ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL CANCER INSTITUTE

Fiscal Year 1981

Part VI-B

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

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE (U.S.)

Fiscal Year 1981

Part VI-B

Division of Cancer Treatment

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 to September 30, 1981

The Laboratory of Molecular Pharmacology is engaged in 2 areas of investigation: (1) studies of the effects of DNA-reactive drugs in mammalian cells in relation to cytotoxic mechanisms, and (2) studies of nuclear proteins and chromatin. In both areas, new methodology has been developed in this Laboratory and is being applied to several problems.

The effects of drugs on DNA in mammalian cells are being studied by means of filter techniques, developed in this Laboratory, for the measurement of DNA single-strand breaks, double-strand breaks, DNA-protein crosslinks, DNA interstrand crosslinks and alkali-labile sites. The methods have the sensitivity, precision and convenience required for meaningful studies of drug mechanisms in cells. During the past year, a detailed study of the DNA intercalating agent, m-AMSA, was carried out. Our Laboratory had previously discovered that other intercalating agents (e.g. adriamycin and ellipticine) produce in mammalian cells a specific DNA effect, consisting of single-strand breaks associated with an equal number of DNA-protein crosslinks. m-AMSA was well suited for a detailed study of this phenomenon, because large numbers of the specific DNA lesions could be produced with relatively little cytotoxicity, and the lesions rapidly reached a steady state and were rapidly reversible. Studies of kinetics and temperature dependence were in accord with an enzymatic origin of the DNA lesions. Two different enzymes may be involved, one producing single-strand breaks and the other double-strand breaks. The ratio of double-strand to single-strand breaks differed for different agents, increasing in the order X-ray < m-AMSA < adriamycin. Studies in subcellular system are in progress to determine whether enzymes of the topoisomerase type are responsible for these effects. Studies are also in progress to determine whether there is a relationship to DNA repair. Future work will aim at the question of whether the intercalator-induced protein-associated DNA breaks are associated with cell killing or whether they help cell survival. This work may define factors that determine susceptibility of tumor cells to intercalating agents.

Our previous work had shown that a particular DNA repair defect affecting removal of O^6-alkyl-guanine lesions confers sensitivity of cells to chloroethylnitrosoureas. Some human tumor cells have this defect, whereas others do not. Repair-defective cells (designated Mer^- phenotype) treated with chloroethylnitrosoureas, produce increased amounts of interstrand crosslinks. This repair defect was found to be specific for nitrosoureas and did not govern interstrand crosslinking by other classes of drugs, such as cis-platinum or nitrogen mustards. The implication is that the susceptibility of a given strain of tumor cells to a particular type of DNA-damaging drug may depend on the presence of a particular DNA repair defect. Attempts are planned to identify other DNA repair deficiencies in human tumor cell strains, and, if such are found, to identify DNA-damaging drugs that could take advantage of this vulnerability.

Nitrosourea derivatives differing in alkylation and carbamoylation mechanisms are being studied in order to determine how these reaction mechanism differences

affect the production and repair of DNA lesions in different cell types.

Studies have continued on the significance of DNA interstrand crosslinking on the sensitivity of cells to cis-platinum. A pair of sensitive and resistant mouse leukemia L1210 cells were studied in detail. The results were interpreted in terms of a kinetic model suggesting that the resistant cells contain increased amounts of a substance, probably a thiol, that inactivate platinum-DNA monoadducts and prevent them from forming interstrand crosslinks.

A fluorometric method was devised to permit DNA alkaline elution assays without the use of radioactive DNA labeling. With the use of these techniques, it was possible to show that, contrary to reports from other laboratories, the chemically induced in vitro differentiation of erythroid or myeloid leukemia cells is not accompanied by the production of DNA strand breaks.

In the nuclear protein area, we are studying the structure and function of histones and their potential role in chemotherapeutic mechanisms. New methods, described in the previous Annual Report, were developed to facilitate these studies. The methods include discontinuous electrophoretic separation of histones and peptide analyses on polyacrylamide gels. In the course of this work, previously unknown histone variants were discovered which were the subject of work carried out during the current year.

The new histone variants, designated H2A.X and H2A.Z, were characterized in terms of peptides which they contain in common with the predominant H2A species. Improvements in the separation techniques for phosphorylated and acetylated histones were devised and used to determine the types of modifications which the new histone species undergo.

Another finding of importance is that the amino acid sequence of H2A.Z is highly conserved in phylogenically diverse organsims. This applies to the non-H2A-like part of the sequence, which is even more highly conserved than H2A itself. This indicates that H2A.Z has a highly specific function.

A major recent discovery is that, contrary to the common histone species, whose synthesis is restricted to the DNA synthesis phase of the cell cycle, the new variants H2A.X and H2A.Z are synthesized at other phases of the cell cycle. This basal synthesis of histones also include the variant H3.3 and the normal H2B and H4 species, but the main H2A species are not made. Inhibition of DNA synthesis with hydroxyurea inhibited the synthesis of the common H2A species while the synthesis of H2A.X and H2A.Z continued. We plan to study the S phase specific drugs, methotrexate and 5-fluorouracil, in this system, with the question in mind why these DNA synthesis inhibitors are effective antitumor agents, whereas hydroxyurea has only small effects.

Histone H2A was known to become covalently linked to the protein, ubiquitin. The newly developed methods facilitated studies of this phenomenon. It was found that, not only the main H2A species, but also H2A.X, H2A.Z and H2B become ubiquitinated. The peptide separation gels made it possible to resolve the synthesis and turn-over of the ubiquitin and histone portions of the linked proteins. It was found that inhibition of DNA synthesis blocks the synthesis of the main H2A species but does not block the incorporation of newly synthesized ubiquitin into linkage with preexisting H2A molecules. A current project is determining the ubiquitin-histone turnover cycle as a function of cell cycle.

An area of investigation planned is to study the behavior of histone species during drug-induced DNA damage and repair. Studies of the behavior of certain non-histone proteins will also be undertaken.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06112-11 LMPH

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Chemotherapeutic Agents on DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.8	5.6	3.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of DNA-reactive drugs on DNA in mammalian cells is being characterized and related to cytotoxic mechanisms. Alkaline elution and related filter methods are being utilized to measure DNA single-strand breaks, DNA double-strand breaks, DNA interstrand crosslinks, DNA-protein crosslinks, and alkali-labile sites. The DNA intercalating agents, m-AMSA and adriamycin, produced protein-associated DNA single-strand and double-strand breaks, probably by a topoisomerase mechanism. The DNA effects of m-AMSA, but not adriamycin, were rapidly reversible.

The fluoro analog of CCNU produced DNA crosslinking effects and differential cytotoxicity dependent on O^6meG repair capacity in human cells. The effects were similar to those of CCNU except that interstrand crosslinking was much more delayed. The effect of carbamoylation on differential cytotoxicity was studied in hydroxy-CCNU derivatives. Resistance to cis-Pt(II) in a line of L1210 cells was attributed to quenching of Pt-DNA monoadducts, probably due to increased cell thiol content, thereby preventing DNA crosslink formation.

Project Description:

Objectives:

(1) Develop sensitive methods for measurement of DNA lesions in mammalian cells treated with pharmacologically reasonable doses of DNA damaging agents.

(2) Determine the types of DNA lesions that are produced by various agents and the kinetics of lesion formation and removal. Examine interference with lesion removal by agents which may be repair inhibitors.

(3) Search for relationships between DNA lesions and cytotoxicity by comparing the effects of various agents on various cell types.

(4) Follow up mechanistic leads that may emerge.

(5) Provide conceptual and experimental foundations for possible applications to:

a) development of improved chemotherapeutic agents or strategems

b) development of predictive drug sensitivity testing of individual tumors

c) development of methods for carcinogen screening

Methods Employed:

(1) Cell culture of various rodent and human cell lines--treatment with chemical agents in vitro; radioactive labeling of macromolecules.

(2) DNA damage analysis by filter elution methods developed in this laboratory.

(3) X-irradiation of cell cultures.

(4) Preparative ultracentrifugation.

(5) Measurement of DNA single-strand length distributions by alkaline sucrose gradient sedimentation.

(6) Agarose gel electrophoresis of DNA

Major findings:

1) DNA Intercalating Agents.

a) Background

Previous work in this laboratory has shown that the intercalating agents, ellipticine and adriamycin, produce a previously unrecognized type of DNA lesion in mammalian cells, characterized by strand breaks associated with

an approximately equal number of DNA-protein crosslinks. The protein was hypothesized to be a nuclease such as a topoisomerase, which in response to the drug cuts the DNA and becomes covalently linked to a strand terminus. During the current year, we have studied several aspects of this phenomenon, particularly in relation to the DNA-intercalating drugs, m-AMSA and adriamycin. Mouse leukemia L1210 cells used for most of these studies.

b) DNA Single-strand Breaks (SSB)

SSB's were measured by DNA alkaline elution, a filter method developed in this Laboratory. DNA-protein crosslinks are removed in this assay by treatment with proteinase-K.

m-AMSA produced much higher frequencies of SSB's than were achievable with adriamycin. Since large numbers of SSB's could be obtained with relatively low cytotoxicity, the system was suited to a detailed study of these DNA effects. The SSB's were of the characteristic type for intercalating agents in that they were observable only if proteinase-K was used to remove DNA-protein crosslinks; the DNA-linked protein otherwise adsorbs to the filters and prevents the alkaline elution of the DNA.

The biologically inactive isomer, o-AMSA, produced no SSB's up to concentrations 100 times greater than the effective concentrations of m-AMSA. At yet higher concentrations, o-AMSA produced SSB's that appeared to be due to disintegration of cells and that were not associated with DNA-protein crosslinks.

With increasing concentrations of m-AMSA, SSB frequency saturated at a maximum of approximately 60,000 per cell. This was not due to limited drug uptake, since m-AMSA uptake was strictly proportional to concentrations. This points to the existence of a limiting quantity of an enzyme or other substance that is required for SSB production.

Adriamycin produced SSB frequencies in proportion to concentrations, but the maximum frequency attained was only 20% of that attained with m-AMSA, even though at the highest concentrations used, adriamycin killed 6 logs of cell whereas m-AMSA killed only 2-3 logs (colony formation measurement).

c) Kinetics of SSB Formation and Resealing

m-AMSA and adriamycin differed greatly from each other in the kinetics of SSB formation and reversal. The difference in reversal rate accounts in large part for the large cytotoxicity difference between the 2 drugs.

m-AMSA-induced SSB's appear rapidly and reach a concentration-dependent plateau within 10 min. After washing the cells to remove the drug, the SSB's rapidly disappear (half-time 5-15 min at 37°). In contrast to m-AMSA, adriamycin produced a linear increase in SSB's with time, and reversal after cell washing was very slow (half-time about 24 hr).

Further evidence for an enzymatic process was obtained from the temperature-dependence of the formation and disappearance rates of m-AMSA-induced SSB's. At 4° C, the formation or resealing of SSB's was completely blocked, while

entry and exit of drug occurred to the same extent (although at slower rates) as at 37°. Subsequent warming of the cells then gave rapid SSB formation of resealing. The formation of SSB's by adriamycin also was highly temperature dependent, as SSB formation was almost stopped at 25°.

The strong temperature dependance indicates that the intercalator-induced SSB's have an enzymatic origin.

d) Equivalence Between SSB's and DNA-protein Crosslinks (DPC)

m-AMSA provided a further test of the generality of the equivalence between intercalator-induced SSB's and DPC's, which was previously observed to hold for adriamycin and ellipticine.

DPC frequencies were measured by means of the filter technique and computation method previous developed in this Laboratory.

Repeated determinations over a wide range of m-AMSA concentrations showed that SSB and DPC frequencies were always well within a factor of 2 of equivalence. The mean value for the SSB/DPC ratio was approximately 1.2; the small deviation from equivalence could be due to a less that 100% efficiency of protein trapping by the filters.

A kinetic experiment showed that DPC's appeared and disappeared in parallel with SSB's following treatment with m-AMSA.

The results support the idea that there is a 1:1 association between intercalator induced SSB's and DPC's.

e) Does the DNA-linked Protein Bridge the Strand Break?

Intercalator-induced SSB's are not observed in alkaline elution experiments unless a proteolytic enzyme is employed. One possibility would be that a protein bridges across a strand break and holds the ends of the break together. This possibility was excluded by alkaline sucrose sedimentation measurements which showed the presence of SSB's at approximately the frequencies indicated by the proteinase-alkaline-elution assays. Control experiments verified that, under the same solvent conditions, and without proteinase, m-AMSA-induced SSB's are detected by alkaline sedimentation but not by alkaline elution.

These findings support the previously proposed model that a protein is linked to one terminus (3' or 5') of the strand break.

f) Double-strand Breaks (DSB)

A DNA elution procedure for DSB measurement was previously devised in this Laboratory. The elution is carried out using a non-denaturing solvent. The assay was characterized and calibrated using X-irradiated cells as standards. The assay was found to have more complex properties than the alkaline elution assay in that the elution kinetics are not first-order and are dependent on cell number. Furthermore, it was not possible to use internal standards in the manner employed in the alkaline elution assay, because DNA strands of different

lengths did not always behave independently. Thus, unlike alkaline elution, the presence of unirradiated cells in this assay affects the elution rate of DNA from irradiated cells. These difficulties were managed by carrying out replicate experiments without the use of internal standards, and by establishing an X-ray calibration curve fit to a second-order polynomial.

Both m-AMSA and adriamycin were found to produce DSB's. The DSB/SSB ratios however were quite different for different agents, and were in the order adriamycin > m-AMSA > X-ray. Like SSB's, m-AMSA-induced DSB's appeared and disappeared rapidly in L1210 cells. The difference in DSB/SSB ratio between adriamycin and m-AMSA suggests that 2 different enzymes may be involved. This is consistent with the known distinct topoisomerases that produce SSB's or DSB's.

g) Subcellular Systems

In order to study the enzyme system presumed to be responsible for the DNA breaks produced in cells by m-AMSA, studies were carried out using subcellular systems. The soluble part of the cytoplasm was removed by means of non-ionic detergent, leaving the cell nuclei and some structural components of cytoplasm. This system was found to respond to m-AMSA by the formation of SSB's and DPC's in nearly equal numbers, and these DNA lesions disappeared after drug removal. In these respects, the effects were similar to those produced in intact cells. The number of SSB's and DPC's however was less than the number produced in intact cells, suggesting that some of the enzyme, or some other essential component, was washed out by the detergent, or that the solvent conditions were sub-optimal. This system will be used to determine the optimum conditions (e.g. solvent ions, pH, cofactors) for the break formation and resealing steps.

h) Enzyme Fractionation Attempts

In order to fractionate the presumed enzyme responsible for the intercalator breaks, an assay was required. It was found that isolated nuclei, deposited on a filter, responded to addition of intercalator by forming SSB's and DPC's. The DPC's were considered the more significant factor, because it avoids the problem of distinguishing the effects of non-specific nucleases. However, in order to have an assay, it was necessary to remove the DPC-forming capacity of the nuclei and to be able to restore the activity by adding back an extract of cell nuclei. It was found that this could be accomplished by extracting the nuclei with a suitable salt concentration. The extract which restored activity to nuclei was found to have topoisomerase activities, assayed using closed circular DNA. However the intercalator effect could not be reproduced using closed circular DNA despite many attempts. It may be that the DNA we used does not contain the right sequence, that some other factor present in cell nuclei is required, or that the pertinent enzyme is not the common topoisomerase of these cells. We are currently proceeding with attempts to fractionate an enzyme using salt-extracted nuclei as the assay system.

