists who also participated in the Consensus Meeting were in remarkable consonance for most of the applications discussed.

MRI and CT are approximately equally useful in detecting and visualizing tumors in the brain, although the multiplane viewing capability of MRI offers additional detailed flexibility in imaging. MRI is superior in viewing demyelinating disease, such as the lesions of multiple sclerosis. It is especially good visualizing in very fine detail the disorders of the cervical spine, so that a majority of myelographic examinations (but not all) formerly used for the spine may be obviated. MRI is excellent in diagnostic imaging of joints and is particularly valued by surgeons replacing hip joints for presurgical three-dimensional planning of sizes and fits. Pelvic regions of male and female are well seen by MRI as well as by CT, but CT is favored in abdominal organs for its speed, eliminating motion artifacts.

Other specific applications where MRI has been found especially useful are in the demonstration of smaller tumors in acoustic neuromas and pituitary tumors and in the detection of a variety of intracranial hemorrhagic conditions after 48 hours and of early changes in aseptic necrosis of bone. CT remains the method of choice for staging bronchogenic carcinoma and in evaluating small lesions of the pancreas and spleen and metastases in the lymph nodes.

ULTRASOUND IMAGING AND CHARACTERIZATION

Several projects have continued under development to improve the technology of ultrasound imaging systems including components. Some of these involve computer ultrasound transmission tomography systems particularly designed for regions of the body that involve

non-bony soft tissues, such as the breast or abdominal organs. Another system uses a combination of reflection and transmission tomography and has also demonstrated the beginning of three-dimensional real time visualization of a rotating object, such as the hand. Another system is also aimed at 3-D real time ultrasound images based on high speed parallel processing of received ultrasound data.

Developments continue on methods for improving ultrasound signal detection by reduction of speckle, by enhancing contrast, by providing computerized image processing to correct for or eliminate sonographic artifacts, and by the use of Doppler ultrasound to detect tumors. Doppler techniques applied to tumor detection in the vascular system in and around tumors has been considerably enhanced by the success in use of two-color Doppler to differentiate forward blood flow from retrograde flow or arterial from venous flow.

Much progress has been made by many investigators in noninvasive characterization of tissues by ultrasound, and in differentiating cyst from solid and other benign and malignant conditions in breast disease. Greater emphasis is being placed on evaluating more than one ultrasonic property of tissues at a time, such as attenuation, speed of sound, and reflectivity, instead of only one parameter. This permits parametric display and multivariate analysis of the signal data that make up the image, enabling differential color encoding of malignant tissues in ultrasound images and assisting the physician in making differential diagnostic correlations.

New statistical approaches to tissue characterization are finding practical success in detecting and assessing tumors of the liver and other organs by ultrasound. The new mathematical technique of fractal geometry is also being used to differentiate malignant from benign liver tissue based on ultrasound data.

Fundamental to all advances in ultrasound imaging and tissue characterization is the increase in knowledge gained from continuing investigations by many grantees studying non-linear propagation and scattering of ultrasound energy in tissues by direct studies in tissues and in phantom models.

Several expert investigators in the longterm programs studying potential bioeffects of ultrasound energy exposure to tissues at levels in the diagnostic range provide new knowledge in this area and the basis for being assured that no significant biological hazard is present in conventional diagnostic ultrasound imaging practices to date. The present emphasis is on achieving a complete physical understanding of the nature of transient and stable cavitation phenomena.

SMALL BUSINESS INNOVATIVE RESEARCH GRANTS AND CONTRACTS

There have been 21 Phase I SBIR grants, 9 Phase II SBIR grants, and 2 Phase II SBIR contracts active during FY88. The grants cover areas of DIRB program interest which include research and development in nuclear magnetic resonance, ultrasound, x-ray instrumentation and

digital radiography, monoclonal antibodies, electronic and software development, microdetectors, and controllers and many other highly useful diagnostic imaging devices

One SBIR grantee has developed the fastest MRI technique, which produces images in less than 60 milliseconds at a rate of 16 images per second, permitting the real time cardiac imaging referred to above. Other SBIR grants are devoted to development of tumor-specific MRI contrast agents and special coils, and one has developed a method for screening MRI patients for the possible presence of implanted magnetic prostheses, similar to an airline baggage scanner.

A computed tomography breast scanner based on reflected ultrasound has been developed with excellent spatial resolution in all directions, sufficient to image small (< 0.5 mm) ultrasound reflecting particles similar to microcalcifications. The same grantee is also developing an "expert radiology assistant" system to facilitate the task of the x-ray mammographer in detecting and classifying lesions. Another development has produced an x-ray sensor for use in quality control and dose reduction in clinical mammography. An interesting linear holographic ultrasound scanner utilizing a band of discrete frequencies has demonstrated increased depth resolution and the possibility of producing a B-scan ultrasound image of an object without mechanical or electronic scanning. A microwave radiometry scanning system is under development to detect breast cancer by detection of thermal anomalies.

WORKSHOPS

The most important aim of the workshops organized by DIRB is to determine the future directions of diagnostic imaging research and to develop initiatives in the areas recognized by the leaders in the field as most desirable to pursue during a fiscal year. Two major workshops were held in FY88.

"Basic Research in Factors Influencing Nuclear Magnetic Resonance (NMR) Relaxation Times in Biological Tissues" -- November 10, 1987

A one-day meeting was held in Bethesda to enable four grantees to present oral progress reports on their studies funded under RFA-84-CA-16. Additional specialists working in related NMR studies shared in the discussion periods. Although phantom models can be constructed and measured with predictable relaxation times (T1 and T2) for non-living materials, the complexities of biological cells and tissues do not yet permit a fundamental theoretical foundation to be constructed to explain magnetic relaxation behavior in living systems; continued basic and applied research are needed.

"Clinical Advances in PET Imaging" -- September 14-16, 1988

The purpose of the workshop is to present the state of the art of currently accepted clinical application of PET Imaging and to define additional areas of importance to clinical management in which further research is needed to define the proper role of PET. In addition to areas of potential medical necessity for PET imaging, efficiency studies, cost benefit analysis, and relationship to other diagnostic imaging modalities including CT and MRI, will also be considered.

INTER-INSTITUTE IMAGING GROUP

The objectives of the Inter-Institute Imaging Group (IIIG) are, 1) to inform members about present and future plans of diagnostic imaging research activities in all NIH Institutes with the purpose of stimulating innovative ideas in the member's programs, 2) to plan joint workshops where there are common interests, and 3) to have an outside speaker at each meeting to discuss various aspects of a new technology or a timely scientific topic.

Since its establishment by DIRB in 1986, the IIIG continues to meet several times each year and the enthusiasm of members is reflected by the high attendance in each planned meeting.

FUTURE DIRECTIONS (DIRB)

1. Multi-Institutional Imaging Trials will need to be expanded beyond their current level. As research in this area expands, both the 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. Head and neck, musculoskeletal, breast, and liver are sites being considered for addition to the RDOG.

2. For decades breast cancer has been the leading cause of death from cancer in women and has shown an apparent recent increase in incidence. Although x-ray mammography is the primary tool for detection, clinical trials by Dempsey et al ("Ultrasound Quarterly," Issue No. 1, Fall 1987) have shown that the use of x-rays plus ultrasound imaging improves the rate of early detection of malignant lesions. However, less than 15% of American women even use breast self-examination or avail themselves of baseline mammography. Several non-ionizing methods of imaging coupled with tissue characterization should be developed further (see next item). MRI is useful for tissue characterization as well as diagnosis, but it cannot be used for screening because of cost and slowness of patient imaging. Ultrasound imaging and Doppler flow, thermal measurement, transillumination, and photon time delay spectroscopy and imaging all have further unexploited potential for non-invasive characterization of breast abnormalities.

3. A DIRB workshop, "Ultrasound Tissue Characterization", held last year indicated that the next stage of development for ultrasound imaging should be the use of multivariate analysis and image processing techniques for improved tissue characterization and the extension of ultrasound systems to higher frequencies for special surface tumor and skin applications and for endocavitary and intraoperative probes. An RFA on "Higher Frequency Ultrasound Tissue Characterization Systems and Techniques" is being developed.