2) Nitrosoureas

a) Background

As detailed in the previous Annual Report, we have found that human tumor cell

lines that are deficient in the ability to repair DNA O^6-meG (designated Mer^- phenotype) have an enhanced response to chloroethylnitrosoureas. Such cells exhibit enhanced interstrand crosslink formation, as measured by alkaline elution, as well as enhanced cytotoxicity. We think that these cells are also deficient in the ability to remove certain 2-chloroethyl DNA adducts that have the potential to form delayed interstrand crosslinks and to kill cells.

b) Specificity of the Repair Process

In order to determine whether this DNA repair deficiency is specific for crosslink formation by chloroethylnitrosoureas, we carried out a similar study using cis-$Pt(II)am_2Cl_2$. Cis-Pt(II), like chloroethylnitrosourea, forms interstrand crosslinks in cells in a delayed reaction requiring about 6 hr to reach its peak. Some of the same Mer^+ and Mer^- cell lines were used as in the chloroethylnitrosourea study. A range of cytotoxic sensitivity was observed that correlated with interstrand crosslink formation. There was however no correlation between either of these properties and Mer phenotype. We hypothesize therefore that there are at least 2 distinct DNA repair processes that govern the sensitivity of cells to different DNA crosslinking agents.

c) Fluoroethylcyclohexylnitrosourea (FCNU)

1) Cell Culture Studies

It was previously found in our Laboratory that replacement of Cl in CCNU by F markedly reduces the rate and/or extent of interstrand crosslinking, both in chemical systems and in cells. Since FCNU retains high antitumor activity (see below), it was of interest to compare FCNU and CCNU in Mer^+ and Mer^- cells. The differential toxicity against Mer^+ and Mer^- cells was similar for FCNU and CCNU. Interstrand crosslinks appeared after 48 hr in the Mer^- cells but were undectable in the Mer^+ cells. Mer-dependent interstrand crosslinking therefore applies to FCNU as well as CCNU, but the time-scale for the formation of the crosslinks is greatly prolonged.

2) Antitumor Activity

Because of the marked difference between FCNU and CCNU in interstrand crosslink formation, the antitumor activity of FCNU was compared with CCNU and other commonly used nitrosoureas against a panel of 6 intracranial tumor systems in mice. This work was done at A. D. Little, Inc. under the direction of Dr. R. K. Johnson, under a contract with the Drug Evaluation Branch, DTP, DCT. FCNU was compared with CCNU, BCNU, MeCCNU and PCNU. FCNU was at least as effective as any of the other compounds in over-all activity against these tumors. An analysis of DTP antitumor data by Dr. J. Plowman of the Drug Evaluation Branch also indicated that the overall effectiveness of FCNU in a variety of systems was at least as good as any other nitrosourea.

The distinguishing feature in the action mechanism of FCNU, i.e. greatly delayed interstrand crosslinking, is of a type that has never been explored clinically, and it is not predictable how this difference will affect clinical response. It is however likely to alter clinical response in some way because DNA repair mechanisms will have a much longer time to remove DNA monoaddcuts before these

monoadducts form interstrand crosslinks. Since mouse and man differ in the dominant DNA repair mechanisms utilized by the cells, it is not impossible that an alteration in action mechanism, such as exists in FCNU, could produce an antitumor effectiveness in man comparable to the effectiveness of CCNU in mice.

As a result of these findings and considerations, we have proposed that FCNU, or another fluoroethylnitrosourea, be considered for clinical trial.

3) Neurotoxicity

A complication in this proposal was the observation by Dr. R. K. Johnson of transient neurotoxicity in FCNU-treated mice. We hypothesized that this toxicity arises from the formation of FCH_2CH_2OH as a decomposition product which would be metabolized to fluoroacetate.[-] Comparison with data reported in the literature indicated that the symptoms observed following FCNU are the same as those produced by a stochiometrically equivalent dose of FCH_2CH_2OH. In accord with previous reports, this toxicity was reduced when FCNU was accompanied by administration of sodium acetate and/or ethanol.

d) Hydroxethyl-CCNU Derivatives

The CCNU ring is hydroxylated at several sites due to metabolism in the liver. The presence of a hydroxyl group at the 2' position prevents carbamoylation reactions, whereas 4'-hydroxylation does not. Carbamoylation reactions are not required for antitumor activity of nitrosoureas, nor does carbamoylation by itself produce antitumor activity. However, previous work in this laboratory has shown that carbamoylation inhibits the ligase step in DNA excision repair. It had also been noted in the comparison between a Mer^+ and a Mer^- cell line that the differential toxicity was greater with chlorozotocin and 1-chloroethyl-1-nitrosourea (CNU), which do not inhibit repair, as opposed to BCNU and CCNU which do. This was attributed to differences in carbamoylation. Therefore, a 2'-OH-CCNU and a 4'-OH-CCNU were compared for differential toxicity and DNA crosslinking in a Mer^+/Mer^- pair of cell lines. As anticipated, 2'-OH-CCNU, the non-carbamoylating derivative, exhibited a greater differential than did 4'-OH-CCNU. The 2 derivatives did not differ in crosslinking in the Mer^- cell line.

It appears therefore that carbamoylating nitrosoureas, such as BCNU, CCNU, MeCCNU and PCNU, may be limiting their own effectiveness by inhibiting DNA repair processes that critical normal cells need in order to exhibit favorable differential cytotoxicity. This factor could be more important in human than mouse cells because of the species differences in DNA repair mechanisms. In view of these considerations, a non-carbamoylating hydroxyethyl-CCNU was proposed as a candidate for clinical trial.

3) Cis-Platinum

Previous work, presented in the previous Annual Report, described studies in 3 sensitive/resistant pairs of L1210 lines and showed that DNA interstrand crosslinking differences were generally correlated with sensitivity differences to the drugs, cis-Pt(II) and L-PAM. One pairwise comparison, however, deviated from the correlation. This was the case of a line that was made resistant to

L-PAM and found to be strongly cross-resistant to cis-Pt(II). Interstrand cross-linking by cis-Pt(II) in the resistant line was approximately 50% of that in the parent line, too high to account for magnitude of the resistance.

This problem was pursued in cloned cell lines isolated from the resistant tumor line and from its sensitive parent line. Interstrand crosslinking peaked in the resistant cell line at about 50% of the peak in the sensitive line. The magnitude of the resistance corresponded to a dose-modification factor of 3.3. As in the in vivo experiments, there was more crosslinking than would have been expected in the resistant line. The kinetics of crosslink formation and removal were studied. Crosslink formation peaked at 6-12 hr (after 1-hr exposure to drug) in both cell lines, with the peak being 2X as high in the sensitive than the resistant line. The apparent loss of crosslinks after 12 hr was significantly faster in the resistant than in the sensitive line, even when doses were adjusted to give equal crosslinking peaks. It was however determined that this apparent difference is not due to a difference in crosslink removal rate. Instead, the difference is attributable to increased quenching of Pt-DNA monoadducts in the resistant line. This was shown by the use of thiourea, which we had previously shown to quench Pt-DNA monoadducts so as to stop their progressive conversion to interstrand crosslinks. When Pt-treated cells were treated with thiourea near the time of peak crosslinking, the subsequent rate of crosslink removal was the same in both cell types. We interpret this experiment as reflecting the true rates of crosslink removal. A kinetic model indicated that the differences in peak crosslinking level and apparent crosslink loss can both be accounted for on the basis of increased monoadduct quenching in the resistant cells. Working with the same cell types, Dr. D. Vistica independently concluded that the resistant cells have increased levels of thiols such as glutathione. All of the available data are consistent with the hypothesis that resistance in this case stems from an increase in cell thiols that quench Pt-DNA monoadducts before they can go on to form lethal crosslinks.

4) Fluorometric Adaptation of DNA Alkaline Elution Assay

In order to permit alkaline elution measurements of cells whose DNA cannot readily be labeled, a fluorometric DNA analysis was devised. The fluorometric assay is essentially that of Kissane and Robins, using diaminobenzoic acid. Conditions were found to trap eluted DNA quantitatively on Durapore filters, hydrolyze the DNA off the filters and assay the DNA fluorometrically. Concurrent fluorometric and radioactive assays established the validity of the method.

5) Absence of DNA Strand Breaks in Bone Marrow Progenitor Cells Induced to Differentiate

It has been reported that the differentiation of mouse erythroleukemia cells stimulated by dimethylsulfoxide is accompanied by DNA strand breaks. This has given rise to the idea that DNA breaks play a role in the differentiation process. We studied this question using the fluorometric alkaline elution procedure in order to avoid the potential problem of radiochemical cell damage. We were able to conclude that there is no significant DNA strand breakage in differentiating mouse erythroleukemia cells, nor in differentiating human HL-60 CML cells, at any time during the differentiation process. The reported strand breaks are attributable to a component of dying cells. These results correct a

misconception.

Proposed Course:

A. DNA intercalating agents.

1. Determine relationship between protein-associated SSB's or DSB's and cell survival. Are these DNA lesions associated with cell killing, or are they related to a compensatory process that aids cell survival? In order to answer this question, several intercalators that exhibit rapid formation and reversal of DNA lesions (including m-AMSA, ellipticine and 5-iminodaunorubicin) will be studied in several murine and human cell types having different sensitivities to these drugs. We will determine whether SSB's and DSB's have different cytotoxic significance.

2. Cells will be carried in progressively increasing concentrations of m-AMSA and/or ellipticine in an attempt to develop resistant cell lines having an altered capacity for protein-associated DNA strand break formation.

3. Determine whether intercalator-induced DNA breaks are like those produced by other DNA damaging agents, such as X-ray, in regard to stimulation of poly-ADPR synthesis.

4. Using cell nuclei, characterize the intercalator-induced SSB and DSB forming reactions in terms of optimum condition for reaction, cofactor requirements and effects of inhibitors (especially of known topoisomerase inhibitors).

5. Fractionate cell extracts in order to isolate the intercalator-induced SSB and DSB forming factors.

6. Carry out the phase I-II clinical trials of continuous infusion m-AMSA over a 3 day period. This project has already been initiated in collaboration with the Medicine Branch. The rationale stems from the observation that constant m-AMSA concentrations produce steady-state levels of DNA break that are rapidly reversible.

B. Sensitivities of human tumor cells to DNA damaging agents in relation to DNA repair defects.

1. The correlation between sensitivity to chloroethylnitrosoureas and Mer^- phenotype is being pursued in neuroblastoma cultures obtained from Dr. Paul Kornblith and his coworkers.

2. Studies are planned with melanoma cells to be supplied by Dr. Frank Meyskens and small cell lung carcinoma cultures to be supplied by Dr. John Minna.

C. Studies of DNA crosslinking agents.

The sensitivity differences among human tumor cell lines to various DNA crosslinking agents will continue to be studied with the objective to identify factors that confer susceptibility of certain cell types to DNA crosslinking. Studies with aziridinylquinone (AZQ) are in progress.

D. Studies of alkali-labile sites produced by DNA methylating agents, including methylmethanesulfonate, methylnitrosourea, procarbazine, DTIC and streptozotocin.

The objective is to relate alkali-labile site measurements to DNA repair and cytotoxic susceptibility. This project is being conducted in collaboration with Dr. Donald Slagel of the University of Kentucky.

E. Antibodies to chloroethylated DNA.

Studies of DNA damage and repair in human tumor cells would be greatly facilitated if specific and sensitive immunochemical assays were available for particular DNA lesions. An attempt to prepare monoclonal antibodies to chloroethylnitrosourea-treated DNA will be carried out in collaboration with the laboratory of Dr. C. Harris, Laboratory of Experimental Pathology, DCCP.

Publications:

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16. Kohn, K.W., Erickson, L.C., Laurent, G., Ducore, J., Sharkey, N.A., and Ewig, R.A.G.: DNA crosslinking and origin of sensitivity to chloroethyl-nitrosourea. Bristol Myers Symposium on Nitrosoureas, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06140-05 LMPH

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protein Interactions in Chromosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. M. Bonner	Sr. Investigator	LMPH	NCI
	M. West	Visiting Fellow	LMPH	NCI
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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using methodology developed in our group over the last several years, we have identified two novel minor variants of histone 2A, named H2A.X and H2A.Z. Using peptide gel analysis, we have shown that one of these, H2A.Z, is quite different in primary sequence and in modification from the other H2A's but at the same time H2A.Z is conserved much more than other H2A's during evolution. H2A.X and H2A.Z are synthesized through the cell cycle in mammalian cells while the major variants H2A.1 and H2A.2 are synthesized only in S phase. The identification of these histones has enabled us to separate histone synthesis into S phase histone synthesis and basal histone synthesis which occurs in G1 and G2 as well as in S. Basal histone synthesis and S phase histone synthesis have different sensitives to hydroxyurea; studies with other antitumor agents of the antimetabolite class are in progress. Histone metabolism during DNA repair is also being studied.

Project Description

Objectives:

The overall objectives are to help elucidate the role of chromosomal proteins in the structure and function of chromosomes, and to apply this knowledge to understanding and treating cancer. Specific objectives are to:

(1) Determine the functional and structural relationship of histones, particularly the recently identified H2A.X and H2A.Z, in chromosomes, and their relationships during the cell cycle.

(2) Determine the effect of chromosomal proteins on the action of chemotherapeutic agents.

(3) Identify and characterize interesting non-histone proteins, and study their role in chromosome function.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional electrophoresis. (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 Hela cells and Chinese hamster ovary cells for studies on cell cycle.

Specific Projects:

We have developed several methods for analyzing histones, and other chromosomal proteins with greater simplicity, speed, and resolution than possible before. With these methods we can also purify proteins by successive electrophoresis in gels of different properties without recovering the protein after each gel. A manuscript describing these techniques was published this year by Eur. J. Biochem.

A. H2A Variants

1. Structural Studies

The methodology described above has led us to the discovery of two novel variants of H2A. The variants are separable from H2A on SDS and acid urea gels, but do migrate very close to major components. Since these two H2A variants, named H2A.X and H2A.Z, differ in size from other H2A's, nucleosomes containing these two variants may have altered conformations, and therefore may exhibit special functions in chromatin.

One variant, H2A.X, is similar to H2A.1 in all respects tested except that it seems to be about 1000 daltons larger. Of the 15 H2A.1 tryptic peptides larger

than dipeptides, 10 are found in H2A.X. H2A.X is phosphorylated, acetylated, and ubiquitinated in a manner similar to H2A.1; the phosphorylation tryptic peptide of H2A.X is the same as in H2A.1, but the peptides involved in acetylation and ubiquitination are not the same.

The other variant, H2A.Z, seems to be 500 daltons smaller than H2A.1 and has only two tryptic peptides in common. Like H2A.1, H2A.Z is ubiquitinated, but unlike H2A.1, H2A.Z is not phosphorylated and has 3 acetylation sites. Therefore H2A.Z is unique in that it differs from the other H2A's in certain functional respects.

Another interesting property of these two proteins is that while both are found in all species and tissues tested so far (from human to sea urchin), they are always minor components (2-10% of total H2A). Since the fraction of DNA carrying transcribable information is also only a few percent of the total DNA, it is possible that the presence of H2A.Z and X are restricted to special classes of nucleosomes, perhaps those on transcribable chromatin. West and Bonner (1980) more fully describe the relationship of all the H2A species from mouse L1210 cells.

2. Phylogeny Studies

In collaboration with Prof. David Nishioka at Georgetown, we have found that the sea urchin contains a protein which co-migrates with H2A.Z. Comparison of the tryptic peptides of these two proteins shows that the sequence of H2A.Z is conserved between mouse and sea urchin to a much greater extent than is the sequence of the other H2A's. Another difference is that the synthesis of H2A.Z continues throughout sea urchin development from fertilization at least through gastrulation, while each of the other H2A variants is synthesized for a particular time period only.

The comparative studies of sea urchin and mammals have suggested that H2A.Z is important in a basic cellular process among multicellular organisms.

In a collaborative study with Professor Morton Bradbury, Head of the Department of Biological Chemistry, School of Medicine in Davis, California, we are doing a similar study with an acellular slime mold, Physarum. The preliminary results indicate that Physarum does contain a histone which migrates with or at least very close to mammalian H2A.Z. We hope to be able to compare the fingerprints of these proteins this summer.

3. Cell Cycle Studies

Dividing cells traverse a cycle consisting of mitosis (M), G1, DNA synthesis (S) and G2 in that order. Histone synthesis is in general tightly linked to DNA synthesis. However, when H2A.X and Z synthesis is studied throughout the cell cycle in synchronized CHO cells it is found that they are synthesized in G1 and G2 as well as S phase even though the major H2A variants 1 and 2 are synthesized only in S phase. Further analysis shows that nucleosomal ratios of the four core histones are synthesized throughout the cell cycle, the amount of histone synthesis in G1 and G2 being about 5% of the S phase synthesis. This synthesis which we call basal synthesis includes the Z and X variants of H2A,

the .3 variant of H3, and a fraction of the only H2B and H4 in Chinese hamster ovary cells. Superimposed on the basal synthesis is S phase synthesis which includes the H2A.1 and .2, the H3.1 and .2, and the remainder of the H2B and H4. The basal histones synthesized in G1 are incorporated into chromatin and are as stable as the S phase histones. However tentative results indicate that the time course of the incorporation of basal histones into chromatin seems to differ from that of the S phase histones.

It is important to note that the histone synthesis seen in G1 and G2 cells cannot be attributed to contamination by S phase cells, because the patterns of histone synthesis are qualitatively different. Our ability to recognize this qualitative difference and thereby separate basal and S phase histone synthesis was made possible by our development of the two dimensional gel system for analyzing histones which in turn enabled us to identify and characterize H2A.X and H2A.Z as histones related to H2A.

4. Linkage of Basal and S Phase Histone Synthesis to DNA Synthesis

Many studies have shown that inhibition of DNA synthesis immediately leads to a similar inhibition of histone synthesis even though total protein synthesis is not significantly inhibited. Our studies with hydroxyurea, a classical inhibitior of DNA synthesis, show that basal and S phase histone synthesis are inhibited to different extents when DNA synthesis is inhibited. While hydroxyurea inhibited the synthesis of the S phase specific variants to less than 10% of their control levels the basal variants still maintained 26% to 34% of their control levels. This approximate threefold difference is also found in other cell types including Hela, a continuous human line, IMR90, a normal human embryonic fibroblast line, BE a human colon tumor line, as well as L1210, a mouse line, and Rueber, a rat hepatoma line. The differential inhibition of basal and S phase histone synthesis could be due to a differential linkage of these two types of synthesis to DNA synthesis or to another effect of the hydroxyurea. Comparing several types of DNA synthesis inhibitors and antitumor antimetabolites could enable us to distinguish between these possibilities. We are presently doing such a study with Dr. Leonard Erickson.

B. Ubiquitinated Histones

Goldknopf et al. (1975) discovered a unique protein complex which they named A_{24}, composed of histone 2A covalently linked to another protein, ubiquitin. Ubiquitin is a protein of highly conserved sequence found in animal cells and bacteria. In vitro, it induces lymphocytes to differentiate and stimulates adenylate cyclase but its function in vivo is unknown.

We have identified ubiquitin adducts not only of the recently discovered H2A.X and H2A.Z but also of H2B. Because of this complexity, we have proposed the prefix "u" to denote a ubiquitinated histone. Therefore A_{24} becomes uH2A.1 and uH2A.2, and the ubiquitin adducts of H2A.X, H2A.Z and H2B become uH2A.X, uH2A.Z and uH2B respectively.

uH2A.1 (A_{24}) is probably not a static entity, but a product of dynamic equilibrium. Dr. Roy Wu, using the methodology described before, is studying the metabolism of ubiquitinated histones during the cell cycle. The ubiquitin

and histone portions of uH2A.1 do seem to turnover independently.

The ubiquitin moiety of uH2A.1 has a half life of approximately 9 hours, while the H2A.1 moiety like other nucleosomal histones has an almost infinite half life. Ubiquitinated histones are absent from metaphase chromosomes.

C. Histone Modification

Histones are modified by acetylation, phosphorylation, methylation, poly-ADP ribosylation, and ubiquitination of various amino acid side chains. Our purpose here is to develop more rigorous procedures for separating and quantitating the various forms in the complex mixtures of modified histones normally present in living cells, and to study whether some insight can be gained as to the functional roles of these modifications. It should be noted that up to now it has only rarely been possible to quantitate various types of histone modifications. Using methods developed in our laboratory, Dr. Pantazis has been analyzing H2A, which is simultaneously acetylated and phosphorylated.

Histone 2A forms three bands on acid urea gels, because of modification by acetates and phosphates. Using our gel methodology these bands can be purified, trypsinized and electrophoresed on peptide gels. The new peptides, AcSer(PO_4)-Gly-Arg or GlyLys(Ac)Gln-Gly-Gly-Lys, due to modification can be located, excised and quantitated. For example, we have found that b_1 H2A.1 (first modified band) is mixture of molecules approximately one-third of which are phosphorylated and two-thirds of which are acetylated. b_2 H2A.1 (second modified band) is a mixture of molecules, many of which contain a new peptide which cannot have arisen from any published modification of H2A.

Recently Dr. Pantazis has discovered that altering the ion content of the growth media can lead to almost quantitative phosphorylation of the H2A. This promises to be a useful tool for studying not only how phosphorylation alters chromatin structure and function, but also how the chromatin may react to environmental influences.

Therefore this methodology is useful in several ways. It makes possible quantitative analysis of known histone modification. It is useful for finding new types of modifications. It can be useful for comparing modifications on the same cell type under various conditions.

D. H4 Expression In An SV40-Mouse H4 Gene Hybrid

Dr. Ajit Kumar of the George Washington University Medical School and Dr. Dean Hamer of the Laboratory of Biochemistry, DCBD have constructed a hybrid between SV40 DNA and a mouse H4 gene. They have shown that the H4 region is transcribed using the late SV40 promoter. The question we are concerned with is whether or not the resulting mRNA is translated to give mouse histone 4. Since mouse H4 and monkey H4 (the host for SV40) are identical proteins, the answer must be a quantitative one rather than a qualitative one. Therefore we compared the ratios of the four nucleosomal histones in Green monkey kidney cells infected with wild-type SV40, a hybrid between SV40 and globin DNA, and the SV40-H4 hybrid. When given a 10 minute pulse of ^{14}C-arginine, the first two infections

showed equimolar synthesis of the four nucleosomal histones. The third infection with the SV40-H4 hybrid showed equimolar synthesis of the H3, H2A and H2B with a 3 to 4 fold excess synthesis of H4. These results are a strong indication that H4 is translated from the mRNA transcribed from the SV40-H4 genome.

Significance to Biomedical Research and Program of the Institute:

When a cell is treated with radiation or a chemotherapeutic agent, whether or not that cell survives may be related as much to its ability to repair what ever damage occurs as to its ability to resist damage in the first place. Many chemotherapeutic agents are known to damage either DNA itself or the synthesis of DNA.

Since histones are intimately involved with the cellular DNA to form chromosomes and since histone synthesis is in general tightly linked to DNA synthesis, the severity of both kinds of damage to the cell as well as the cell's ability to repair such damage may involve the histones. However, the methodology was inadequate to investigate these relationships rigorously. We feel that the methodology developed by us over the last few years now allows us to ask significant questions in these areas. Our findings using this methodology, so far are the identification and characterization of novel H2A like histones, the evolutionary conservation of one of these, H2A.Z, and the synthesis of these as well as other histones at a low level throughout the cell cycle - basal histone synthesis as opposed to S phase histone synthesis. These findings already bring important new aspects to studies of the interactions between histones and DNA.

At present we are studying two aspects of histone-DNA interaction that are directly relevant to chemotherapy. The first is based on our finding that hydroxyurea inhibits S phase synthesis much more than basal histone synthesis, although the latter is significantly inhibited. Using this result as a starting point we are collaborating with Dr. Leonard Erickson to compare the differential effects of various antitumor agents, particularly of the antimetabolite class, on basal and S phase histone synthesis. Such a study could lead to some insight into the differential potencies of these compounds.

The second aspect concerns what happens to histones and histone synthesis when the chromatin is damaged by crosslinking antitumor agents, X-rays, or UV light. We feel that our methodology is sufficiently sensitive so that useful insights may be obtained in this area.

Proposed Course:

1. To elucidate the properties of H2A.Z and basal histone synthesis, and their importance in chromosome structure and function.

2. To gain insight into the different degree of linkage of basal and S phase histone synthesis to DNA synthesis as shown by hydroxyurea and antimetabolites.

3. To study the role of histones in DNA and chromatin repair.

4. To continue studies on differences between normal and neoplastic cells at the chromatin level, and how these differences might be useful.

Publications:

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2. West, M.H.P., and Bonner, W.M.: Histone 2A is a heteromorphous family of eight protein species. Biochemistry 19: 3238-3245, 1980.

3. West, M.H.P., and Bonner, W.M.: Histone 2B can be modified by the attachment of ubiquitin. Nucleic Acids Res. 8: 4571-4680, 1980.

4. Filipski, J., Kohn, K.W., and Bonner, W.M.: The nature of inactivating lesions produced by platinum(II) complexes in phage λ DNA. Chem. Biol. Interact. 32: 321-330, 1980.

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ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 - September 30, 1981

The objectives of the Laboratory of Tumor Cell Biology are to develop, implement, and analyze data obtained from studies of cellular proliferation, cell differentiation, and biochemical growth characteristics of normal and malignant mammalian cells both in vivo and in vitro. Particular attention is given to hematopoietic cells, their normal behavior and especially changes seen during leukemogenesis. Because of unusual access to human blood cells and because of the interest of this group, there is special focus on human leukemias and lymphomas. It is anticipated that an enhanced understanding of cell regulatory mechanisms will permit the optimal use of anti-tumor agents in the therapy of cancer and the development of new approaches.

The Laboratory of Tumor Cell Biology is concerned with several biological and biochemical problems: (1) Studies on the cellular and molecular origin and pathogenesis of human leukemia. Biochemical control mechanisms involved in cell differentiation and neoplastic transformation are examined. Tumor viruses of animals are used both as tools (to define and isolate genes and gene products important for growth in man) as well as for help in understanding mechanisms of naturally occurring animal leukemias. Also, studies designed to determine if humans have similar viruses (retroviruses) are and have been intensively studied. (2) Studies on the biochemical events preceding mitosis. An understanding of these events appears essential to the control of proliferation, information derived from such studies may lead to more effective inhibitors or neoplastic cell growth. Events leading to mitosis are also of interest since many of the effective antitumor agents are useful only when cells are in DNA replication or in mitosis. Phytohemagglutinin stimulated human lymphocytes and tissue culture cells are the principal tools in these studies. (3) Attempts to develop new approaches to cancer chemotherapy using information gained from basic cellular studies. (4) Studies on the development of biochemical nd immunological markers for malignant cells are carried out. Biochemical and immunological studies are also conducted in individuals with disorders associated with an increased incidence of neoplasia. (5) Controls regulating cellular growth and differentiation, and the process of malignant transformation in hematopoietic cells. (6) Growth factors (and their receptors) that control the growth and differentiation of blood cells have been isolated and are under intensive study, e.g., T-cell growth factor (TCGF), CSF, and related hematopoietic growth effecting molecules.

During the past year a number of findings were reported by investigators from the Laboratory;

Major Findings:

1. Two new human virus isolates called human T cell lymphoma (leukemia) virus strains CR and MB ($HTLV_{CR}$, $HTLV_{MB}$) have been extensively characterized. They are not significantly related to any known animal retrovirus. These are the first unambiguous human retrovirus isolates.

2. Infectivity of the newly isolated retrovirus from a patient with cutaneous T cell lymphoma (HTLV) was confirmed by in vitro transmission of the virus to T cells of some normal relatives of a patient with acute lymphocytic leukemia.

3. Primate retroviruses and HTLV have been shown to induce a new antigen (human activated antigen or HAA). This antigen may be related to T cell growth factor (TCGF) receptor.

4. Solid phase radioimmunoassay has been developed to detect natural antibodies to HTLV proteins in sera of patients with T cell malignancies. This is the first evidence of a specific immune response in humans to a retrovirus.

5. Serological studies also show the presence of natural antibodies to internal proteins of HTLV in sera of patients with cutaneous T cell leukemias and lymphoma. These studies suggest that HTLV is an acquired virus.

6. A monoclonal antibody against p19 isolated from HTLV has been produced. This antibody is being used to check HTLV expression in various malignant cells, by immunofluorescence.

7. A persistant high titre antibody to HTLV p24 has been detected in a juvenile gibbon, eleven months after single inoculation of HTLV.

8. Nucleic acid hybridization studies show that HTLV is not an endogenous retrovirus of humans.

9. Binding and penetration of HTLV is specific for T cells and not for B cells.

10. T cell growth factor (TCGF) has been purified to near homogeneity from PHA stimulated lymphocyte conditioned media.

11. T cell growth factor was isolated from the surface of cells from patients with T cell leukemias. This growth factor has been shown to be different from TCGF obtained by lectin stimulation of normal peripheral blood lymphocytes.

12. In vitro translation product of mRNA isolated from TCGF producing cells has been shown to be biologically active.

13. Retinoic acid has been shown to induce terminal differentiation of myeloid cells from patients with acute promyelocytic leukemia.

14. Methods to stimulate erythyroid differentiation in liquid culture system have been developed.

15. Hamster spleen conditioned medium has been shown to promote the growth of human bone marrow cells.

16. Reverse transcriptase negative, virus like particles have been detected in the culture medium of a human B cell clone obtained by exposure of human lymphocytes to simian sarcoma virus. These virus particles contain antigenically functional gp70, p30 and p12 in the form of polyprotein precursors.

17. Virus like particles have been obtained from cell free supernatants of cultured embryonal carcinoma cells. These particles contain a DNA polymerase activity similar to mammalian type C RNA tumor virus reverse transcriptase.

18. The presence of a common antigen shared by human myeloid leukemias and the HL60 cell line has been demonstrated.

19. ELISA (enzyme linked immune serum assay) technique has been developed and applied to the analysis of sera from feline virus negative cats and human leukemia patients for the presence of oncornvirus related antigens and antibodies.

20. Induction of a histone polypeptide (HP) has been observed in acute promyelocytic cells (HL60) after differentiation with dimethyl sulfoxide (DMSO). It can be potentially useful as a marker of differentiation.

21. The histone polypeptide (HP) has been found to be specific for human acute leukemias. HP was not detected in leukocytes from patients with chronic leukemias.

22. Ultraviolet radiation has been shown to selectively inactivate DNA polymerase γ. This method is useful in distinguishing DNA polymerase γ from oncornavirus reverse transcriptase.

23. Affinity chromatography on Cibacron blue has been used to achieve purification of DNA polymerases in a single step.

24. Molecular cloning and analysis of the first human 'onc' genes, i.e., the human cellular homolgue of the sarc gene of simian (v-sis) and feline (v-fes) sarcoma virus has been accomplished.

25. Molecular cloning of the genomes of simian sarcoma virus and the associated helper virus has been accomplished.

26. Transformation specific sequences (v-sis) of simian sarcoma virus as a distinct viral onc gene have been identified.

27. Technology for the sequencing of DNA has been developed. Partial sequence of the env gene and onc gene region of simian sarcoma virus has been determined.

28. Restriction maps of different strains of gibbon ape leukemia viruses have been obtained for cloning.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06117-09 LTCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells and Origin and Pathogenesis of the Leukemias and Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Theodore Breitman	Chemist	LTCB NCI
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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION Sections on Hematopoietic Cellular Control Mechanisms, Hematopoietic Cell Biochemistry and Immunology, and Molecular Genetics of Hematopoietic Cells.

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
40	24	16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for and cloning of viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation in vitro. (This relates to a major interest of the Laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (5) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Projection Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia. Special focus is on the leukmias and lymphomas.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes;

   a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

   c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

2. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization, restriction enzyme analysis and gene cloning.

3. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells were recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

5. Markers: (a) Immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells are being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

6. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus related markers and other biological markers.

7. Techniques are being developed to use monoclonal antibodies, prepared against cell surface antigens for subtyping and separation of peripheral blood and bone marrow cells with the help of a fluorescence activated cell sorter.

8. Antibodies associated with the membranes of human leukemic cells have been isolated. They are under study as to which protein antigens they interact with i.e., are they leukemia cell specific, e.g., viral, etc.

9. Recombinant DNA technique is being utilized to obtain molecular DNA clones of defective and non-defective primate viruses. DNA from these clones will be utilized to carry out transfection experiments and for generation of subgenomic fragments for probes and functional analysis.