4. MRS has important potential for further study and staging of tumors and for monitoring the effects of radiation therapy, hyperthermia, chemotherapy, and immunotherapy. A New York Academy of Sciences conference held last year on Physiological NMR Spectroscopy included only one study in this important area. An NCI workshop "Clinical and Research Uses of Magnetic Resonance Spectroscopy in Staging and Monitoring of Radiation Therapy" will be held in early December, 1988, followed by a Program Announcement soliciting grants for these areas of investigation.

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.

6. The "Clinical Applications of Positron Emission Tomography" will be explored in a DIRB workshop to be held September 14-16, 1988. Advances in the use of this diagnostic modality are bringing to reality the ability to differentiate between normal tissue and tumor tissue, either viable or non-viable.

7. A DIRB workshop "Directions of Research in Diagnostic Imaging is being planned in the coming year.

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, 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 FY87 and the estimated budget for FY88.

FY87 and FY88 RDB Budget

			$(thousands)	
GRANTS	FY87	FY88	FY87	FY88
Traditional (R01)	189	176	31,652	30,605
Program Projects (P01)	11	13	16,313	14,473
Conference and New Investigator (R13 & 23)	9	3	340	75
First Awards	7	11	601	953
Merit Awards	5	7	1,330	1,920
Cooperative Agreement (U01)	0	1	0	1,331
Other	6	0	114	0
SBIR	11	8	908	1,212
TOTAL GRANTS	239	219	51,258	50,569
CONTRACTS				
Regular	21	12	5,750	5,652
SBIR	5	6	1,198	1,244
TOTAL CONTRACTS	26	18	6,948	6,896
TOTAL RDB BUDGET (FY88)	264	237	58,206	57,465

*Not all FY88 SBIR contract awards have as yet been determined.

FY88 Annual Report Summary
Radiation 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. This area of radiation research includes a range of scientific disciplines including biology, chemistry, physics and clinical oncology as well as the specialized treatment modalities of photodynamic therapy and hyperthermia. Radiation 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 radiosensitizers, 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.

Particle Radiotherapy

Radiotherapy with both charged and uncharged particles continues to receive a significant portion of the RDB budget. Neutron therapy Phase III clinical trials continue and charged particle therapy with both protons and heavy ions are used in the treatment of tumors that have no alternative effective therapy. Rare but devastating tumors are now being successfully treated with protons and heavy ions. They are tumors classified as chordomas and low grade chondrosarcomas of the base of the skull and cervical spine. Because of the precision of the charged particle beams, these tumors are treated with much larger doses than are achievable using conventional x-ray radiotherapy. Notorious for their proximity to spinal cord and other central nervous system structures (CNS) which are sensitive to radiation, these tumors cannot be treated with conventional x-ray therapy without damaging the sensitive structures. Using precise treatment planning and the dose localization that is possible with protons and heavy ions, the particle beam is able to treat the tumor to a higher dose, while sparing the sensitive CNS structures. In a survey of the literature, a population of 45 patients had received 55 Gy from x-ray treatment. Of these, 29 patients recurred within a median follow-up of 3.4 years, yielding a local control rate of 36%. Using particle therapy and a 25% higher dose of 69 Gy, a control rate of 78% has been achieved for the first 50 patients in a median follow-up time of 34 months. A similar group of 15 patients with tumors, sarcoma of the paravertebral soft tissues, have also shown good results with the proton beam. Local control has been achieved in 14 and in no patient has there been clinical evidence of radiation damage to the spinal cord.

Uveal melanoma continues to be another successful site lending itself to treatment with protons or helium ions. A recent review shows that data regarding survival and visual function after treatment is confirming earlier and favorable initial impressions using charged particle beams. Disease-free survival at 60 months is 96% in those patients with small tumors. Local control exceeds 98% for those receiving doses of 70 Gy. Treatment of such malignancies with particles is an alternative to enucleation.

A Phase III trial comparing protons with x-ray therapy in the treatment of patients with carcinoma of the prostate stage T-3 and T-4 is also underway. Other sites of interest include lung, head and neck and meningioma. Proton beam radiotherapy is carried out at the Harvard Cyclotron by the Radiation Medicine Department of Massachusetts General Hospital, Boston. Therapy with helium and neon ions is conducted at Lawrence Berkeley Laboratory, Berkeley, California.

Neutron therapy clinical trials continue with Phase III protocols in the head and neck, prostate, lung and radio-resistant histotypes, such as sarcomas of the soft tissue and bone. Protocols for tumors of the rectum and uterine cervix were closed due to a lack of patient accrual. The protocol for treatment of salivary glands was closed in March 1986, with neutrons as the treatment of choice. Accrual to the head and neck study is approximately 25% complete; the prostate, 30%; lung, 50%; and radioresistant histotypes, 23%.

Facilities that are supported to complete the clinical trials through contract mechanisms include the University of Washington, Seattle; University of Texas Cancer Center, M.D. Anderson Hospital, Houston, Texas; and University of California, Los Angeles. M.D. Anderson's cyclotron suffered a major breakdown and will not resume treatments until October 1988. The other two facilities report no major problems and were responsible for the bulk of the patient accrual in this fiscal year period. New funding mechanisms are expected to be implemented in the next fiscal year, designed to encourage patient accrual to the clinical trials and to capture third-party reimbursements for the treatments. The new financial arrangements will enable continuation of the clinical studies beyond the current cut-off date of September 1989 to their completion.

Hyperthermia

The high level of interest in hyperthermia by the research community is evidenced by the large number of grant applications and contract proposals representing all aspects of pre-clinical and clinical studies in this field. Two major directions in the area of hyperthermia are the development of appropriate devices for the optimum application of hyperthermia to a tumor mass and the elucidation of the molecular or biochemical mechanism(s) of hyperthermia-induced cytotoxicity and radiosensitization. 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 seen clinically and to correlate the temperatures achieved with clinical results. Efforts to develop new and more effective hyperthermia applicators and devices are in progress.

Several research efforts have been directed toward the effects of hyperthermia on blood flow. As investigation into blood flow in canine muscle in response to 43 C. fractionated hyperthermia indicated that blood flow in normal canine muscle increases in response to hyperthermia during the first treatment but then subsequently decreases back to control values. Another investigator studied the response of human tumor blood flow in six patients with head and neck tumors using a thermal clearance method. Although the work is still in progress the preliminary data show that: 1) tumor blood flow remains relatively constant during hyperthermia although changes on order of 25% are noted in some tumors, 2) the magnitude of the blood flow rates obtained varies from 0 to 60 ml/100 gm/min, 3) there appears to be no correlation between the blood flow rate and tumor volume, 4) in one case there is evidence of a thermo regulatory fluctuation in blood flow. The possibility of increasing the temperature differential between malignant or normal tissue by modifying tumor blood flow has been investigated. Using vasoactive agents which act on the tumor vessels i.e., norepinephrine, sodium nitroprusside and 5-hydroxytryptamine, substantially reduce (>50%) tumor blood flow, was noted which resulted in a preferential increase in the tumor temperature. Additionally, calcium entry blockers (e.g., verapamil and flunarizine), at drug concentrations well tolerated by humans, increased both primary and metastatic tumor blood flow by 50%.

Another investigator has shown that tumor vessels are more sensitive to heat then normal vessels and that glucose and galactose lead to a reduction in tumor blood flow than in normal tissue. A greater reduction in tumor blood flow was noted when the glucose was administrated interpertioneally rather than intervenously. A portable, self-contained, modular clinical instrument for the local, simultaneous quantification of tissue thermal properties and blood flow (perfusion) in small tissue volumes has been developed and prototypes distributed for clinical evaluation. This instrument is reported to be able to quantify tissue perfusion levels below 10ml/100 gm-min.