10. Human T cell growth factor (TCGF) has been purified to almost homogeneity for further characterization. Studies are in progress to determine receptors on activated T cells for TCGF.

Major Findings

1. Two new human virus isolates called human T cell lymphoma (leukemia) virus strains CR and MB ($HTLV_{CR}$, $HTLV_{MB}$) have been extensively characterized. They are not significantly related to any known animal retrovirus. These are the first unambiguous human retrovirus isolates.

2. Infectivity of the newly isolated retrovirus from a patient with cutaneous T cell lymphoma (HTLV) was confirmed by in vitro transmission of the virus to T cells of some normal relatives of a patient with acute lymphocytic leukemia.

3. Primate retroviruses and HTLV have been shown to induce a new antigen (human activated antigen or HAA). This antigen may be related to T cell growth factor (TCGF) receptor.

4. Solid phase radioimmunoassay has been developed to detect natural antibodies to HTLV proteins in sera of patients with T cell malignancies. This is the first evidence of a specific immune response in humans to a retrovirus.

5. Serological studies also show the presence of natural antibodies to internal proteins of HTLV in sera of patients with cutaneous T cell leukemias and lymphoma. These studies suggest that HTLV is an acquired virus.

6. A monoclonal antibody against p19 isolated from HTLV has been produced. This antibody is being used to check HTLV expression in various malignant cells, by immunofluorescence.

7. A persistant high titre antibody to HTLV p24 has been detected in a juvenile gibbon, eleven months after single inoculation of HTLV.

8. Nucleic acid hybridization studies show that HTLV is not an endogenous retrovirus of humans.

9. Binding and penetration of HTLV is specific for T cells and not for B cells.

10. T cell growth factor (TCGF) has been purified to near homogeneity from PHA stimulated lymphocyte conditioned media.

11. T cell growth factor was isolated from the surface of cells from patients with T cell leukemias. This growth factor has been shown to be different from TCGF obtained by lectin stimulation of normal peripheral blood lymphocytes.

12. In vitro translation product of mRNA isolated from TCGF producing cells has been shown to be biologically active.

13. Retinoic acid has been shown to induce terminal differentiation of myeloid cells from patients with acute promyelocytic leukemia.

14. Methods to stimulate erythyroid differentiation in liquid culture system have been developed.

15. Hamster spleen conditioned medium has been shown to promote the growth of human bone marrow cells.

16. Reverse transcriptase negative, virus like particles have been detected in the culture medium of a human B cell clone obtained by exposure of human lymphocytes to simian sarcoma virus. These virus particles contain antigenically functional gp70, p30 and p12 in the form of polyprotein precursors.

17. Virus like particles have been obtained from cell free supernatants of cultured embryonal carcinoma cells. These particles contain a DNA polymerase activity similar to mammalian type C RNA tumor virus reverse transcriptase.

18. The presence of a common antigen shared by human myeloid leukemias and the HL60 cell line has been demonstrated.

19. ELISA (enzyme linked immune serum assay) technique has been developed and applied to the analysis of sera from feline virus negative cats and human leukemia patients for the presence of oncornvirus related antigens and antibodies.

20. Induction of a histone polypeptide (HP) has been observed in acute promyelocytic cells (HL60) after differentiation with dimethyl sulfoxide (DMSO). It can be potentially useful as a marker of differentiation.

21. The histone polypeptide (HP) has been found to be specific for human acute leukemias. HP was not detected in leukocytes from patients with chronic leukemias.

22. Ultraviolet radiation has been shown to selectively inactivate DNA polymerase γ. This method is useful in distinguishing DNA polymerase γ from oncornavirus reverse transcriptase.

23. Affinity chromatography on Cibacron blue has been used to achieve purification of DNA polymerases in a single step.

24. Molecular cloning and analysis of the first human 'onc' genes, i.e., the human cellular homolgue of the sarc gene of simian (v-sis) and feline (v-fes) sarcoma virus has been accomplished.

25. Molecular cloning of the genomes of simian sarcoma virus and the associated helper virus has been accomplished.

26. Transformation specific sequences (v-sis) of simian sarcoma virus as a distinct viral onc gene have been identified.

27. Technology for the sequencing of DNA has been developed. Partial sequence of the env gene and onc gene region of simian sarcoma virus has been determined.

28. Restriction maps of different strains of gibbon ape leukemia viruses have been obtained for cloning.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanism and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect on Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72; Proc. Nat. Acad. Sci. 69: 2879-2884, 1972; Proc. Nat. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976, 479: 198-206, 1977, 564: 235-245, 1979.

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units, focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter techique with RNA covalently attached (Proc. Nat. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (e) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients, free flow electrophoresis and centrifugal elutriation. (Lancet 1: 508-509, 1976).

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 - September 30, 1981

The scope of activities of the Cancer Therapy Evaluation Program (CTEP) has continued to increase. In previous annual reports details were given of the range of activities which included the clinical evaluation of new anticancer agents and the coordination of extramural clinical research programs testing combined modality approaches. The current report will update these areas as well as focus on organizational changes, activities of the Office of the Associate Director, summary of program activities, and highlights.

I. Organizational Changes

Major organizational changes were accomplished within the CTEP during the last year. The total CTEP program was physically consolidated in September, 1980 with the move of the Office of the Associate Director and the Investigational Drug Branch to the Landow Building. Now all CTEP personnel are located on the fourth floor of that building. The CTEP now consists of the Office of the Associate Director (OAD) and four branches: Clinical Investigations Branch (CIB), Investigational Drug Branch (IDB), Radiotherapy Development Branch (RDB), and Biologics Evaluation Branch (BEB). The latter branch was created to serve as the coordinating administration structure for clinical trials in biological response modifiers. Dr. Macdonald is the Acting Branch Chief and Drs. Bruno and Poster have been transferred from IDB to staff the branch. Other changes within the CTEP include the assignment of Dr. Daniel Kisner as Deputy Associate Director, CTEP, the appointment of Dr. Daniel Hoth as Chief, IDB, and the retirements, effective July 1, 1981, of Drs. Vincent Bono and Roger Halterman. The activities of the RDB have expanded significantly since Dr. David Pistenma became Chief in August, 1979. Organizational changes include the addition of Dr. Edward Gilbert, a skilled clinical radiation therapist to the program. The IDB has undergone two organizational changes with Dr. Hoth becoming Branch Chief and Dr. Silvia Marsoni Section Chief of the Drug Evaluation and Reporting Section. Dr. Vincent Bono has been transferred to the OAD as Special Assistant for Information Management.

II. Activities of the Office of the Associate Director

The OAD is responsible for integrating the efforts of all four program branches. This is primarily achieved through participation of the Associate Director (AD) in the branches' activities and through twice-monthly staff meetings and weekly branch chief meetings.

Major reponsibilities and achievements of the OAD have been: A) program supervision and budgetary allocation, B) review and development of therapeutic strategies, C) reorganization of clinical trials, and D) coordination of clinical activities in all international agreements.

Personnel in the OAD are as follows:

John S. Macdonald, M.D.	-	Associate Director, CTEP
Daniel L. Kisner, M.D.	-	Deputy Associate Director, CTEP
Mary Jane Mathews	-	Secretary to AD, CTEP
Judith Uyehara	-	Secretary to Deputy AD, CTEP
William Soper	-	Technical Information Specialist
Elise Mackie	-	Program Analyst
Jan Wright	-	Technical Assistant

A. Program Supervision and Budgetary Allocation

A series of orientation sessions is instituted every July for the staff. In addition, the staff is encouraged to attend similar sessions in the Clinical Oncology Program. These sessions constitute a period of training for new staff of the CTEP. The Office of the AD is also responsible for supervising the management of all program areas in concert with the branch chiefs. Budgetary allocations to each area within the program are worked out by direct coordination with program directors, project officers in each of the branches and the Administrative Officer. These are subsequently discussed with the Director, DCT and decisions are transmitted back to the entire staff of the CTEP.

B. Development and Progress of Specific Therapeutic Strategies

1) The clinical trials being executed under the direction of CTEP through the Biological Response Modifiers Program (BRMP) are being performed using an entirely new support mechanism, the task order contract. This mechanism allows flexibility and speed in response in the initiation of clinical trials since a single group of master contractors are available to quickly compete for BRM clinical trials proposed by DCT staff. The master contracting group contains 25 institutions. Clinical trials including Phase I-II studies of leukocyte and fibroblast interferon, Phase I studies of thymosin fractions 5 and 1, and a Phase I trial of MVE-2, a pyran copolymer were funded in September, 1980 and initiated in FY 81.

2) The Testicular Cancer Intergroup Study is accruing adequate numbers of patients and will add information to address the critical question of the need for adjuvant chemotherapy after surgery in Stage II testicular cancer.

3) Task order contracts were negotiated to perform Phase I chemotherapy studies in pediatric patients. Two drugs are now being evaluated and a third drug will undergo clinical trial starting July, 1981.

4) A new intergroup study to correlate response to chemotherapy in acute myelogenous leukemia with in vitro tests of the biochemical sensitivity of leukemic cells to antineoplastic agents was developed by Dr. H. Preisler of Roswell Park Memorial Institute with the aid of CTEP. This proposal has been funded and is a longitudinal study of the mechanisms by which human leukemic cells become resistant to antineoplastic drugs.

5) After acceptance of the amygdalin IND by the FDA, Phase I trials and Phase II trials of amygdalin have been completed. These studies have critically

evaluated the potential role of laetrile in cancer therapy and have shown the material to be of no value in cancer treatment.

6) The CTEP, after evaluating the results of trials of tetrahydrocannabinol as an antiemetic, petitioned the FDA to permit movement of this drug to the Group C distribution scheme. Over 600 pharmacies have now been registered to distribute THC and over 500,000 capsules have been distributed since November, 1980.

7) Under the direction of Drs. Kisner and Macdonald, a CTEP forward planning program has been initiated. Disease areas are being reviewed by CTEP staff members at "mini-retreats" held by the staff and invited extramural consultants to devise a series of options on what should be the next steps in clinical trials in particular diseases. This should allow CTEP to assist investigators in developing a coordinated approach to treatment research.

8) Drs. Bono and Kisner have developed a project plan for a CTEP data based information management system. This has been approved by the Cancer Treatment Program Staff and will be presented to the June, 1981, Board of Scientific Counselors. This system will allow computer assisted access to all protocol information within the CTEP clinical trials program. The ready availability of updated information will be invaluable to the CTEP, particularly for the forward planning process.

9) An RFA for surgical oncology development planning grants was issued from the Surgery Section of the CIB. The same branch also issued program announcements for R01 and P01 grants in surgical oncology.

C) Organization of Clinical Trials

In October, 1980, Dr. Macdonald presented a proposal for changes in the administration of clinical trials to the Board of Scientific Counselors. This request was prepared in response to the Clinical Trials Review held in 1979. CTEP proposed that all research clinical trials (cooperative group grant and research clinical trials contracts) be transferred to the cooperative agreement funding mechanism. Cooperative agreements are similar to grants in regard to review processes and define carefully the relationship between the cooperative agreement holder and the Government. CTEP also proposed the development of 2-3 regional clinical trials groups to be funded under the cooperative agreement mechanism. These proposals to modify the manner in which clinical trials are performed were accepted by the DCT Board of Scientific Counselors.

D. Representation in International Activities

The OAD is responsible for providing the clinical input for treatment research activities of the DCT including international agreements (Table 1). All protocols are channeled to the CIB for review. NCI-PAHO (Pan American Health Organization) treatment research programs were initiated in 1978 and are coordinated through the AD office. EORTC protocol activities are also similarly reviewed; the AD is additionally on the Protocol Review Committee of the EORTC. Participation in the U.S. - France, U.S. - U.S.S.R., U.S. - Hungary, U.S. - Italy, U.S. - Germany, and U.S. - China agreements took place in 1980-81, with major emphasis on new drug testing. Of particular note is the recent participation of the CTEP in the newly developed U.S. - Italy Agreement. Dr. Macdonald traveled

to Italy in November of 1980, to participate in the initial meeting of this Agreement. Cooperation in the areas of biological response modifiers, clinical chemotherapy trials, drug development, and pediatric oncology was discussed. CTEP is also actively involved in other international activities as outlined in Table 2. These cooperative efforts are not formal international agreements, but are informal collaborative efforts aimed at drug development.

Dr. Macdonald has continued his activities with the UICC. He is one of the four chairpersons for the UICC chemotherapy courses which are given on a world-wide basis and he is also a member of the Organizing Committee of the UICC Clinical Oncology Conference which will be held in Lausanne, Switzerland in October, 1981.

III. Summary of Program Activites

Tricyclic Nucleoside (NSC 280594) will be soon introduced by the IDB into clinical Phase I trial. IND's were filed for Methyl Tetrahydro Homofolate (NSC 139490) and Spirogermanium (NSC 192965) in 1980. There are seven other new drugs in toxicology which should enter clinical trial in the next year.

The CIB protocol review has included all studies in grant-supported cooperative groups (Table 3) and contract-supported studies (Tables 4, 5, and 6). CIB investigators also served as program director initiated R01 grants. Eighty-five grants were funded for $7.0 million. The value for 35 P01 grants is over $22 million. Similar values for the Radiotherapy Development Branch are shown under the Branch's report.

A number of guest workers visited the CTEP to accomplish work on specific projects. These included: Dr. Charles Pruet (one year, beginning August, 1981), Dr. Nikolay Dimitrov (one year, beginning September, 1981), and Dr. Maurice J. Staquet (two months, beginning July, 1981).

Conferences set up by the CTEP are included in Section VIII.

IV. Highlights

A. Organizational

- Dr. Daniel Hoth became Chief of the Investigational Drug Branch.

- Dr. Edward Gilbert was hired as a radiotherapist in the Radiotherapy Development Branch.

- Dr. Daniel Kisner was appointed Deputy Associate Director, CTEP.

- Dr. Richard Ungerleider became Section Chief, Pediatric Section, Clinical Investigations Branch, CTEP.

- Dr. Silvia Marsoni was appointed Section Chief, Drug Evaluation and Reporting Section, Investigational Drug Branch.

- The Biologics Evaluation Branch was established.

- The function of the Program Analyst was transferred from the Clinical Investigations Branch to the Office of the Associate Director.

- The responsibility for the DCT Decision Network Committee was transferred from the Developmental Therapeutics Program to the Cancer Therapy Evaluation Program. Dr. Macdonald serves as Chairman and Dr. Hoth as Vice-Chairman.

B. Scientific Program Highlights

- The GI Tumor Study Group reported results of its study in the adjuvant chemotherapy of gastric cancer. 5-FU + Methyl CCNU was shown to significantly increase the time to recurrence in a study of 165 patients with resected gastric cancer.

- The GI Tumor Study Group reported its rectal cancer study in which it was shown that the combination of postoperative radiation + 5-FU + Methyl CCNU was significantly superior to surgery alone patients with rectal cancer. The recurrence rate in patients receiving combination chemotherapy plus radiation was 21% vs. 52% in patients treated with surgery alone. This controlled randomized study for the first time documented the objective benefit of postoperative therapy in rectal cancer.

- The National Surgical Adjuvant Breast Cancer Program reported the results of its chemo-hormonal therapy adjuvant study. In patients with Stage II breast cancer a combination of phenyalanine mustard, fluorouracil, and tamoxifen was shown to be superior to phenyalanine mustard + fluorouracil alone. This benefit in disease free survival corresponded directly with increasing estrogen receptor protein level measured in the resected tumor tissue. This study has defined a role for an antiestrogen therapy in the adjuvant treatment of breast cancer. The National Surgical Adjuvant Breast Cancer Program has also initiated a new protocol to examine treatment of Stage I (node negative) patients with breast cancer. Patients who are estrogen receptor positive will be randomized to either tamoxifen or no further therapy. Patients who are estrogen receptor negative will be randomized to either methotrexate + fluorouracil or no further therapy. This study is of considerable interest since it utilizes antihormonal therapy as a single agent and also utilizes chemotherapy without an alkylating agent. The latter strategy may decrease long term complications of chemotherapy such as second tumors.

- Biological Response Modifiers Program clinical trials within CTE have initiated studies in 14 institutions. Seven institutions are studying leukocyte, lymphoblastoid and fibroblast interferon in Phase I/II clinical trials; five institutions are studying thymosin, alpha 1, and fraction 5; and two institutions are studying non-specific immunomodulator, MVE-2. These studies are closely coordinated with the Biologic Response Modifier Program and will allow us both to look at clinical results and the effect of these agents on the modification of biologic responses in these patients.

- Two chemoprevention projects have been initiated. Retinoids used topically will be applied in patients at high risk for the development of cervical cancer and oral retinoids will be used in patients at high risk for the

development of skin cancer. These are the first two chemoprevention studies carried out within the Division of Cancer Treatment.

- The first organized pediatric Phase I studies were initiated this year. The Pediatric Task Order Group is currently studying three drugs, indicine-n-oxide, DON, and dihydroxyanthracenedione. This is the first organized funded effort in Phase I evaluation of antineoplastic agents in pediatric age group patients.

- The Group C distribution of THC has been carried out and over 600,000 capsules have been shipped to an excess of 500 pharmacies around the country.

- The amygdalin Phase I and II clinical trials have also been completed with 160 patients on study and amygdalin has been shown to have no benefit in the management of patients with disseminated cancer.

- The Brain Tumor Study Group published (N. Eng. J. Med. 1980; 303: 1323-1329) the results of its prospective randomized trial (BTSG 72-01) testing the efficacy of Methyl CCNU used as a single agent, radiotherapy alone, the combination of BCNU plus radiotherapy and the combination of Methyl CCNU plus radiotherapy in patients with malignant gliomas following maximal surgical resection. Radiotherapy used alone or in combination with a nitrosourea significantly improved survival in comparison with Methyl CCNU alone. The group receiving BCNU plus radiotherapy had the best survival, but the difference in survival between the groups receiving BCNU plus radiotherapy and Methyl CCNU plus radiotherapy was not statistically significant. The combination of BCNU plus radiotherapy produced a modest benefit in long-term (18 month) survival as compared with radiotherapy alone, although the difference between survival curves was not significant at the 0.05 level. The results suggest that radiotherapy plus BCNU is the best treatment at the present time.

- The Brain Tumor Study Group evaluated the possible oncolytic activity of high dose corticosteroids and the effectiveness of Procarbazine in patients with malignant gliomas following maximal tumor resection and radiotherapy. This controlled, prospective, randomized study (BTSG 75-01) demonstrated that corticosteroids did not exhibit an oncolytic effect (no effect on survival), despite the dramatic acute effect that corticosteroids have on brain tumor patients. This information now permits steroids to be used freely in brain tumor protocols without concern about their effect on survival. The toxicity of the high dose corticosteroid schedule used in this protocol was minimal and mild, indicating that the adverse effects of high dose steroids may not be as great as generally believed. Procarbazine was shown to be almost as effective as BCNU in treating patients with malignant gliomas.

TABLE 1

DIVISION OF CANCER TREATMENT

INTERNATIONAL ACTIVITIES

I. BILATERAL AGREEMENTS

Country	Treatment Research Areas
France	Clinical Pharmacology, Phase I/II Trials, GI Cancer
Japan	Drug Development, GI Cancer
U.S.S.R.	New Drugs, Lung, Breast & Ovarian Cancers
U.K.	Drugs Development, Clinical Pharmacology
Poland	Pediatric Oncology
Germany	New Drugs
China	Epidemiology, New Drugs, Clinical Trials
South America (PAHO)	Clinical Trials, New Drugs
Hungary	Drug Development, Immunology
Italy	Medical Oncology, Drug Development, Pediatric Oncology

TABLE 2

DIVISION OF CANCER TREATMENT

INTERNATIONAL ACTIVITIES

II. MISCELLANEOUS PROGRAMS

Program	Treatment Research Area
NCI - Canada	New Drug Trials
NCI - Cairo University	Bladder Cancer, Head & Neck Cancer
Cancer Chemotherapy Center - Japan	Information on Drug Development and Clinical Trials
Institute Jules Bordet - Belgium	Information on Drug Development

TABLE 3

GRANT-SUPPORTED COOPERATIVE GROUP PROGRAM

Multimodal Multidisease Groups

Cancer and Leukemia Group B (CALGB)
Eastern Cooperative Oncology Group (ECOG)
Southeastern Cancer Study Group (SEG)
Southwest Oncology Study Group (SWOG)
Childrens Cancer Study Group (CCSG)

Multimodal Single Disease Groups

Gynecological Oncology Group (GOG)
National Wilms' Tumor Study Group (NWTSG)
Polycythemia Vera Study Group (PVSG)

Single Modality Group

Radiation Therapy Oncology Group (RTOG)

Single Modality, Single Disease Group

Radiotherapy Hodgkin's Disease Group (RHDG)

Regional Groups

Northern California Oncology Group (NCOG)
North Central Cancer Therapy Group (NCCTG)

Special Activities Groups

Lymphoma Pathology Reference Center (LPRC)
Radiologic Physics Center (RPC)
Cooperative Clinical Coordinating Center (CCCC)
European Organization for Research on Treatment for Cancer (EORTC) Data and Statistical Center

Multimodal Single Disease Group
(partially contract funded)

National Surgical Adjuvant Breast and Colorectal Project (NSABP)

TABLE 4

DIVISION OF CANCER TREATMENT

I. CLINICAL EVALUATION OF NEW DRUGS:

CONTRACT-SUPPORTED ACTIVITIES (1980)

Institution	Principal Investigators	Phase
Georgetown University	P. Woolley, P. Schein*	I, II*
Mayo Clinic	J. Kovach, J. Edmonson, C. Moertel*	I/II, II/III
M.D. Anderson	G. Bodey	I/II, II/III
Memorial Sloan-Kettering Cancer Center	C. Young, J. Burchenal	I/II, II/III
Mount Sinai Cancer Center	T. Ohnuma	I
Sidney Farber Cancer Institute	E. Frei, G. Canellos	I, II/III
University of Kansas	B. Hoogstraten	I
University of Vermont	I. Krakoff	I
University of Wisconsin	H. Davis	I
Wayne State University	L. Baker	I/II, II/III

*Gastrointestinal cancer only

TABLE 5

DIVISION OF CANCER TREATMENT

II. DISEASE STUDY GROUPS
Contract-supported Clinical Trials (1980)

NAME	NO. OF CONTRACT	DISEASE	PHASE
BTSG	11	BRAIN	II/III
GITSG	8	COLON, RECTAL	III
GITSG	6	GASTRIC	II/III
GITSG	4	PANCREAS	II/III
LCSG	7*	LUNG	III
NSABP	1*	COLON, RECTAL	III
HNCSG	8*	HEAD & NECK	III
WHO	1*	MELANOMA	III

*Additional subcontracting institutions not shown.

TABLE 6

DIVISION OF CANCER TREATMENT

III. MISCELLANEOUS STUDY RESOURCES
Other Non-Grant Supported Clinical Trials (1980)

Group or Institution	Principal Investigator	Study Area
Georgetown	P. Schein	GI, Nutrition Research
VASOG	G. Higgins	Adjuvant GI, Lung
NCI-Milan	U. Veronesi G. Bonadonna	Breast Cancer

CLINICAL INVESTIGATIONS BRANCH (CIB)

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7.0 Conferences

The Clinical Investigations Branch (CIB) is responsible for the scientific administration of the national cooperative clinical trials groups (the Cooperative Group Program); for scientific monitoring of the Phase II/III contracts, the disease-oriented contracts, the individual investigator-initiated clinical oncology grant program, the nutrition grant program, the interagency agreements, the surgical oncology grant program, and the Intergroup Testicular study; and for scientific administration of the Program Project grants in clinical cancer treatment.

1.0 Personnel

1. William D. DeWys, M.D - Acting Chief and Head, Nutrition Section
2. Edwin M. Jacobs, M.D. - Associate Chief
3. Raymond B. Weiss, M.D. - Special Assistant to the Associate Director
4. Richard S. Ungerleider, M.D. - Head, Pediatric Section
5. John Y. Killen, Jr., M.D. - Head Medicine Section
6. Bimal C. Ghosh, M.D. - Head, Surgical Oncology Section
7. Roger H. Halterman - Program Director, Program Project Grants
8. Daniel L. Kisner, M.D. - Special Assistant for Nutrition (prior to 2-1-81)
9. Thomas T. Kubota, M.D. - Special Assistant for Nutrition
10. Gary B. Witman, M.D. - Senior Investigator
11. Elise Mackie, M.M. - Program Analyst
12. Brenda Edwards, Ph.D. - Statistical Consultant
13. Barbara Shepherd - Secretary to the Chief and Grants Assistant
14. Elaine Lewis - Secretary to the Associate Chief
15. Jan Kostyk - Secretary to Drs. Killen, Ungerleider and Witman (prior to 3-19-81)
16. Wilma Kline - Secretary to Drs. Ghosh and Kubota
17. Anne Gooding - Secretary to the Nutrition Section (prior to 2-81)
18. Jan Wright - Technical Assistant (Contracts) (Secretary to Dr. Halterman prior to 11-3-80)
19. Margaret Howard - Secretary to Drs. Killen, Ungerleider and Witman (beginning 4-20-81)
20. Mira Milic - Stay-in-School
21. Robert Goldman - Summer Student

Dr. DeWys is responsible for the overall administration of the Branch and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, the Cancer Clinical Investigation Review Committee (CCIRC), and the National Cancer Advisory Board. He also supervises the Project Officers on clinical contracts, and the Program Directors on grants. He is the Project Officer for a contract studying anorexia (section 3.3). He is Chairman of the Clinical Oncology Review Committee (CORC) and Head of the Nutrition Section, CIB.

Dr. Jacobs serves as Associate Chief of the CIB and Program Director for the Clinical Cooperative Group program. He coordinates the program review with the Executive Secretary of the CCIRC, the review body for the Cooperative Group Program. He is administrator for the Group protocols. He is also the Project Officer for the Memorial Hospital Phase II/III contract and serves as study chairman for the National Intergroup Testicular Adjuvant protocol.

Dr. Ungerleider is the Project Officer for the Phase II pediatric task order contracts. He is the CIB pediatric liaison to the Cooperative Groups which are conducting studies of pediatric cancers. He also spends 25% of his time working in the Pediatric Oncology Branch laboratories.

Dr. Killen is Head of the Medicine Section of the CIB. He is Project Officer for the Ovarian Cancer Study contract, and the Gastrointestinal Tumor Study Group contract, the NSABP colorectal and breast cancer contract, the Istituto Nazionale, Milan contract and the Emmes Corporation Statistical contract. Dr. Killen is also Program Director for the R01 Clinical Treatment grants.

Dr. Ghosh is Project Officer for the Head and Neck contracts, and Program Director for the R01 Surgical Oncology grant program. He is CTEP consultant for surgical oncology. He also has been working in the Surgery Branch especially with patients with esophageal cancer.

Dr. Halterman is Program Director for the Program Project grants. He coordinates review of these grants with the Clinical Cancer Program Project Review Committee

Dr. Kisner was on an extended assignment in Brussels, Belgium as CTEP representative to EORTC until February 1981 when he became Deputy Associate Director, CTEP.

Dr. Kubota is Program Director for the R01 Nutrition grant program, and Project Officer for the Nutritional Assessment contracts and the Small Cell TPN contracts.

Dr. Witman is Project Officer for the Mayo Clinic, Wayne State, M.D. Anderson and University of Michigan Phase II/III contracts, the Lung Cancer Study Group contracts, the Cervix Chemoprevention contract, and the Phase II task order contract.

Ms. Mackie undertakes special projects and analyses which are needed for the CIB program. As Executive Secretary for the Clinical Oncology Review Committee (CORC) she is the liaison and coordinator for all contract reviews. In addition, she supervises review of protocols and maintenance of protocol files. She is also Project Officer for the ECTO contract and liaison for the NCI-PAHO contract.

Dr. Edwards is CTEP consultant for statistics and works part-time in the CIB helping with protocol review. Her main affiliation is with the Biometry Branch of the Division of Cancer Treatment (DCT).

Mrs. Shepherd acts as administrative secretary for the Branch and is the grants administration coordinator for the R01 grant program (Clinical Treatment, Nutrition, and Surgery).

Ms. Lewis is secretary to the Associate Chief, and coordinates cooperative group protocol review and grant administration.

Ms. Wright assists the Program Analyst with protocol review and maintenance of program files and administrative work for the CORC.

Ms. Kline and Howard serve as secretaries to senior staff and Ms. Milic and Mr. Goldman assist senior secretaries in the Branch as needed.

2.0 Grant Programs

2.1 Clinical Cooperative Groups

The Cooperative Group Program was initiated by the Cancer Chemotherapy National Service Center to test the new agents from the NCI drug development program (1955-66). The program underwent several administrative changes and most recently has been in the Division of Cancer Treatment (1975 - present) where the major emphasis has been on combined modality approaches to cancer treatment.

The accomplishments of the Cooperative Groups were reviewed by the DCT Board of Scientific Counselors in March 1979. Much of the material presented by the groups has been published (see Hoogstraten et al (Eds): Cancer Research, Impact of the Cooperative Groups. New York, Masson Publishers, 1980.

2.11 Listing of the Cooperative Groups

Multimodality Multidisease Groups

Cancer and Leukemia Group B (CALGB)
Eastern Cooperative Oncology Group (ECOG)
North Central Cancer Treatment Group (NCCTG)
Northern California Oncology Group (NCOG)
Southeastern Cancer Study Group (SEG)
Southwest Oncology Group (SWOG)

Multimodality Groups Devoted to a Major Oncologic Area

Children's Cancer Study Group (CCSG)
Gynecologic Oncology Group (GOG)
Pediatric Oncology Group (POG)

Single Modality Group

Radiation Therapy Oncology Group (RTOG) (see section on RBD)

Single Disease Groups

Intergroup Ewing's Sarcoma Study (IESS)
Intergroup Rhabdomyosarcoma Study (IRS)
National Surgical Adjuvant Breast and Bowel Project (NSABP)
National Wilms' Tumor Study Group (NWTG)
Polycythemia Vera Study Group (PVG)
Radiotherapy Hodgkin's Disease Group (RHDG)

Special Activities Groups

Clinical Coordinating Center for Statistics (CCC)
European Organization for Research on Treatment for Cancer (EORTC)
Operations and Statistical Office
Lymphoma Pathology Reference Center (LPRC)
Radiologic Physics Center (RPC) (see section on RDB)

2.12 Description of the Cooperative Groups

The Cancer and Leukemia Group B (CALGB), founded in 1955, studied primarily hematologic malignancies until the 1970's when it also developed multimodal studies in solid tumors. It has made major contributions in the chemotherapy of breast cancer as well as the leukemias and lymphomas. A new area of interest in psychiatry is unique in the protocols of CALGB.

The Eastern Cooperative Oncology Group (ECOG), founded in the 1950's, developed and remains committed to multimodal solid tumor studies, but has increased studies in the hematologic malignancies. The group has made major contributions in breast and gastrointestinal malignancies in particular.

The North Central Cancer Treatment Group (NCCTG) is recently organized and consists of the Mayo Comprehensive Cancer Center and ten clinics in the North Central region. The objectives of the group are to make the most promising cancer research accessible to patients in their region, and to conduct clinical research of high quality in a community setting.

The Northern California Oncology Group (NCOG) has recently been developing multimodal programs in brain tumors, high LET radiation and radiosensitizer studies, and are participating in the head and neck contract. They represent a regional type cooperative group.

The Southeastern Cancer Study Group (SEG), previously involved in hematology studies, underwent reorganization and have now developed multimodal studies in lung cancer and melanoma, and have major potential in genitourinary cancers. They also have a strong nursing committee.

The Southwest Oncology Group (SWOG) has been involved in multimodal programs. One of the largest groups, with an annual accrual of about 5000 patients, it has the resources to rapidly complete Phase II and III studies. They have made significant contributions in AML, myeloma, lymphoma, and breast cancer.