The relationship between extracellular pH, intracellular pH, cell energy status and sensitivity to hyperthermia was evaluated in the Chinese hamster cell line CHO. Intracellular pH and thermal sensitivity remain unchanged over an extracellular pH range of pH 7.0 to 8.0. Above and below this range, intracellular IpH varies with change in extracellular pH, and the magnitude of thermal sensitization closely correlates with the magnitude of the intracellular pH change above or below an intracellular pH of ~7.1. Substantial variation in intracellular (and extracellular) pH did not significantly effect the concentration of ATP, ADP, or AMP; creatine phosphate levels decreased under very acidic conditions. The absence of any change in ATP (the directly utilizable high energy phosphate) indicates that the mechanism of pH sensitization does not involve energy depletion. These results suggest that intracellular pH is the most appropriate indicator of thermal sensitivity. These data have further practical implications since 31P-NMR is a non-invasive method for measuring pH in human subjects, a method which is believed to measure tissue intracellular pH. Another study investigated intralesional pH following TCE10 or TCD90 "doses" of hyperthermia at time periods ranging from 4 hours to 7 days post-treatment. The preliminary data show that the average pH immediately after 43.5˚C. for 60 minutes fell to 6.55 and recovered to pre-treatment values within 24-36 hours post-hyperthermia. After 43.5˚C. for 120 minutes the average pH fell to 6.2 and did not return to pre-treatment values within 7 days post-hyperthermia.

Several studies have investigated the development of thermotolerance and its relationship to heat shock protein systhesis. The magnitude of thermotolerance and the level of heat shock protein (HSP) expression were measured in chinese hamster ovary cells after gradual temperature transients from 37˚ or 39˚ to 42˚ or 43˚C. When the level of thermotolerance was measured by clonogenic survival after challenging temperatures between 42˚CC and 43˚C, substantial thermotolerance was observed. However, when the challenging temperature was raised to 45˚C, proportionally less thermotolerance was apparent. Scanning densitometry revealed that low levels of heat shock proteins were synthesized during the heating gradients, but less that after a heat shock at 45˚C that delivered an equivalent heat dose. The immunoassay of HSP 70 levels measures both pre-existing and newly synthesized protein, and showed that there was no net increase in HSP 70 during two of the heating gradients tested, despite the increase in synthesis noted on the gels. Higher turnover of HSP 70 at the elevated temperatures possibly accounted for the failure to detect a net gain in total protein. In contrast, the total amount of HSP 70 doubled during the 6 hrs following a heat shock of 45˚C for 10 min, an equivalent heat dose to one of the gradients where no net increase in HSP 70 was measured by immunoassay.

The data indicates that tolerance to hyperthermia at 43˚C or below may occur under some conditions in the absence of elevated levels of HSP 70, but tolerance to higher temperatures is more closely correlated with increased levels of heat shock proteins. However, even at higher temperatures, the data show disparities between the levels of HSP measured and the thermotolerance expressed. Other investigators examined heat survival and the synthesis of heat shock proteins of mouse embryos at various stages, e.g., 1-cell, 2-cells, 4-cells, 8-cells, morula and early blastocyst stage found that one-cell embryos were extremely heat sensitive and synthesized hsp's at very low levels or not at all. At that developmental stage, neither thermotolerance nor hsp synthesis could be induced by heat shock. In contrast, unheated blastocysts synthesized hsp's constitutively, were comparatively heat resistant, and both thermotolerance and enhanced rate of hsp synthesis were induced by a non-lethal heat exposure.Their data demonstrate a correlation between hsp synthesis, thermal sensitivity, and thermotolerance in this system. The results strengthen the suggestion that gene activation of hsp synthesis is closely related to the differentiation process. Furthermore, they found that up to morula stage, even though the 70 kD protein was synthesized constitutively, heat can neither induce enhanced synthesis of this protein nor thermotolerance. Another study, was directed toward heat shock recovery. It was shown that individual elements within the

microfilament bundle (MFB) are not disrupted but rather that the bundling process of the microfilaments is heat labile. The recovery of the bundling can be observed but results in an altered morphology of the filament bundles. The altered morphology observed during heat shock recovery was attributed to the overexpression of proteins within a 45,000 molecular weight range. The investigator's current hypothesis is that the adhesion plaque (the area to which MFBs terminate) is heat labile and that the composition of that attachment area of the MFBs to the membrane is altered during heat shock recovery.

The induction of DNA lesions by radiation has been well established and is accepted widely as an explanation for radiation-induced cytotoxicity. In contrast, no specific explanation for hyperthermic cytotoxicity is, as yet, indicated or accepted. This is due in part to the generalized disruption of subcellular structures and anzymatic reactions which accompanies the absorption of a considerable amount of energy by cells at hyperthermic temperatures. In general, several studies by different investigators have shown that hyperthermic cytotoxicity can be expected to be a consequence either of thermal alteration or denaturation of macromolecular (i.e., protein) structure or thermal disruption of the rates or equilibria of enzymatic reactions. this general explanation does not identify a specific macromolecule or enzyme reaction which is rate limiting in hyperthermic cytotoxicity. Only in the case of S phase cells does hyperthermic exposure result in a lesion (chromosome aberration induction) indicating a subcellular mechanism (i.e., nuclear lesion induction) potentially responsible for cytotoxicity. Since the major metabolic difference between S and other cell cycle phases is DNA replication, studies of the effect of hyperthermia on DNA replication are in progress to help elucidate how nuclear lesions can result in hyperthermic cytotoxicity in S phase cells.

It is well known that temperatures in the 41.5-42.0°C produce a cell survival of approximately 50% with the killing of S phase cell contributing most to lethality. One study tried to if chronic thermotolerance can develop when CHO cellswere heated in S phase at 41.5-42.0°C for up to 12 hours. The results indicated that exponential cell killing continued while cells progressed through S, suggesting that chronic thermotolerance cannot be expressed in S phase. This is particularly interesting since reports in the literature indicate that when S phase cells are exposed to high temperatures (about 45°C) for a short period of time followed by incubation at 37°C they can develop thermotolerance. However, as the cells divided and moved into the heat resistant G1 phase during heating at 41.5°C, chronic thermotolerance was observed after several hours of heating. Additional studies were conducted to investigate mechanisms of killing when cells are exposed to higher temperatures, i.e., about 45°C where survival can decrease below 10% and where G1 killing becomes predominant, CHO cells were heated when they were in plateau phase, i.e., mostly G1. Plateau-phase cells sterilized by heat died by one of two distinct modes of death. A "rapid" mode, which predominated during the first few days post-heating, was characterized by cell detachment and inhibited rates of protein, RNA and DNA synthesis. A "slow" mode of death became evident after the cells had fully recovered from the heat-induced inhibition of macromolecular synthesis, and cell detachment had ceased. These populations had reduced plating efficiencies relative to nonheated populations, and contained a large fraction of cells with multiple nuclei. The multinucleated cells did not form colonies, but heated populations also contained uninucleated cells which were nonclonogenic. As the heat dose was increased and the surviving fraction decreased, the rapid mode of death predominated. These data show that heat damage is expressed in two ways. This might result from the existence of two separate targets for heat killing, or a single target which manifests its effects in different ways as the damage it sustains increases.

The effects of drug exposure duration and of heat and drug sequencing on hyperthermic potentiation of mitomycin-C (MMC) and cisplatic (DDP) were studied. One hour heating at 42°C was combined with drug exposure times of 1, 2, 4, and 8 hr. For DDP, hyperthermic

potentiation was greatest when heating was done during the first hour of drug exposure, while potentiation of MMC was greatest when heating was done at the end of the drug exposure interval. For both DDP and MMC, the dose enhancement ratios (DERs) at 1% survival were highest with the shortest drug exposure times and decreased as the drug exposure time increased from 1 to 8 hours. For DDP, the DER decreased from 1.9 with a 1 hour exposure to 1.2 with an 8 hour drug exposure. For MMC, the DER decreased from 1.8 to 1.5 as the drug exposure duration was increased from 1 to 8 hours. These results suggest that thermochemotherapy in vivo is likely to be most effective with rapid infusion of DDP or MMC, just before heating. If longer infusions are used, heating should be done at the beginning of the drug infusion for DDP and at the end of drug infusion for MMC.