The Children's Cancer Study Group (CCSG) is a multimodality organization concerned exclusively with pediatric malignancies. They have initiated major Phase II and III studies in hematologic and solid tumors, and have begun to collect information about the long term effects of cancer therapy. They recently decided to give more emphasis to innovative pilot studies (e.g., bone marrow transplantation) among select institutions while maintaining their interest in broad-based studies.

The Gynecologic Oncology Group (GOG) has involved the specialties of gynecology, radiation, medical oncology, and pathology for research in gynecologic cancers. They have done a systematic analysis of Phase II drug activity in several gynecologic malignancies.

The Pediatric Oncology Group (POG) is a newly formed multimodality organization composed of former members of the pediatric divisions of SWOG and CALGB. While continuing to accrue patients on SWOG pediatric protocols, they have initiated several new Phase II and III studies, with a major interest in the classification of childhood leukemias through the use of cell markers.

The Intergroup Ewing's Sarcoma Study (IESS) is conducted by members of the two pediatric cooperative groups, CCSG and POG. Since its inception in 1973 it has accumulated the largest group of patients with Ewing's sarcoma in any study in the world. IESS-1 demonstrated that addition of either adriamycin or bilateral pulmonary irradiation to chemotherapy with VAC improves response and survival of children with nonmetastatic disease. IESS-2 has as its objective improved survival and relapse-free survival rates with the fewest long-term complications.

The Intergroup Rhabdomyosarcoma Study Group (IRS) is composed of members of POG and CCSG. Their first study developed a staging system, demonstrated varied prognoses depending on site, and evaluated the effect of multimodal therapy. Their next study has incorporated special treatment considerations relating to primary site of disease. A third study is currently being devised for initiation within the next 12 months.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is a pioneer multimodality group. In the past, it focused exclusively on primary treatment of breast cancer, but now it is also involved in studies of primary colorectal cancer. Major contributions to our theory and practice of adjuvant chemotherapy have been accomplished by this group. A current major study compares segmental mastectomy with or without radiotherapy to total mastectomy. In 1980-81 they have developed protcols to study adjuvant chemotherapy in Stage I breast cancer.

The National Wilms' Tumor Study Group (NWTG) is an intergroup organization incorporating the pediatric cooperative groups along with several independent investigators. Their third study (NWTS-3) is primarily concerned with refinement of therapy. NWTS-1 and 2 conclusively demonstrated that most children with this tumor can now be expected to survive if they are managed by combined modality therapy from the outset, and that prognosis is closely related to histopathologic findings.

The Polycythemia Vera Study Group (PVSG) has protocols to determine the natural history, course, and optimum therapy of polycythemia vera and currently is funded for followup and final analysis of their primary protocol which has shown an increase in incidence of leukemia in patients treated with chlorambucil as compared to radioactive phosphorous or phlebotomy.

The Radiotherapy Hodgkin's Disease Group (RHDG) has studied whether survival in localized Hodgkin's disease was different when patients received involved fields of radiation, or extended fields. The trial is presently in followup.

The following special activities groups provide support services for groups:

The Clinical Coordinating Center (CCC) is a statistical center located at the Sidney Farber Cancer Institute which provides data processing and statistical services and consultation for ECOG and RTOG. It has made some noteworthy contributions to statistical science in clinical trials.

The Operations and Statistical Office of the EORTC is funded by the DCT.

The Lymphoma Pathlogy Reference Center (LPRC) provides expert review of pathological material for the groups performing therapeutic research in malignant lymphoma.

2.13 Summary of the Cooperative Groups

Phase I and broad Phase II trials comprised a substantial effort in the past, but now this emphasis has changed toward Phase III and combined modality studies with curative intent (adjuvant studies). Change in direction is also indicated by specialties represented by group members. There has been a steady increase in the groups' medical oncolgists and pediatric oncologists over the years, and the large increase in numbers of pathologists, radiotherapists, surgeons, and other physicians in the past three years is impressive and is a direct reflection of the move toward the multidisciplinary clinical research of cancer.

The scientific progress of the groups is reflected in publication of numerous papers and abstracts.

Some specific areas where noteworthy contributions have been made by the cooperative groups include:

1) Improved statistical methods for conducting clinical trials.
2) Definition of prognostic factors in childhood leukemia.
3) Progressively improved therapy in leukemia with improved survival.
4) Intergroup trials in Wilms' tumor widely extending the benefit of combined modality therapy.
5) Combined modality therapy of Ewing's sarcoma, rhabdomyosarcoma, and osteosarcoma with improved survival in these tumors.
6) Delineation of the natural history of polycythemia vera.
7) An understanding of cell kinetics and tumor burden in myeloma.
8) Further refinement of the combined modality and chemotherapy of Hodgkin's disease.
9) Development of testing of combination chemotherapy of non-Hodgkin's lymphoma.
10) Large scale adjuvant trials in operable breast, colon, and rectal cancer which should define the current promise of long-term chemotherapy of micrometastases.

11) Evaluation of adriamycin and daunorubicin in AML with improved therapeutic results.
12) Demonstration of the role of l-asparaginase in ALL treatment.
13) Large scale trials of combined mdality therapy of small cell lung cancer.
14) Conducted studies where exaggerated results of preliminary studies were refuted by carefully-done randomized multi-institutional trials.
15) Initiation of an Intergroup (national) Stage I and II testicular cancer trial.
16) Initiation of an Intergroup Mesothelioma study.

2.2 Program Project Grants (P01)

At the present time there were 27 active program project grants with a total expenditure of $25,615,000. Program project grants provide research support for broadly based programs that blend pre-clinical and clinical activities.

Each grant involves a number of investigators each of whom conducts a research project designed to elucidate one or more aspects of a common goal. These efforts are conducted in an organizae fashion in order to facilitate the interactions of these participating investigators. This approach is designed to acquire knowledge more effectively than would a simple aggregate of research projects operating without organization and thematic integration.

Historically, the program has supported highly successful research projects that have made significant contributions. By bringing together basic and clinical investigators, the program has been able to provide excellent patient care and also explore basic elements in tumor biology.

Although clinical research is the main thrust of all programs, substantial efforts in more basic elements are present. These activities include drug development and pharmacology, cell kinetics, immunobiology, marrow transplantation, histopathology and hematology.

The development of potentially curative strategies based on investigation of the kinetic basis for drug responsiveness of common tumor types has been a high priority. A variety of new methods have been developed and some older techniques have been used in new or different ways to predict kinetic patterns.

The aggressive use of bone marrow transplantation as an adjunct to other forms of treatment continues. Monoclonal antibodies directed against human hemopoietic and immunologic precursors and leukemic cell antigens are being used as research tools. Progress has been made in efforts to define the cellular and clinical characteristics of malignant lymphomas and related leukemias in terms of T and B lymphocyte systems. These efforts have lead to new studies on control mechanisms in lymphoma induction and progression, membrane and cytoplasmic markers, and cell surface receptors and antigens.

In vitro assays using explanted tumr tissue in culture to measure the effectiveness of drugs and other types of treatment methods have been correlated with in vivo activity. Overall, many of these grants are supporting investigative teams which are demonstrating that the whole can be greater than the sum of its parts.

2.3 RO1 Grant Programs

2.31 Clinical Oncology

Description

The thrust of this program is to foster development and evaluation of techniques for treating cancer patients. This includes the use of chemotherapy, radiation therapy, immunotherapy, and surgery alone, or in combination. Improved experimental designs and statistical analyses are integral to the program. The program also supports investigators who are searching for improved methods of supportive care and related work in protected environments and bone marrow transplantation. 72 grants are included in this program.

Accomplishments

A number of clinical trials or preclinical feasibility studies have been completed or are well underway. A few examples are:

1. McCredie et al have studied the role of prophylactic granulocyte transfusion during induction chemotherapy of adult acute leukemia patients and have observed complete remissions in 29 out of 34 (85%) which is an improvement compared with the same chemotherapy but without prophylactic granulocyte transfusions.

2. Several groups of investigators have been studying in vitro assay of sensitivity of tumor cells to chemotherapy agents and have reported significant correlations between in vitro and in vivo (patient response to therapy) results. These techniques will permit more appropriate and effective cancer treatment for individual patients. A number of techniques have resulted in dramatic increases in clonogenicity and the frequency of successful growth of tumors in vitro.

3. D. L. Lamm, et al have demonstrated that intavesical BCG is effective therapy for patients with recurrent superficial carcinoma of the bladder. There was a statistically significant decrease in recurrence rates for treated patients compared to concurrent randomized controls.

4. Dr. Bloomfield and associates at the University of Minnesota have presented preliminary data showing that the presence of glucocorticoid receptors in malignant lymphocytes is correlated with response to steroid therapy.

5. Rowley, et al, have developed a large body of data on chromosomal abnormalities associated with acute leukemia in adults, and have shown correlations between certain specific lesions and prognosis. Furthermore, they have identified certain specific abnormalities of chromosomes 5 and 7 which are associated with leukemia complicating other cancer treatment, and which may well be related to cause.

6. Workers at a number of institutions are studying the use of autologous bone marrow transplantation as a means of circumventing the myelosuppressive toxicity of various chemotherapy agents. In this way much higher doses of drug can be administrered, permitting the exploitation of dose-response effects. Early data indicate favorble results in several otherwise refractory tumor types, for example melanoma, as well as in lymphomas.

7. Preliminary information from Whitemore, et al, indicates that proplylactic retroperitoneal lymph node dissection in Stage I nonseminomatous testicular cancer may not be required. They have also confirmed the frequently cited <15% false negative error in the detection of retoperitoneal lymph node metastases and a <15% false negative rate in the detection of distant metastasis.

8. Dr. Ensiminger has successfully developed an implantable, fully portable pump for the purpose of infusing the hepatic artery of patients with either primary or metastatic hepatic malignancy.

9. M.F. McNeally, et al have initiated a trial of autologous tumor cell vaccine as a novel form of immunotherapy. Preliminary results indicate that such treatment is feasible and well tolerated. Therapeutic trials are being initiated. This investigator has also suggested that different batches of BCG may differ in effectiveness.

10. Mankin and others have documented the role of "limb-sparing" procedures in patients with bone tumors.

11. Using a canine model, E.A. Neuwelt has developed a method for disrupting the blood-brain barrier, permitting increased penetration of drugs into the central nervous system. They have also shown that the CT scan can be used to judge effectiveness of disruption. Early clinical trials are underway.

12. Several investigative teams used lithium as a probe to study regulation of bone marrow proliferation. Lithium has been found to increase the release of colony stimulating activity (CSA) from monocytes. Increased CSA stimulates proliferation of the granulocyte compartment. This may attenuate chemotherapy induced neutropenia and may decrease the risk of infection in cancer patients.

13. Thomas, et al, are developing new and improved methods of granulocyte harvesting. They and others are also studying the use

of these cells in the treatment of infection associated with the leukopenia resulting from therapy of acute leukemia.

14. Antiviral and antitumor effects of interferon are under investigation in several institutions. There is unequivocal evidence of antitumor effect. The frequency and and severity of side effects has been a problem, however.

2.32 Nutrition

A separate grant program was created and an RFA released in September of 1979. Grants may be divided into clinical and preclinical studies into the pathophysiology of cancer and nutrition, nutritional assessment, and nutritional intervention. There are currently 21 active grants.

Accomplishments:

The major accomplishment has been the creation of working liasons between medical subspecialties and research disciplines which have heretofore never collabaorated on common problems. For example, Dr. Duck's study on therapy induced growth disorders in childhood cancer involves the close collaboration between the pediatric oncologist and endocrinologist. Dr. Bernstein's grant in learned food aversion involves close collaboaration between the pyschology and oncology departments. Dr. Long's project in energy metabolism in cancer patients involves close collaboration between a biochemist and a surgeon.

2.33 Surgical Oncology

A grants program in surgical oncology is being established this year to encourage more research in surgical oncology. This program will include traditional R01 and P01 grants and an exploratory studies program (P-20).

2.4 P01 Nutrition Grants

There are two core grants supporting the establishment of Clinical Nutritional Research Units in major cancer centers with collaboration between clinicians and laboratories in geographically related hospitals and universities. A variety of projects are underway including zinc and immunity, nutritional rehabilitation in cancer of the head and neck, and pharmacologic aspects of nutrition.

3.0 Contract Programs

3.1 Medicine Section

3.11 Gastrointestinal Tumor Study Group (GITSG)

The GITSG has shown benefit for adjuvant therapy of rectal and gastric cancers.

This consortium consists of 11 institutions which have contracts for the treatment of gastric, pancreatic and colorectal cancer and a statistical support contract (Emmes Corp.). The six gastric and pancreatic contracts were recently awarded after recompetition. The colorectal contracts are now being recompeted and awards are anticipated for the autumn of 1981. Important events in the past year have included:

1. Closure of the adjuvant rectal cancer study (7175). Interim analysis has demonstrated that the combination of post-operative radiation and chemotherapy results in improved disease-free survival compared to a concurrently randomized surgery-only control group. This is the first randomized study showing efficacy for adjuvant therapy of rectal cancer.

2. Opening of a new adjuvant rectal cancer study (7180), comparing the best arm of the previous study (7175) with a less intense regimen of chemotherapy plus irradiation.

3. Closure of adjuvant gastric study (8174). This important trial showed for the first time that chemotherapy is beneficial for patients with surgically resected gastric cancer.

4. Opening of a new gastric adjuvant trial (designed to assess the efficacy of adriamycin in this setting). The group had previously demonstrated that this drug is active in advanced disease.

5. Closure of a randomized phase II trial in advanced gastric cancer. This trial has identified a new agent, triazinate, as an active drug in gastric cancer.

6. Opening of a new advanced disease trial which will compare the addition of two newly identified agents, cis-platinum and triazinate, to the proven combination of 5-FU plus adriamycin.

7. New protocols are being designed for the therapy of patients with locally unresectable gastric and rectal cancer.

3.12 National Surgical Adjuvant Breast and Bowel Project (NSABP)

The NSABP has shown that post-menopausal women with positive estrogen receptors and involved lymph nodes are benefited by the addition of tamoxifen to L-PAM plus 5-FU.

NSABP Breast Contract

This contract supports the study of adjuvant treatment in breast cancer. Currently, protocols B-04, B-05, B-07 B-08 and B-09 are closed to patient accrual although all patients are still being followed. Protocol B-06 (segmental mastectomy) B-09 and B-10 (C-Parvum) are still open for patient accrual.

Preliminary data from protocol B-09 indicate that Tamoxifen adds to the efficacy of the combination of LPAM plus 5-FU in the subset of postmenopausal women with positive estrogen receptors. New protocols in stage II disease will evaluate the efficacy of adriamycin in the adjuvant setting. New studies have also been launched in stage I breast cancer.

NSABP Colo-rectal Contract

This contract is designed to support randomized controlled studies using adjuvant therapy in treatment of colo-rectal cancer. Present studies include C-01 which randomizes patients having Stage B+C colon cancer between no treatment, chemotherapy, and immunotherapy and R-01 which randomizes patients having Stage B+C rectal cancer between non-treatment, radiotherapy and chemotherapy.

3.13 Phase II-III Drug Evaluation Contracts

This contract is for Phase II-III studies to detect useful therapeutic effects of new drugs alone and in various combinations in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, ovary, endometrium, cervix, head and neck, stomach, pancreas and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. At each of the 4 participating institutions a minimum of 200 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator. During this past fiscal year, the Sidney Farber Cancer Center and the Mayo Clinic have been phased out through recompetition, and the University of Michigan began a contract.

3.14 Lung Cancer Study Group

This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study completed patient accrual in October, 1980 and has been supplanted by a natural history registry (to identify patients with limited disease who are at high risk of relapse.) Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III epidermoid carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated for incompletely resected advanced Stage III disease and in partially resected locally advanced non-small cell cancer. New protocols

accepted for activation by the group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

During this fiscal year, clinical center contracts held by Vanderbilt University and the M.D. Anderson Hospital were phased out during re-competition and a contract with the Illinois Cancer Council was initiated.

3.15 Isitituto Nazionale por le Studio e la Cura dei Tumori

A major effort in breast cancer has been through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received world-wide attention. The Istituto has recently shown an improved overall survival for premonopausal patients treated with CMF. They also recently reported that 12 months of CMF is no more effective than 6 months. A re-analysis of disease-free survival among postmenopausal patients showed a clear advantage for patients receiving an average >75% drug dose compared to those with <75% drug doses.

3.16 WHO Melanoma

This contract provides for data management and statistical operations for the clinical trials of the WHO International Melanoma Group. This group initially demonstrated, in a prospective randomized study, that prophylactic node dissections have no therapeutic value for clinical Stage I malignant melanomas. During the last 5 years the WHO melanoma Group has performed studies to determine: (1) the value of DTIC, BCG or DTIC plus BCG as adjuvant therapy of Stage II melanoma, and (2) the value of DTIC versus DTIC plus BCG versus C. parvum for metastatic melanoma. The last two studies have been performed under an NCI contract which provides support for the Group's Secretariat but no patient care costs. Each study has been closed to further patient accrual and results will be published when suitable followup is available.

In the past year the Group has initiated two new studies: (1) a study to determine whether 4-5 cm margins are necessary in resecting primary melanomas, and (2) a study of intralymphatic BCG for treatment of regional lymphatic disease. The previous adjuvant therapy trial will be replaced by a study of poly A-poly U versus a control group.

3.17 Phase II Gastrointestinal Cancer

This contract is designed to carry out Phase II studies in gastric, pancreatic, and colonic cancer. There are 2 participants. The Georgetown University program is responsible for a systematic investigation of new agents in gastric and pancreatic cancer and the development of new combinations of agents. The Mayo Foundation is

evaluating new drugs placed in clinical trials in patients having advanced gastrointestinal cancer. The Human Clonogenic Stem Cell Assay (HCSCA) is being analyzed in some of the 175 colon cancer patients placed on studies as a prospective means of selecting chemotherapy.

3.18 Ovarian

These contracts (M.D. Anderson, Roswell Park, and Mayo Clinic) as well as that for a pathology reference center (M.D. Anderson) are currently in phase-out status. The protocols (7601 and 7602) and pathology review have been taken over by the Gynecologic Oncology Group.

3.19 Chemoprevention of Cervical Cancer

This contract is supporting a Phase I evaluation of topical retinoids, a vitamin A derivative, applied directly onto the surface of cervical mucosa. After a careful evaluation of toxicities, a Phase III trial will be initiated, which shall attempt to identify the role of vitamin A analogs as chemoprevention agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial shall try to reproduce the finding in laboratory animals that cellular differentiation and maturation can be triggered by vitamin analogs. The clinical trials are ongoing at the University of Arizona and Albert Einstein College of Medicine. Pathologic support is through a Central Pathology Unit at Georgetown University.

3.2 Pediatric Section

Pediatric Phase I-II Task Order

This task order contract is for Phase I and II testing of new agents in pediatric oncology patients. There are 9 master contractors who constitute a pool of investigators to study new agents as selected by CTEP. This contract is currently in its second year, with Phase I studies of the following drugs being conducted: DON (M.D. Anderson Hospital), indicine-n-oxide (Children's Cancer Research Foundation), and AZQ (Childrens Hospital of Los Angeles and Memorial-Sloan Kettering Cancer Center.) During the current year, contracts are to be awarded for the study of metoxantrone, homoharringtonine and a third to-be-selected agent.

3.3 Nutrition Section

The TPN Small Cell Group is in its second year of accrual on a study designed to evaluate the value of total parenteral nutrition as an adjunct to aggressive chemotherapy in a very malignant but treatable tumor. A group of studies in improving means of nutrtional assessment and the study of metabolic features of the cancer patient are in their final year of funding. Contracts will soon be awarded to study

calorimetry in patients with advancing neoplasms and a longitudinal study of nutritional and metabolic parameters in patients with malignant disease.

Small Cell TPN Contracts:

Five institutions are involved in a cooperative effort to determine the value of total parenteral nutrtion as an adjunct to aggressive therapy in anaplastic small cell carcinoma of the lung. With 99 patients entered to date, this is the largest randomized study of the value of aggressive nutritional support. Dietary, metabolic, and pharmacologic questions are also being evluated at individual institutions.

Nutritional Assessment Contracts:

This series of contracts is evaluating methods for the assessment of the of the nutritional status of patients with cancer. These contracts are in their final year of funding. A new contract to study the nutritional and metabolic pertubations in a longitudinal fashion in a homogeneous group of patients with advancing malignancy is currently being competed. A new contract to study calorimetery in patients with cancer is also under competition.

Evaluation of Pharmacologic Agents for the Treatment of Anorexia in the Cancer Patient:

This is a preclinical contract testing agents for antianorectic activity in an animal tumor model. Weakly positive results were observed with cyproheptidine and methysergide but many other agents have given negative results.

3.4 Surgical Section

Head and Neck Contracts Program:

This is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the additon of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy.

The participating institutions are University of South Florida, University of Texas-Galveston, University of Cincinnati, University of Maryland, Memorial Sloan-Kettering Hospital, University of Michigan, Radiation Therapy Oncology Group, Northern California Oncology Group.

This study was activated for patient accrual October 28, 1978. The current status as of April 30, 1981, is 361 patients on the study. The future plans include patient accrual for one more year and then followup for two years.

4.0 Miscellaneous

4.1 Intergroup Testicular Study This is a collaboration

between five cooperative groups and three large institutions having an interest in testicular cancer. The protocol is (1) a randomized controlled study of adjuvant chemotherapy of stage II resectable testicular cancer and (2) a 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 relapses) 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 tying, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Accrual statistics include:

Stage	As of 4-14-80	As of 5-12-81
I	18	79
II	37	100
Total	55	179

At this rate of patient entry the original accrual objectives will be met with two additional years of patient entry. To date 12 relapses have been recorded, 2 in stage I and 10 in stage II. All but one relapse have achieved complete remission on chemotherapy and one patient has died.

4.2 Extramural Clinical Trials Office (ECTO)

This contract provides administrative support to contract-funded clinical research projects: Lung Cancer Study Group, Gastrointestinal Cancer Study Group, Head and Neck Contract Project, and the Intergroup Testicular Study. Services include: assistance in protocol and forms design; patient randomization; quality control of data; coordination of scientific activities of clinical invetigators and statisticians; planning of meetings; preparation of meeting agenda, minutes and other reports; and related administrative tasks. This contract is being recompeted, with award to be made by September 30, 1981.

4.3 Clinical Oncology Review Committee (CORC)

The Clinical Oncology Review Committee (CORC), the DCT technical review committee, reviews proposals for new resource contracts and recompetitions of resource contracts. The CORC performs annual reviews of all

incrementally funded clinical research and resource contracts. During FY'81 there were nine CORC meetings for review of 36 contracts and 45 proposals for 14 contract projects. Dr. DeWys is Chairman; Ms. Mackie is Executive Secretary.

4.4 NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

Since May, 1978 the CCTRP, an outgrowth of the Latin American Cancer Research Information Project, has supported development of clinical cancer research programs in ten Latin American Centers in seven countries and seven U.S. cancer centers--each Latin America center has a specific U.S. collaborating center. There were 28 active protocols and patient accrual of approimately 550 in the past year. Each Latin American center has one or two combined modality studies and additional pilot studies for development of new therapies. Each study is designed by the L.A. investigator and the U.S. co-investigator, reviewed by the Cancer Therapy Evaluation Program staff protocol review group, and conducted either independently by the L.A. centers or in collaboration with the U.S. center. In current clinical studies, a study prospectively comparing the two most commonly used chemotherapeutic regimens (BACOP and C-MOPP) in histiocytic lymphoma is nearing completion. The new antitumor agent, Cis-platinum, has been agressively evaluated in advanced pancreatic and gastric cancer. A multi-center adjuvant osteogenic sarcoma protocol is in the planning stage. The third annual meeting/workshop was held in Washington, D.C. April 24-25, 1981.

4.5 VA Surgical Oncology Group (VASOG)

The VA Surgical Oncology Group is receiving phase-out support via an Interagency Agreement.

4.6 Patient Accrual - 1980

Total patients entered on Phase III, IV (adjuvant) studies: 18,997*
(coop. group: 16,391; contract: 4,629; VASOG: 118; WHO Melanoma: 141
EORTC: 1651)
Total patients entered on Phase I, II, and pilot studies: 9,211*
(coop. group: 6,465; contract: 2,885; EORTC: 1,029)
Total patients on study and in follow-up: 81,242*
(coop. group: 72,113; contract: 8,285; VASOG: 1,854;
WHO Melanoma: 1,144)
*total does not include the WHO Melanoma Group or EORTC Group - these contracts provide funding only for the operations offices and not for patient entry.

Comparing accrual data of 1980 to that of 1979, it is interesting to note that, although the total number of patients entered in 1980 showed a decrease of about 3,000, there was a slight increase in total number of patients on study and in follow-up. This may indicate the increase in duration of survival.

5.0 Staff Publications

1. DeWys, W.D.: Nutritional care of the cancer patient. JAMA 244:374-376, 1980.

2. DeWys, W.D.: Pathophysiology of cancer cachexia. Cancer Res. (in press).

3. DeWys, W.D., Allegra, J.C., Simon, R., Lippman, M.E.: A proposed model for the prediction of response to endocrine therapy in breast cancer from the estrogen receptor status of one site and the number of metastatic sites. Cancer Res. 40:2423-2427, 1980.

4. DeWys, W.D. and Bathina, S.: Synergistic antileukemic effect of theophylline and 1,3-Bis(2-chloroethyl)-1-nitrosourea. Cancer Res. 40:2202-2208, 1980.

5. DeWys, W.D., Begg, C., Lavin, P.T., et al.: Prognostic effect of weight loss prior to chemotherapy in cancer patients. Amer. J. Med. 69:491-497, 1980.

6. DeWys, W., Kisner, D.: Maintaining caloric needs in the cancer patient. Contemp. Surg. 15:25, 1979.

7. DeWys, W.S. and Kubota, T.: Enteral and parenteral nutrition in the care of cancer patients. JAMA (in press).

8. DeWys, W.D., Muggia, F.M., Jacobs, E.M.: Staging of testicular cancer. A proposed clinical-surgical schema. Cancer Treatment Reports 64:669-674, 1980.

9. Donaldson, S.S., Wesley, M.N., DeWys, W.D., Suskind, R.M., Jaffee, N., van Eys, J.: A study of the nutritional status of pediatric cancer patients. Am. J. Dis.Child. (in press).

10. Ghosh, L., Nassauer, J., Faiferman, I., Ghosh, B.C.: Pathology of metastasizing tumors in nitrosomethyl urea-induced rat mammary carcinoma.

11. Ghosh, L., Nassauer, J., Faiferman, I., Ghosh, B.C.: Ultrastructural study of membrane glycocalyx in primary and metastatic human and rat mammary carcinoma. J. Surg. Onc. (in press).

12. Killen, J.Y.: Systemic therapy of colorectal carcinoma. Current Concepts in Oncology (in press).

13. Killen, J.Y., Holyoke, E.D., Mittleman, A., et al.: Adjuvant therapy of adenocarcinoma of the colon following clinically curative resection: an interim report from the Gastrointestinal Tumor Study Group (GITSG). In Jones, S.E. and Salmon, S.E. (Eds.): Adjuvant Therapy of Cancer III. New York, Grune & Stratton, 1981 (in press).

14. Killen, J.Y., Macdonald, J.S.: Investigational new drugs in the treatment of advanced small cell carcinoma. In Greco, F.A. and Bunn, P.A. (Eds.): Small Cell Lung Cancer. New York, Grune & Stratton, 1981 (in press).

15. Kisielius, T., Bianco, A., Merrill, J.M., Taylor, S.G., IV, and DeWys, W..D.: Bleomycin in combination chemotherapy of metastatic Wilms' tumor: a late recurrent case, refractory to conventional therapy. Med. Pediatr. Oncol. 6:11-14, 1979.

16. Kisner, D.L., DeWys, W.: Anorexia and cachexia in malignant disease. In Newell, G.R., Ellison, N.M. (Eds.): Cancer and Nutrition. New York, Raven Press, 1981, pp. 355-365.

17. Macdonald, J.S., Weiss, R.B., Poster, D., Hammershaimb, L.: Subacute and chronic toxicities associated with nitrosourea therapy. In Prestayko, A.W., Crooke, S.T., Baker, L.H., Carter, S.K., and Schein, P.S. (Eds.): Nitrosoureas: Current Status and New Developments (in press).

18. Smith, F.P., Cambareri, R.J., Killen, J.Y., Schein, P.S.: Chemotherapy of gastrointestinal malignancies. In Pinedo, H.M. (Ed.): Cancer Chemotherapy Annual, 1980. Amsterdam, Exerptia Medica, 1980, 284-298.

19. Ungerleider, R.S.: Antitumor activity of chlorozoticin. Cancer Treat. Rev. 7:191, 1980.

20. Ungerleider, R.S.: An introduction to cell markers in leukemia. Cancer Res. (in press).

21. Ungerleider, R.S.: Working conference on neuroblastoma treatment trials. Cancer Treat. Rep. (in press).

22. Weiss, R.B., Bruno, S.: Hypersensitivity reactions to cancer chemothereutic agents. Annals Intern. Med. 94:66-72, 1981.

23. Weiss, R.B., Henney, J.E., DeVita, V.T., Jr.: Multimodal treatment of primary breast carcinoma. Analysis of accomplishments and problem areas. Am. J. Med. 70:844-851, 1981.

24. Weiss, R.B., Poster, D., Penta, J.: The nitrosoureas and pulmonary toxicity. Cancer Treat. Rev. (in press).

25. Witman, G., Cadman, E., Braverman, I.: Cis-platinum treatment in cutaneous T-cell lymphoma. Cancer Treat. Rep. (in press).

26. Witman, G., Cadman, E., Chen, M.: Misuse of scalp hypothermia. Cancer Treat. Rep. (in press).

27. Witman, G., Davis, R.: A lupus erythematosus syndrome induced by clonidine hydrochloride. R.I. Med. J. 64:147-k50, 1981.

6.0 Staff Presentations

William D. DeWys, M.D.

1. Nutritional Problems of the Cancer Patient. Association of Pediatric Oncology Nurses. Philadelphia, Pennsylvania, September 26, 1980.

2. Nutritional Problems of Cancer Patients. Baltimore Cancer Research Program. Baltimore, Maryland, December 5, 1980.

3. Pathophysiology of Cachexia. Pediatric Cancer and Nutrition Workshop. Bethesda, Maryland, December 11-12, 1981.

4. Nutritional Assessment of the Cancer Patient. European Organization for Research on Treatment of Cancer. Brussels, Belgium, January 8-9, 1981.

5. Nutritional Care of Cancer Patients. George Washington University Medical Center Grand Rounds. Washington, D.C., February 19, 1981.

6. The Nutritional Status of Cancer Patients; Food Intake. Nutrition and Cancer Update 1981 Symposium, University of Louisville, Louisville, Kentucky, March 27, 1981.

7. Nutrition and the Cancer Patient. East Carolina Medical School, Greenville, North Carolina. April 16, 1981.

8. Nutrition and the Cancer Patient. Cancer Therapy in the Community Symposium. New Bern, North Carolina, April 17, 1981.

9. Bladder Cancer. Veterans Administration Hospital Grand Rounds. Washington, D.C., June 4, 1981.

10. NCI Extramural Programs. Visitors from the International Congress on Nutrition. Bethesda, Maryland, August 24, 1981.

Edwin M. Jacobs, M.D.

1. Visiting Lecturer in Oncology. University of Hawaii Cancer Center. Honolulu, Hawaii, October 20-24, 1980.

2. History of Clinical Trials. SWOG Nurses Workshop. Albuquerque, New Mexico, March 12, 1981.

3. NCI Administrative Presentation. Pathology Center for Lymphoma Clinical Studies. New York, New York, March 30, 1981.

John Y. Killen, Jr., M.D.

1. Current Status of Adjuvant Therapy for Breast Cancer. Breast Cancer Task Force, Treatment Working Group. Bethesda, Md. December 9, 1980.

2. Chemotherapy of Gastrointestinal Cancer. Swedish Surgical Oncology Society and Swedish Society for Radiation Therapy. Hemavan, Sweden. March 30-April 3, 1981.