Biochemical and biophysical characteristics of a cell's membrane are considered to be critical factors in a cell's response to heat and/or irradiation. One investigator has examined the use of dietary-manipulated changes in membrane lipid composition and membrane-active anesthetics to potentiate heat killing. He found that cell membrane lipids organized at low temperatures become disorganized as temperature increases. This disorganization alters membrane properties leading to passive changes in trans membrane permeability, shifts in surface charge and altered sterio-organization of membrane-associated macromolecules (proteins). The results of this study provide support for the theory that lipids may be the initial target in cells exposed to hyperthermia.

Attempts to model the heat transfer properties of tissues with the aim of improving the control and predictability of hyperthermia treatments have been reported. One investigator has compared a two dimensional treatment planning model with a three dimensional approach. He reported that when blood flow is low, thermal conduction dominates and hence the thermal boundary conditions in the third dimension are important. Thus, three dimensions are necessary for accurate results. When blood flow is high, the losses are dominated by it and so two dimensional computations are reasonably accurate.

Another investigator studied ability of microwave antenna arrays, as well as ferromagnetic seeds, to heat idealized three-dimensional tumors. The finite element method was used to solve the bioheat transfer equation, and the specific absorption rate (SAR) was calculated analytically for microwave antenna arrays. Both homogeneous and nonhomogeneous blood perfusion models were considered. Temperature distributions were calculated for various implant spacings in cylindrical and ellipsoidal tumors. The effects of tumor size, geometry, and blood perfusion models on the performance of each heating modality were studied using isotherm plots. Percentage of tumor volume heated to 43°C or greater was calculated to assign a hyperthermia equipment performance (HEP) rating to each case. Previous two-dimensional calculations and the three-dimensional calculations of this investigation were compared. a method of estimating complete three-dimensional temperature distributions in heated tumors using temperature measurement data was evaluated. Based on simulations of real tumors, it appears that the complete 3-D temperature fields can be successfully predicted. The development and testing of a computer controlled system for heat therapy of solid tumors that extirpates minimal intratumoral temperatures and continually re-adjusts power applied to multiple applicators to create an optimal heating pattern has been described.

The clinical work in hyperthermia is focused on evaluation of new hyperthermia devices. Interstitial devices which include spiral coil microwave antennae and ferromagnetic seeds are being tested in both animals and man. The Phase I studies of whole body hyperthermia have been completed and new Phase II-III trials which combine hyperthermia with radiation or chemotherapy are being initiated. Similarly, spontaneously arising tumors in animals are being treated according to protocols which combine fractionated external beam radiotherapy with microwave induced hyperthermia.

Photodynamic Therapy

Photodynamic therapy (PDT) is rapidly gaining acceptance as a potential treatment modality for solid malignancies. PDT is based upon the principal that systemically administered photosensitizers are 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. Progress in PDT has been delayed by the problems with availability of Photoforin II the only photosensitizer thus far approved for clinical trial testing. Photoforin II is a complex mixture of porphorins, a property which makes standardization of syntheses difficult. 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.

Purpurin derivatives, a group of synthetic photosensitizers, were tested for their photodynamic activity against transplantable N-{4-(5-nitro-2-furyl)-2-thiazolyl} formamide-induced urothelial tumors growing in male Fischer 344 rats. Histological examination of tumors in animals treated with the purpurin derivatives and red light (<590nm, 360 joules/cm2) revealed tumor cell necrosis 24 h after completion of therapy. Control tumors showed no histological change. Metallic purpurin derivatives, metallopurpurins, were found to be even more effective as photosensitizers than their non metallic counterparts. The metallopurpurins have their major absorption peak in the red region (> 650 nm) of the visible spectrum, a region with good tissue penetration. Tin etiopurpurin I dichloride appears to be the most active of the metallopurpurins and it can be easily prepared from the corresponding purpurin with a high degree of purity. In 30-day tumor regrowth studies, 70% of animals treated with the metallopurpurin derivative SnET2 were free of tumors while 50% of the animals treated with the corresponding free-base purpurin were free of tumor. Histological studies have shown that the disruption of tumor vasculature appears to contribute to the early stages of cell necrosis.

Other studies have identified new cationic photosensitizers and have characterized their photosensitizing efficacy and selectivity in a panel of malignant and nonmalignant cell lines, and determined the kinetics for uptake and release.

The synthetic "picket fence" (derivatives of tetraphenylporphine with acrylamide substituents at the ortho-position of the phenyl groups) porphyrins exhibit several attractive features that make them promising candidates as biological photosensitizers. The parent compound, tetraphenylporphine, is characterized by a high quantum yield (0.84 in normal propylalcohol) for intersystem crossing. The acrylamide derivatives are amphoteric molecules with a polar head group (porphine) and lipophilic side chains that favor their incorporation into membrane-mimetic assemblies such as micelles, monolayers, and vesicles. Tetra (o-acetamidophenyl) porphine (TAc), has been evaluated both in vitro and invivo in mitochondria from the R3230AC mammary tumor. Studies in vitro, consisting of incubation of mitochondria with TAc at a concentration of 4.0 ug/ml followed by photolysis, resulted in the inhibition of cytochrome c oxidase, proton translocating ATPase, succinate dehydrogenase, and malate dehydrogenase. The diminution in activity of the first three enzymes was approximately 2-fold greater than seen with Photofrin II under the same conditions.

Mono-1-aspartyl-chlorin E6, a chlorophyll derivative, was compared to hematoporphrin derivative (HpD) and was found to be chemically pure and more selective than HpD in targeting tumors. The isolation and purification of this natural product, however, may limit its usefulness as a clinical photosensitizer.

Other porphrin related compounds, verdins and benzochlorins have been synthesized and studied. The absorption maximum were shown to be approximately 700 nm and 650 nm respectively. Both classes of sensitizers appear to be spectroscopically superior to HpD in terms of their potential for photosensitization.

A precise understanding of the mechanism of tumor destruction by photodynamic therapy is critical to the further development of this treatment modality for clinical use. Several studies have been undertaken which contribute to the understanding of the mechanisms of PDT. One study is examining the structure-activity relationships relating to tumor localization and photosensitization by derivatives of chlorophyll. It was determined that the best tumor-localizing sensitizer was a molecule with -SO , H groups on adjoining methylene bridges. This result indicates the importance of charge distribution as a determinant of tumor localization. Ester and ether linkages were consistent with localization and photosensitization, while all carbon linkages were not. Preliminary studies on pheophorbides A and B show these to be potent sensitizers, but poorly retained in vivo by neoplastic cells. In contrast, pheophytins A and B (containing the phytyl residue) were effective sensitizers and localizers.

The role of oxygen in tumor destruction by PDT was investigated using a RIF tumor model. Since this tumor, under the treatment conditions used, had no detectable pre-existing hypoxic fraction, tumor hypoxia was induced chemically by administration of the vasoactive drugs isoproterenol, chlorpromazine, and epinephrine, 30 min before PDT treatment. The hypoxic fraction of tumors was monitored by a radiobiological assay and found to be 10%, 3.4% and 2.2% respectively. These findings suggest that pre-existing tumor hypoxia does not significantly limit photodynamic therapy, at least in this tumor model.

In a sequel study the administration of Fluosol-DA 20% and carbogen breathing was found to delay the onset of PDT-induced hypoxia through the first hour post-treatment. Progressive values of tumor hypoxia were observed after 4 hours post-treatment. The time period in which tumors remained well oxygenated coincided with observations of reduced tumor cell cytotoxicity. Decreases in tumor cell clonogenicity were observed only after tumor cells became hypoxic. These findings were consistent with the 24 h delay in complete tumor response in animals given Fluosol-DA 20% and carbogen breathing before PDT. There were only significant differences in long-term tumor response and cure observed between the two groups tested. These results again indicated that under the given treatment conditions the primary factor leading to tumor cell death was disruption of the vascular supply to the tumor.

The metabolic effects of PDT under conditions analogous to a clinical setting have been examined using 31 P-NMR spectroscopy, a non-invasive technique that allows phosphate-containing metabolic components in tissues to be monitored in situ. Earlier this investigator had shown that mitochondria are a major target of porphyrin photosensitization and the damage to mitochondria will lead to reduction in cellular ATP, which would be required for cellular metabolism and replication. The 31 P-NMR spectra obtained from tumors in vivo showed a dramatic decrease in the B-ATP/Pi ratio within 1 hr after illumination, the ratio dropping in 2 to 8 hours to 0 to 20% of that found prior to photoradiation. Concomitantly, the Pi resonance increased markedly. Disrupted cells and pycnotic nuclei were observed in tumors 48 to 72 hours after photoradiation to a depth of about 5 mm. Together with his earlier studies, the investigator concluded that the reduction in tumor ATP levels was an early biochemical response to photodynamic therapy.

The cytotoxicity that ensues photosensitization by hematoporphyrin derivative (Hpd) has been attributed to production of singlet oxygen, a reactive oxygen species that can oxidize various chemical components of phospholipids, proteins and nucleic acids. Many of the affected cellular constituents are localized to membranes, hydrophobic environments conducive to

partitioning of hydrophobic porphyrins. To test whether efficacy of Hpd-induced photosensitization depends on entry of Hpd into membranes, one investigator immobilized Hpd (and more recently, Photofrin II) on Sepharose beads, which are about 500 times larger than mitochondria. He determined that immobilized Hpd produced singlet oxygen when photoradiated in yields similar to those for Hpd in solution. Using intact mitochondria, he then showed that immobilized Hpd was able to inhibit cytochrome c oxidase, an enzyme that spans the inner mitochondrial membrane, but had little effect on mitochondrial enzymes that are located on the interior portion of the inner membrane. These results suggest that photosensitization by Hpd most likely arises from entry of the photosensitizer into the biological membrane, although proteins on the exterior membrane surface may be susceptible to damage by singlet oxygen produced in proximity to their location. This investigator extended his studies to define mitochondrial target sites of enzymes that are located in distinct sites within the mitochrondrion. He studied adenylate kinase (AK), found in the intermembrane space between the outer and inner mitochondrial membranes, and monoamine oxidase (MAO), which is attached to the outer face of the outer mitochondrial membrane. Utilizing intact mitochondria in vitro, Photofrin II photosensitized both MAO and AK, the inhibitions of activity displaying both drug-dose and light-dose relationships. However, MAO activity was inhibited to a much greater extent that AK under the same experimental conditions. Using an in vivo-in vitro protocol and sampling at 2 to 72 hours after systemic administration of Photofrin II, photosensitization of MAO was seen at 2 hours after drug treatment, but inhibition of activity was not observed at later times. AK activity was unchanged over the entire time course. Compared to cytochrome c oxidase, located in the inner mitochondrial membrane and which displayed a sustained inhibition of activity, he suggested that inhibition of MAO or AK probably does not contribute to tumor cytotoxicity under conditions of clinical phototherapy. However, these results lend further support for preference for membrane environments as sites of action of porphyrin-induced photosensitization.

Studies designed to develop and optimize a system of dosimetry for predicting and monitoring effective absorbed dose in PDT of cancer have been conducted. As a result of these studies a calibrated isotropic detector capable of measuring energy fluence as well as energy fluence rate is now available commercially. Studies to further refine the capabilities of this device are in progress. Another investigator has reported an in vivo fluorescence detector which is capable of detecting a very small number of tumor cells containing dihematoporphrin ether (DHE).

Two studies have individually reported that lighter dose rates which induce hyperthermia may be advantageous in PDT.

The photodynamic effect on subcutaneously implanted mouse bladder tumors with HpD injected intratumorly (I.T.) and intraperitoneally (I.P.) was examined. Tumor cell killings, measured by cell survival, were observed in both the I.T. and I.P. administered animals, and was dependent on fluence and HpD dosage. However, although there was significantly higher porphyrin in the I. T. injected tumors, there was no significant enhancement in cell killing in these tumors. Histological examination of the effect of PDT on the blood vessels indicated that while cell death accompanying severe hemorrhage in I.P. injected tumors, there was much less hemorrhage and intact blood vessels remained in the I.T. injected tumors. This observation suggests that in the latter, direct photodynamic action may play a more significant role in tumor cell death. This is in contrast to tumors with HpD injected systemically, in which destruction of blood vessels is believed to be the main cause of tumor destruction.

A study was carried out to achieve selective tumor cell killing with a monoclonal antibody (Mab) as the carrier for chromophores followed by photo-irradiation. This approach has numerous advantages over conventional serotherapy, the most notable of which is double

selectivity; i.e., Mab for delivery of chromphores to specific target cells and laser illumination for specific drug activation. Because chromophores are only active when illuminated, the Mab need not be completely tumor-selective.

In this work, a Mab, 3G2-C6, to human bladder tumor-associated cell surface antigen was conjugated site-specifically to a chromophore chlorin-e6 as an intermediary. This conjugation technique yielded conjugates with high chromophore:Mab ratios without significantly altering the antigenicity of the Mab. Binding activity and specificity for target cells (a human bladder tumor cell line, MGH-U1) were typically 80-90% of the unconjugated Mab. When target cells were treated with the Mab-chlorin-e6 conjugate and photoirradiated at 660 nm (absorption maximum for chlorin e6) using an argon ion-pumped dye laser, cell killing was observed, which was dependent on fluence and chromophore concentration. Experiments in the presence of D 0 suggested a singlet oxygen-mediated mechanism. This conjugate showed selective phototoxicity to target cells, and was non-toxic in the absence of photo-irradiation. Conjugates with irrelevant Mab or free chlorin-e6 at comparable concentrations showed no toxic effect. These results suggest that Mab-chromophore conjugates and photo-irradiation may represent a potential treatment for malignant tumors with high degree of specificity.

The potential utility of benzophenoxazines as photosensitizers using cultured tumor cells and animal tumors as experimental systems was evaluated. An extraction procedure, to quantitate the uptake and retention of one of the dyes (Nile Blue 1) by tumor cells in culture and by tumors in animals was developed. Preliminary results obtained showed that with 10 mole of the dye added, about 40% of the dye was taken by the cells within 20 minutes. The efflux of the uptake dye was very slow. After 24 hours of efflux condition, 70% of the dye remained within the cells.

Radiation Modifiers: Sensitizers and Protectors

Both the pre-clinical and clinical research areas associated with radiosensitizers continue to be active. Further pre-clinical data is being gathered by Stanford University to obtain an IND for nicotinamide (NSC 604583). Previously it had been reported that nicotinamide had produced a significant enhancement of radiation damage in three different tumor systems: the EMT6, RIF1 sarcomas and the Lewis Lung carcinoma. Enhancement ratios of 1.2-1.7 were obtained. Much smaller enhancement ratios (1.0-1.2) were obtained in two normal tissue assays. Preliminary evidence indicated that the radiosensitizing effect of nicotinamide was produced by an increase in tumor oxygenation. This investigator has extended his studies to the SCCVII carcinoma, and has shown that nicotinamide (100 mg/kg) sensitizes this tumor whether it is implanted subcutaneously (in the back) or intramuscularly (in the leg). He has also shown that similar radiosensitization (DMF = 1.5) is obtained if nicotinamide is given orally, intraperitoneally, or intravenously. Mechanism studies have focused on the effect of nicotinamide on tumor blood flow. This investigator used the Hoechst stain 33342, together with analysis of mean tumor fluorescence by a cell sorter, to obtain a measurement of tumor perfusion given without nicotinamide. His data show a 30-40% increase in tumor perfusion, which extends from 30 minutes to 4 hours after nicotinamide injection. He also measured tumor blood flow using clearance of ^{133}xenon from the tumor. Using the technique, he found only a small and not statistically significant increase in tumor blood flow for nicotinamide. All of these experiments were performed with SCCVII carcinoma. The investigator to resolve this difference in results between the two techniques using a modified version of the Hoechst method, which will not be susceptible to possible changes in the pharmacokinetics of the dye following nicotinamide injection.

The data accumulated to date indicate that nicotinamide may be an important radiosensitizer for clinical application. This investigator, in conjunction with the clinical staff of the Division of Radiotherapy, is developing a clinical protocol to study blood flow and oxygen status of human tumors. The results of this clinical study will be used to support an IND application for this radiosensitizer.

The in vitro and in vivo mechanistic studies of NSC 130181 (SR 4233) have concentrated on DNA damage and repair after drug exposure, modification of cellular toxicity by the use of other drugs and radiation, and biochemical assays of drug metabolism and free radical damage to macromolecules. Recent studies have shown that there is a rapid rejoining of single stranded DNA breaks if cells are removed from drug and if given sufficient "repair time" at 37 degrees. Double stranded DNA breaks induced by SR 4233 appear irreparable in preliminary experiment using the neutral elution procedure. As part of his modifiers studies, the investigator attempted to either enhance or reduce the toxicity of SR 4233, using the survival of hypoxic CHO cells as an endpoint. Treatments which sensitized cells to killing by SR 4233 included severe glutathione depletion by DEM or BSO, incorporation of 5-bromodeoxyuridine into cellular DNA, co-treatment of cells with SR 4233 in the presence of excess misonidazole and addition of iron-EDTA to cells during SR 4233 treatment. Treatments which reduced the hypoxic toxicity of SR 4233 included the presence of radical scavengers such as DMSO or DMTU during drug exposure, and co-treatment with SR 4233 in the presence of desferal, an iron-chelating agent.

Although the magnitude of sensitization or protection achieved by the use of these modifying agents was not thought to be sufficient to warrant their use in combination with SR 4233 in vivo, these results did lend further support to the free radical theory of SR 4233 action. In an attempt to simulate the conditions which may exist in tumors when drug is injected at different times relative to a radiation dose the interaction of SR-4233 and radiation was studied using SCCVII mouse tumors cells in vitro. Cells receiving a fairly toxic treatment with SR 4233 (reducing survival to about 0.01-0.05) under hypoxic conditions were sensitized to subsequent irradiation under aerobic conditions. The sensitization took the form of a removal of the shoulder of the x-ray survival curve. Similarly, hypoxic cells irradiated immediately before SR 4233 treatment (a single dose of 20 Gy, which reduced survival to 0.009-0.2) were sensitized to drug. The SR 4233 cytoxicity curve was found to be steeper in cells previously irradiated. Both of these "preincubation effects" may contribute to the enhanced killing observed in SCCVII tumors when SR 4233 combined with radiation

Studies of the metabolism of NSC 130181 have shown that SR 4233 reduction can be conveniently estimated by following the formation of SR 4317 using a fluorimeter. Using this technique several inhibitors of known bioreductive enzymes were tested to try to determine a principal pathway for SR 4233 reduction by CHO cells. Neither allopurinol, dicoumarol, carbon monoxide, or inhibitors of mitochondrial electron transport decreased the rate of SR 4233 reduction.

No single enzyme or pathway was therefore found which was responsible for CHO cell metabolism of SR 4233. Activation of SR 4233 by microsomes could be catalyzed by either NADPH or NADH as the electron donor and were not inhibited by carbon monoxide. Substances which interfere with NADPH generation, dehydroepiandosterone, or compete for NADPH electrons, such as MISO, decreased SR 4233 reduction rate. Reduction rate was also closely correlated to exposure glucose concentration in cell incubation.

The effect of creating additional tumor hypoxia using hydralazine and other hypotensive drugs on the toxicity of SR 4233 toward tumor cells in vivo was investigated. Hydralazine (10 mg/kg i.p.) produces an immediate fall (to 20-30% of normal) in the blood flow and a

simultaneous increase (to approximately 100%) in the radiobiological hypoxic fraction in the EMT6 and SCCVII mouse tumors. This increase in tumor hypoxia produces an increase in cell killing by a single dose of SR 4233 (0.3 mmole/kg) from approximately 40% to nearly 99.9% of the tumor cells. However, a small reduction in the acute LD_{50} of the drug was also produced (from 0.5 to 0.3 mmole/kg). DMF is for the increased cell killing by SR 4233 and the decreased LD_{50} were 4.5 and 1.7, leaving a therapeutic gain factor of 2.8. This selective production of tumor hypoxia combined with SR 4233 (or similar bioreductive cytotoxic drug) represents a potentially powerful new method for selective targeting cytoxic drugs to tumors, a method that may prove extremely useful in clinical chemotherapy.

A new series of 2-nitroimidazole analogues containing an aminobenzamide moiety has been synthesized in attempts to employ a dual mechanism of radiosensitization involving (a) electron affinity of the nitro function and (b) increased diffusion of oxygen in hypoxic cells perhaps due to the inhibition of cellular oxygen utilization by the benzamide group.The radiosensitizing efficiency of these agents was determined in exponentially growing asynchronous Chinese hamster (V-79) cells under hypoxic conditions. The o- and p- substituted benazamides were more potent radiosensitizers than m-submitted analogue. The sensitizer enhancement ratios were 2.1 and 2.2 respectively at 1 mM concentrations in comparison to 1.9 for misonidazole under these conditions. These agents were also less toxic than misonidazole with LD_{50} values of >2.4 g/kg.

Studies have examined the effects of perfluorochemical (PFC) emulsions on the radiation response of tumors and normal tissues in rats and mice. Using an exchange transfusion technique, the hematocrits of rats were reduced to 50% of normal (i.e., ~25) or to a very low value (~1), with the blood replaced by Fluosol-DA, a procedure which does not alter the viability of BA1112 tumor cells assayed 24 hrs later. The effects of these exchange-transfusions on the tumor cell radiosensitivity are now being evaluated. Additional Studies using EMT6 tumors in BALB/c mice are examining in detail the relationship among the PFC dose and time of PFC administration, the hematocrit, and fluorocrit of the mice, and the viability and radiosensitivity of the tumor cells. These studies will defined in greater detail the mechanisms by which PFC-emulsions alter the tumor radiation response. Studies with normal tissues are also being conducted by this investigator. Histologic and bronchoalveolar lavage techniques are being used to examine pulmonary changes after thoracic irradiation; no augmentation of reactions ascribable to PFC-treatment have been observed thus far. Studies of artificial lung metastases in mice receiving whole-thorax irradiation have been completed. Administration of Fluosol plus carbogen during irradiation with graded radiation doses from 5 to 15 Gy did not change the number of lung colonies from those observed in saline-injected, air-breathing animals irradiated at the same doses.

A clinical trial was initiated to investigate the usefulness of intravenous Fluosol infusion and concomitant hyperbaric oxygen breathing as an adjunct to radiation therapy in the treatment of glioblastoma multiforme. Patient accrual began in February, 1987. Sixteen patients have now been enrolled in this clinical trial. The presently used treatment schedule consists of treating patients over a six week period. In weeks 1,2, 5 and 6, patients receive a single treatment of 600 rads whole brain following Fluosol administration and under hyperbaric oxygen conditions. During week 3, patients receive routine radiation therapy, 200 rads x 5 whole brain, and during week 4, 200 rads x 5 to a cone down port. Presently a Fluosol dose escalation schedule is in progress. To date there have been no severe side effects seen with the Fluosol/hyperbaric oxygen treatments. There has been no transient episode of oxygen toxicity seen in the more than 50 pressurizations which have taken place. This is consistent with the experience with hyperbaric oxygen, 3 ATA, without Fluosol which has been observed in other clinical trials.

The compound dimethylfumarate (DMF) has been studied as to its efficacy and mechanism in radiosensitizing hypoxic cells. The investigators reported what they believe to be the largest radiosensitization of hypoxic cells yet seen in vitro under non-toxic conditions solely through the action of a thiol-depleting agent. They found that dimethylfumarate (DMF) depletes intracellular glutathione (GSH) in a reaction mediated by GSH-transferase, as well as by spontaneous reaction. In Chinese hamster ovary (CHO) cells this depletion is rapid, e.g., 0.5 mM DMF depletes GSH to less than 10% of control in 5 min at room temperature. DMF is a very effective hypoxic cell radiosensitizer of CHO cells, giving an enhancement ratio (ER) of about 3 with a 5 min exposure of cells to 5 mM DMF at room temperature, a nontoxic exposure. At this same concentration of drug, there is a small radiosensitization of aerobic cells (ER = 1.2 compared to untreated aerobic cells) by DMF. However, 5mM DMF results in nearly a complete collapse of the hypoxic dose response curve to the same level as in cells irradiated in air with DMF. In other studies designed to investigate the mechanism of action of DMF the results suggested that 1) DMF is not sensitizing via electron-affinic mechanisms. Thus, the enhancement of radiation sensitivity appears to be related, largely, to the drug's ability to deplete thiols 2) DMF treatment depletes protein thiols to about 60% of control, suggesting that part of the radiosensitizing action of DMF is due to radiochemical reactions related to lowering of intracellular thiol and part of the effect may be due to DMF causing interference with post-irradiation repair of hypoxic radiation damage by depleting critical protein thiols 3) that neither the radical scavenging nor the induced hypoxia (thiol-mediated oxygen consumption) hypotheses are satisfactory explanations for the the reversal of BSO-induced radiosensitization by either GSH, cysteine or cystine. 4) that temperature independent thiol/disulfide exchanges occur with the addition of exogenous GSH, but that a temperature-dependent (catalytic?) process(es) is more closely tied to the reversal of the radiosensitized state. These investigators also developed radical-repair model for the oxygen effect to study the effect of varying the concentration of damage-restitution species in thiol depleted conditions. Good agreement of the model with experimental data in the published literature was obtained for cells with glutathione (GSH) levels decreased due to genetic deficiency with sharp disagreement seen laboratory at low concentrations of oxygen for cells depleted of their GSH by buthionine sulfoximine (BSO). These and other data in the literature suggest that the intracellular spatial distribution of thiols in the depleted state may be significant with the nature of such distribution not being revealed by measurement of average cellular thiol level.

Compounds are being screened for their ability to produce stable fluorescence in hypoxic cells. Preliminary analysis of the ability to use hydroethidine to sort radiation-treated cells indicated as good resolution of hypoxic (radiation-resistant) cells as that obtained using the best stain, Hoechst 33342. AF-2, a fluorescent nitrofuran that binds preferentially to hypoxic cells, also exhibits temperature dependence of binding. Aerobic cells exposed to AF-2 at 37 degrees C. bind about 5 times less AF-2 than cells exposed at 45 degrees C. AF-2, could be considered a prototype of a fluorescent probe to measure intracellular temperature during hyperthermic exposures. AF-2, was found to enhance the binding of another hypoxia probe, 3H-misonidazole by up to 10 fold. Experiments were conducted to characterize this effect, and an attempt made to determine possible reasons. Electron transfer processes were implicated.

A variety of compounds, representing different chemical classes are being studied both pre-clinically as potential radiosensitizers Perflurocarbons, nicotinamides, ubiquinones, benzotriazine dioxides and a new series of 2-nitroimidazole have shown sufficient in-vitro activity to warrant further pre-clinical evaluation and/or preliminary clinical trials. The specific hypoxic cell cytotoxic agent NSC 130181 (SR-4233) which was identified through the NCI Radiosensitizer Screening contract has undergone extensive pre-clinical testing, as well us, some of its analogues which were synthesized in an effort to optimize this compound. Nicotinamide (NSC 604583), another potential radiosensitizer, also identified through the NCI

Radiosensitizer Screening contract, has shown sufficient in-vitro and in-vivo activity to consider it for clinical trial. Stanford University has interest in getting an Investigational New Drug (IND) certification for this compound. Pilot clinical trials are being planned. A ubiquinone type compound, isolated from a natural product and in use clinically in China as a radiosensitizer - anticancer drug, has been synthesized and is undergoing in-vitro and in-vivo testing as a potential radiosensitizer. There continues to be interest in pre-clinical and clinical studies evaluating radiation sensitizers (misonidazole) as chemosensitizers in combination with chemotherapeutic agents. Toxicology studies with the radioprotector WR 2721 are continuing. WR-2721 has also been shown to be a useful agent in reducing the chemotoxicity of antineoplastic agents.

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is being tested clinically using Photofrin II as the photosensitizing drug. This compound is the only photosensitizer that currently has an Investigational New Drug (IND) status. Photofrin II is a mixture of porphrins which has presented synthesis and formulation problems in the past and financial interest in this photosensitizer has changed hands twice in the past three years. Photofrin II has its absorption maximum around 405 nm, a wave length in the electromagnetic spectrum (visible portion) with very limited tissue penetration. A secondary weaker absorption peak exists at 627 nm, a wave length with better tissue penetration. In practice, Photofrin II is generally activated with light at 630nm. Newer photosensitizers are being proposed which have their major absorption peak between 650 and 715 nm, an area of the visible spectrum with excellent tissue-penetrating properties. One such compound , a metallopurpurin derivative, tin etiopurpurin I dichloride (NSC #619679) was accepted by the NCI-DCT Decision Network for further development. The preclinical data on this compound indicated that it is more cytotoxic than Photofrin II and lacks the skin photosensitivity of the latter. Through the grant, contract and small business programs, support has been extended for research in many of the aspects associated with PDT. In addition to the development and synthesis of new photosensitizers there is activity in trying to: determine the mechanisms by which photosensitizers are localized, the mechanisms associated with cytotoxicity, the development of a dosimetry system for monitoring light absorption, methods for determining tissue levels of the photosensitizer, the development of more effective and efficient light delivery systems and the development of newer light sources capable of producing the appropriate wave lengths of light needed for the new photosensitizers.

State of the Art of Radiolabeled Antobody 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 upporting this technology that is only now receiving significant federal funding. Part of the research is in the immunology area where the search for improved tumor specific antibodies is the goal. Other research is inthe development of radionuclides for imaging and therapy. For imaging of tumors, a low energy gamma emitter of about 150 KeV is optimum. However, 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 antobodies for greatest stability in in vivo.

Phase I and II clinical trials are being carried out at some 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 has accrued in 175 patients since October 1987.

In September of 1987, DCT NCI began support of a Dosimetry Center, through RTOG, at Johns Hopkins University. The Center is training other RTOG members in the computerized calculations of tumor volume and dose necessary to perform the objective evaluation of treatment. The volume calculation will be useful in objectively evaluating the effects of any type of cancer therapy on tumor masses.

As more is understood about the inhomogeneous distribution of radiolabeled antibodies, it is clear that more research is needed on the dosimetry of therapy. A workshop held by RRP recommended the funding of dosimetry research and approval was given by the June, 1988, Board of Scientific Counselors for RRP to advertise a Request for Applications (RFA) to fund several grants in this area.

A workshop on the Radiobiology of Radiolabeled Antibodies is planned for the fall of 1988. This will provide a forum to recommend research directions for radiobiology to RRP.

A strong central coordination of all of these research areas is necessary to thoroughly explore radiolabeled legands/conjugates for the diagnosis and therapy of cancer.

Radiation Therapy Treatment Planning

A number of contract-supported studies that organize several institutions into Collaborative Working Groups are investigating the many facets of radiation therapy treatment planning and treatment delivery. One group evaluating photon beam treatment planning completed its contracts in FY88. Eight disease sites were investigated and the report summarizing the results and conclusions of the group will be published. This Working Group emphasized that the three-dimensional (3D) nature of treatment planning and treatment delivery process must be considered in the development of an optimal plan. Only if 3D planning is carried out can the adequacy of the target volume coverage and the effects on normal tissues be evaluated. This is extremely important in assessing potential complications in the irradiation of a critical organ or tissue. Current practices of 3D treatment planning are labor-intensive and time-consuming. A new class of computer programs will most likely be necessary before 3D treatment planning can be introduced into clinical use.

Another Working Group is charged with making recommendations for interstitial radiotherapy in the calibration and dosimetry of radioisotope sources, computer calculations and a quality assurance program. A third group is evaluating electron beam dose distributions with presently available beams, imaging techniques, and computerized treatment planning systems.

These groups of investigators are breaking new ground in the definition of three-dimensional treatment planning, treatment plan optimization and evaluation of treatment delivery. Their recommendations will be an important contribution to the planning and delivery of radiation therapy and, ultimately, to local tumor control.

Radiation Biology

The NCI, primarily through the Radiotherapy Development Branch (RDB), continues to support a major portion of radiation biology research in the United States. Radiation Biology research funded by the RDB 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) Recombinant growth factor induced modulation of the radiation response of human bone marrow progenetor cells. (This may provide a basis for increasing the therapeutic index for cancer patients by reducing the hematologic toxicity of radiotherapy.)

(2) Identification of genetic markers associated with radiation resistant tumors (These markers might aid in the development of rapid predictive assays of radiation therapy and in the determination of molecular mechanisms of radioresistance.)

(3) The interactions of polyamines with bacterial transforming DJNA (Polyamines are located near DNA in the cell and they might modify radiation sensitivity by scavenging free radicals or by stabilizing DNA backbone structure).

(4) The influence of irradiated volume on CNS tolerance by irradiation of various lengths of rat spinal cord, covering the total cervical and thoracic cord (Reduction in the length of cord irradiated below 10 mm shows a step increase in the tolerated dose.

(5) Endocrine deficiencies in humans after radiotherapy for tumors of the head and neck (They are common with the higher incidence of complications occurring 1 to 5 years after radiotherapy).

(6) Investigations into sublethal and potentially lethal damage repair (SLDR and PLDR) in a spectrum of malignant human cell lines and in normal human fibroblasts and endothelial cells (There was no correlation between the magnitude of PLDR and the inherent radiosensitivity for the tumor cell lines but SLDR appears to correlate well with radiosensitivity in the tumor cell lines.)

SMALL BUSINESS INNOVATIVE RESEARCH (SBIR) GRANTS AND CONTRACTS

The RDB funded 6 SBIR contracts (1 Phase I and 5 Phase II) and 7 SBR grants (4 Phase I and 3 Phase II). Funded SBIR research includes a fiberoptic fast neutron dosimeter, a photon dosimeter and a PDT dosimeter, lasers for PDT, microwave, and ultrasonic hyperthermia equipment development, hyperthermia dosimetry, modeling and equipment, and computer controlled multileaf collimaters for photon radiotherapy.

WORKSHOPS

"Small Field Stereotactic Radiotherapy" -- June 8-9, 1988.
Workshop participants presented current experience and reviewed the several modalities used for precision treatment of small lesions. After a presentation of the radiobiology and physics involved, the participants listed research goals and called for a standing working group to coordinate the research in the treatment of small brain tumors and arteriovenous malformations (AVM).

"Boron Compounds Suitable for Cancer Therapy" -- May 3-4, 1988
Participants presented data on compounds suitable for concentrating boron in certain malignant tissues, such as melanoma and glioblastoma for subsequent potentially curative therapy using a boron-neutron capture process which emits a highly localized, heavy charged particle to surrounding tissues. New initiatives arising from the workshop are anticipated to explore new biochemical methods for tagging tumor-seeking molecules with boron compounds as well as testing existing compounds for their localization potential for both therapy and imaging studies.

"Future Directions of Computer-Assisted Radiotherapy" -- August 13, 1988

This workshop will take place at the end of the World Congress on Medical Physics and Biomedical Engineering, San Antonio Texas, through a joint U.S. and Scandinavian collaboration. Participants will hear latest developments in three-dimensional treatment planning, knowledge-based systems for radiotherapy, imaging handling and treatment optimization. New initiatives in the field of medical informatics and knowledge-based systems are anticipated as a result of this workshop.

"Hyperthermia Clinical Trials Workshop" -- May 12-13, 1988

Workshop participants updated the state-of-the-art in hyperthermia clinical trials. Problem areas were emphasized. It was clear that a lot of work is needed in the area of delivery systems development before major advances will be forthcoming from the field of hyperthermia. However, it was pointed out that there are at least two anatomic sites that are ready for a well controlled Phase III clinical trial. The need for quality assurance and investigator compliance for all clinical trials were stressed.

FUTURE DIRECTIONS (RDB)

Now available only at limited institutions, three-dimensional treatment planning is a growing research activity in the nation's radiotherapy departments. New contract awards from RDB in December 1988 will fund a Working Group to develop new software tools that will automate and streamline many of the activities in three-dimensional treatment planning that are now too labor intensive for routine radiotherapy.

Dynamic conformal radiotherapy is a new and exciting research area in which complex treatment plans using conventional photon and/or electron beam accelerators design treatments 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.

Medical informatics is a new and growing science 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 radiotherapist. 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.

Further research and development of radiolabeled monoclonal antibodies and cell specific receptors is necessary to explore the possibility of cellular radiotherapy with these agents. The dosimetry of these radionuclide tagged compounds is an important research area of this rapidly developing therapeutic approach and requires further support. A Dosimetry Center for radiolabeled radiotherapy agents is being supported within the Radiation Therapy Oncology Group, a clinical trials group. The Board of Scientific Counselors of the Division of Cancer

Treatment recently approved a request for applications (RFA) to support several applicants in research on the dosimetry of radiolabeled antibodies. The results of these grants will be provided to the Dosimetry Center for implementation into clinical trials. Radiolabeled immenoconjugates will continue to be a high priority research area of the Radiation Research Program for both diagnosis and therapy.

A "Patterns of Care Study" (PCS) in radiation oncology was funded in June 1988 with the American College of Radiology to determine a consensus of the best current management (BCM) methodology in five cancer sites; breast, cervix, Hodgkin's disease, prostate and recto-sigmoid. The PCS will survey the BCM compliance of a representative sample of the U.S. radiation oncology facilities. The outcome of the treatment of patients in those facilities will be studied in order that recommendations can be made to improve patient survival and reduce complications nationwide.

Hyperthermia as an adjunct to radiation and chemotherapy continues to interest the clinical research community. Further research and development needs to be forthcoming for devices capable of deep heating and of non-invasive thermometry. the usefulness of hyperthermia needs to be confirmed by randomized clinical trials for specific disease sites and anatomic sites. These trials need to have strict quality assurance requirements that are closely adhered to by all participants, for the results of these trials to be valid.

The major problem facing Photodynamic Therapy as an emerging modality for cancer therapy is the depth of penetration of the activating light in tumor tissue. Newer photosensitizers and better light sources and light delivery methods could resolve this problem. Currently these areas are under active investigation.

Radiosensitizers have demonstrated an ability to increase the sensitivity of neoplastic tissue to radiation beams and attempts will be made to improve the efficacy of these agents and to decrease their toxicity. New compounds need to be developed. This development is depended heavily on the capability to screen a large number of compounds for radiosensitizing activity. A more rapid screening system with large capacity needs to be instituted if the area of radiosensitizers is to progress.

A field conceptually related to PDT in being binary in nature, but involving ionizing radiation is boron neutron capture therapy (BNCT). Agents which localize in tumors are tagged with the 10B isotope of boron. Irradiation of such a compound with low energy neutrons results in emission of a short range alpha particle, which deposits an intense radiation dose at the cellular level. Many facets of this potentially valuable treatment modality are ripe for research including 1) the boron chemistry of localizing compounds such as porphyrins and antibodies, 2) the phorneucology of such compounds; and 3) the physics and dosimetry of thermal and epithermal neutron beams suitable for therapy.

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