3. High Dose Methotrexate is Ineffective as CNS Prophylaxis for Patients with Small Cell Bronchogenic Carcinoma. American Society of Clinical Oncology. Washington, D.C. April 30, 1981.

4. The Investigational Drug Program of the NCI. National Bladder Cancer Project. Hershey, Pa. June 10, 1981.

Gary B. Witman, M.D.

1. The Drug Development System - New Agent Use in Clinical Trials. Investigational Agents Committee - NCI Canada. Toronto, January 22, 1981.

Raymond B. Weiss, M.D.

1. Adjuvant Therapy of Cancer. Sacred Heart Hospital. Cumberland, Maryland, November 10, 1981.

2. Pulmonary Toxicity of Chemotherapeutic Agents. SUNY Upstate Medical Center. Syracuse, New York, March 5, 1981.

3. Diagnosis and Medical Management of Hodgkin's Disease. American Cancer Society. Silver Spring, Maryland, March 11, 1981.

7.0 Conferences

1. Pediatric Cancer and Nutrition Workshop, 12/11-12/80.

2. Neuroblastoma Workshop, 1/14/81.

3. Autologous Bone Marrow Transplantation Workshop, 1/27-28/81.

4. Thyroid Cancer Cooperative Study Group Meeting, 4/22/81

5. Phase II/III Contractors Meeting, 6/24/81.

6. Acute Myelogenous Leukemia Workshop, 7/6-7/81.

7. Surgical Oncology Workshop, 9/9/81.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of sponsoring new investigational drugs for clinical trials and of evaluating them for antitumor efficacy. It does this by pursuing several objectives: (1) obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA), (b) by managing the Phase I/II Working Group, (c) monitoring the clinical trials conducted by these investigators, (d) meeting FDA regulatory affairs responsibilities for all active IND's and, (e) regulating the distribution of investigational new drugs.

The permanent professional staff of the Branch includes one physician, one pharmacologist, one biochemist, and one pharmacist. In addition, there are a staff of three physicians, who have a one or two year appointment with the Branch.

During the past year the recently developed system of drug classification and policies for DCT sponsorship of clinical protocols continued to operate with evident efficiencies. Briefly, drugs are classified as either Group A, B, or C. Group A drugs are those in Phase I clinical trials or in their initial Phase II trials. Protocols are accepted from members of the Phase I/II Working Group, DCT Contractors, or Cooperative Groups. Group B drugs are those which have been evaluated for efficacy in several Phase II trials in pertinent tumors and which have shown in these studies to be tolerated by patients. Protocols are accepted from DCT Contractors, Cooperative Groups, and through the New Drug Studies Mechanism. Group C drugs have reproducible efficacy in specific types of tumors. These drugs are provided to registered physicians for treatment of patients with specific diseases in accord with our "Guideline" protocols. Safety of use is the research objective of this mechanism.

During the past year the following major projects were continuing. The FDA approved the initiation of the Group C THC program which is now in large-scale distribution. This is described is further detail below. A Phase II trial of amygdalin was completed, which demonstrated one responder in 174 patients. The Clinical Trials Monitoring Service initiated its monitoring of Phase I studies during this year and now provides a computerized data base for all NCI Phase I trials.

PHASE I/II WORKING GROUP

Institute	Principal Investigator
Baltimore Cancer Research Program, NCI	P. Wiernik
Children's Hospital of Los Angeles	S. Siegel
Clinical Pharmacology Branch, NCI	B. Chabner
Georgetown University	P. Woolley*
Mayo Clinic	J. Kovach*
M. D. Anderson	G. Bodey*
Medical Oncology Branch, NCI	R. Young
Memorial Sloan-Kettering Cancer Cneter	C. Young*
Mount Sinai Cancer Center	T. Ohnuma*
NCI-VA Medical Oncology Branch	J. Minna
Ohio State University	J. Neidhart
Pediatric Oncology Branch	A. Levine
Roger Williams General Hospital	F. Cummings
Roswell Park Memorial Institute	A. Mittelman
Sidney Farber Cancer Center	E. Frei*
St. Jude's Children's Hospital	C. Pratt
U.C.L.A.	J. Block
University of Kansas	B. Hoogstraten*
University of Miami	C. Vogel
University of Vermont	I. Krakoff*
University of Wisconsin	D. Tormey*
Wayne State University	L. Baker*
Yale University	J. Bertino

*Contract Supported

DRUG EVALUATION AND REPORTING SECTION

Phase I Studies: Three investigational drugs sponsored by the DCT were newly introduced into Phase I clinical trials:

WR-2721	NSC-296921
Methyltetrahomofolic Acid	NSC-139490
Desmethylmisonidazole	NSC-261036

In addition Phase I studies were continued from last year on the following drugs:

m-AMSA 24 hour infusion	NSC-249992
AT-125	NSC-163501
AZQ	NSC-182986
Dihydroxyanthracenedione	NSC-301739
PCNU	NSC-95466
ICRF-187	NSC-169780
DON	NSC-7365
Aclacinomycin	NSC-208734
Thymidine	NSC-21548

Phase I trials exploring the biochemical modulating effects of various drugs were conducted, including:

PALA/5FU	NSC-224131/19893
Thymidine/5FU	NSC-21548/19893
Thymidine/PALA/5FU	NSC-21548/224131/19893

Two new investigational agent IND's were approved at the DN-4 level Phase II

Spirogermanium	NSC-192965
CL 216942 (ADAH)(Orange Crush)	NSC-337766

Drug	Acute Leukemia	Breast	Colon	Lung SC	Lung N-SC	Lymphomas	Melanoma
Spirogermanium	0	0	0	0	0	0	0
CL 216942 (ADAH) (Orange Crush)	+	+	+	+	+	+	+

0 = Not tested

Seven compounds entered the program and were submitted to the Food and Drug Administration during the current calendar year 1980.

The remaining five are:

Ara-A	NSC-404241
Levonantradol	NSC-331615
MeTHHF	NSC-139490
Desmethylmisonidazole	NSC-261036
WR-2721	NSC-296961
Thymosin Fraction 5	NSC-(no number assigned as yet)
Thymosin Alpha 1	NSC-(no number assigned as yet)

One IND, 6 MMPR, NSC 40774, was scheduled to be reopened.

Eleven IND's were discontinued due to low clinical activity/interest:

Acronycine	NSC-403169
Azapicyl	NSC-68626
Azaserine	NSC-742
Camptothecin	NSC-100880
Chromomycin	NSC-58514
Dibromomannitol	MSC-94100
Dichloroallyl lawsone	NSC-126771
IMPY	NSC-51143
Methyl Prednisolone	NSC-19987
Nafoxidine	NSC-70735

Closure was requested for 19 IND's for commercially available drugs. Sixteen completed discontinuation during 1980:

Adriamycin	FUDR
BCNU	Hydroxyurea
Bleomycin	Medroxyprogesterone
Cyclophosphamide	Methotrexate (low dose)
DTIC	Mithramycin
Fluoroxymesterone	Prednisone
Thiotepa	Spironolactone
Vincristine	Testolactone

Closure is still pending for the following three:

Diethylstilbesterol
Ara-C
Mitomycin C

The DRAS also supported IND-related activities of the Biologics Evaluation Branch, CTEP, during 1980. This support includes preparation and maintenance of IND's and drug distribution support and monitoring.

A procedure for the reregistration of investigators was initiated during 1980. As agreed with the FDA, reregistration will be conducted on a regular basis. The process requires the coordination of two contractors and involves more than 3,000 investigators. Due to budgetary restrictions, and with the approval of the DCT Board of Scientific Counselors, distribution of commercially available drugs was discontinued.

Group C Distribution of Tetrahydrocannabinol (THC)

During 1980, the drug delta-9-tetrahydrocannabinol (NSC-134454) was placed under the NCI's Group C mechanism for use as an antiemetic with chemotherapy. Guidelines for THC use and a procedures document for pharmacy registration and implementation of distribution were developed. The approvals of both the Oncology Advisory Committee and the Food and Drug Administration were secured.

Because THC is a Schedule I drug, concurrence on distribution procedures and a Schedule I distribution license were obtained from the Drug Enforcement Administration. The DRAS also interfaced with the DEA to verify physician's DEA registration and modified the existing computer drug distribution system to accommodate the registration numbers.

Distribution of THC as an antiemetic under state and individual investigator studies was transferred to the NCI from the National Institute of Drug Abuse. Procedures were also developed to direct the NIDA's distribution of marijuana cigarettes for use with chemotherapy.

The DRAS coordinated two NCI support service contractors to implement the registration and distribution activities. The existing computer system was modified to allow recording of pharmacy and physician registration and distribution authorizations. Routine procedures were established for investigator registration and pharmacy correspondence, and hard copy records systems were set up for all THC data. THC capsule distribution began in November 1980. By the end of the year, more than 400 hospitals and 1000 physicians had been approved and were participating in the program. More than 500,000 THC capsules had been distributed in the first six months of the program.

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RADIOTHERAPY DEVELOPMENT BRANCH

I. Introduction

The Radiotherapy Development Branch (RDB) is concerned with the basic, applied (pretherapeutic), and clinical development of cancer treatment modalities utilizing ionizing and nonionizing radiations and the investigation of means of enhancing the biological effects of these radiations. The RDB supports the basic and applied pretherapeutic research and development activities by grants or contracts and uses similar funding mechanisms to introduce new or improved conventional radiotherapeutic modalities or techniques into clinical trials.

II. Personnel

1. David A. Pistenma, M.D., Ph.D. - Chief
2. Francis J. Mahoney, Ph.D. - Deputy Chief/Program Director for Radiation
3. Thomas A. Strike, Ph.D. - Project Officer for Brain Tumor Study Group
4. Edward H. Gilbert, M.D. - Program Director for Radiotherapy
5. Ms. Bonnie L. Rabbitt - Secretary to Chief
6. Mrs. Ethel C. Wheeler - Secretary to Deputy Chief
7. Ms. Maureen L. Volz - Statistical Assistant for Brain Tumor Study Group
8. Ms. Irene R. Leonard - Stay-In-School

III. Radiotherapy Development Branch Research Program

The Radiotherapy Development Branch research program is composed of individual investigator grants, program project grants, cancer research emphasis grants, and contracts. The total costs of this program in FY 1980 are shown in Table 1. The total costs for FY 1981 will be very similar.

A. Contracts

A brief summary of contracts and FY 1981 awards are as follows:

1. Phase II Study of Photoradiation Therapy (NO1-CM-97311)

   The contract for Phase II studies is in its final year and will be recompeted hopefully with award of two contracts. The present contract is to Roswell Park Memorial Institute where this modality was pioneered. The purpose of the contract is to determine the scope and limitations of photoradiation therapy as a primary modality or in combination with other modalities. Progress this year has been satisfactory.

2. Radiosensitizers and Radioprotectors

   a. Radiosensitizer Synthesis and Testing (NO1-CM-77139 and NO1-CM-87206)

      Two contracts for the development of improved radiosensitizers are in their final year and will be recompeted. The Institute

for Cancer Research, Sutton, England (NO1-CM-77139) and Stanford University--Stanford Research Institute (NO1-CM-87206) both have developed analogs of the nitroimadazole, misonidazole. These analogs have the potential to improve therapeutic ratios. These compounds are being evaluated by the contractors, by NCI, and by other investigators.

b. Screening of Radiosensitizers (NO1-CM-07257)

A contract for screening compounds for potential radiosensitizing activity was awarded to Arthur D. Little, Inc. The objective of this contract was to identify new hypoxic cell sensitizers or other types or classes of radiosensitizers which act by a different mechanism. Compounds may undergo in vitro and/or in vivo testing after various physical-chemical properties are determined and evaluated.

c. Screening of Radioprotectors (NO1-CM-07330)

A contract for screening compounds for potential radioprotector activity was awarded to the Fox Chase Cancer Center. The objective of this contract was to identify compounds or classes of compounds which increase the therapeutic ratio of radiotherapy more than the reference radioprotector WR-2721 or compounds which protect tissues not protected by the reference compound. Various in vitro and in vivo testing will be utilized in this screen to further evaluate promising compounds.

3. Clinical Neutron Therapy Program

The facility at Fox Chase Cancer Center (NO1-CM-97314) is nearing completion. The gantry and ancillary components of the DT generator have been shipped and are being installed. Shipment of the DT generator tube will be made in May or June 1981 provided the Nuclear Regulatory Commission issues a license therefore. The construction aspects of the contracts at the University of Washington (NO1-CM-97282) and the University of California, Los Angeles (NO1-CM-97315) will be awarded in late FY 1981 or early FY 1982. The University of Washington has subcontracted with Scanditronix to procure a cyclotron whereas the University of California, Los Angeles, has contracted with The Cyclotron Corporation for a cyclotron. Both cyclotrons are scheduled for delivery in late calendar year 1982.

B. Clinical Research Activities

1. Brain Tumor Study Group

The Brain Tumor Study Group (BTSG), a contract-supported, clinical trial cooperative effort consisting of 7 medical centers, evaluates multimodality treatments of malignant brain tumors. Patient accrual into Phase II and III protocol studies continued to be excellent during this period. More than 200 newly diagnosed patients having malignant gliomas were randomized into the Phase III protocols

(BTSG 77-02 and 80-01). The former protocol was closed to accrual in December 1980. An additional 200 patients with recurrent malignant gliomas or metastatic brain tumors were randomized to the Phase II protocol (BTSG 78-20). Analysis of the previous Phase III protocol (BTSG 75-01) has been completed and the final draft will be submitted for publication.

2. Radiation Therapy Oncology Group

The Radiation Therapy Oncology Group is a grant-supported clinical cooperative group which emphasizes radiation therapy clinical research. Although protocols do involve chemotherapy and/or surgery, the primary thrust of the RTOG is radiation therapy-related research. Major areas of effort include (1) improvement of low LET radiotherapy through radiosensitizers and radioprotectors as well as hyperthermia and (2) high LET radiotherapy research. The latter activity is supported in part through a separate P01 grant. The clinical high LET radiotherapy research program is integrated insofar as possible into the overall plan for the Group.

3. Radiological Physics Center

The Radiological Physics Center, well established as a most important resource for the clinical cooperative groups, checks calculations and treatment plans and assists in the calibration of radiotherapy machines and other equipment in radiotherapy facilities.

C. New Initiatives in FY 1981

1. Phase I Evaluation of Equipment for Hyperthermic Treatment of Cancer (RFP NCI-CM-17480)

This contract-supported activity will incorporate two tasks. Task A is the assessment of the performance of heat generating and thermometry systems in major anatomic sites (brain, head and neck, lung, mediastinum, upper abdomen, pelvis, superficial and deep lymph nodes, trunk, and extremities). In Task B the working group will develop consensus guidelines for heating tumors in the different anatomic sites. This information should accelerate the transition from pilot studies to definitive clinical trials of the use of hyperthermia as an adjunct to treatment with radiotherapy or chemotherapy.

2. Intraoperative Radiotherapy (RFP NCI-CM-17481)

This project shall consist of two tasks. Task A is the investigation of the role of intraoperative radiotherapy in the treatment of intraabdominal malignancies according to carefully defined surgical, pathological, and radiotherapy criteria and the development of guidelines for intraoperative radiotherapy techniques and their use for the irradiation of intraabdominal malignancies. Task B (optional) is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy in the treatment of intraoperative malignancies.

3. Evaluation of Treatment Planning for Particle Beam Radiotherapy (RFP NCI-CM-17482)

This project consists of two tasks. Task A is treatment planning for particle beam radiotherapy in each major anatomic site (brain, head and neck, lung, mediastinum, upper abdomen, pelvis, trunk, extremities, and superficial and deep lymph nodes) utilizing state of the art imaging and computer treatment planning systems. Task B is the evaluation of particle beam treatment capabilities which will involve dosimetry and microdosimetry measurements in patients or in phantoms to confirm the treatments planned in Task A. Anticipated participants include institutions with capabilities for radiotherapy with pions, heavy ions, and cyclotron- and DT generator-produced neutrons.

IV. Working Groups and Committees

A. Radiosensitizer/Radioprotector Working Group

This working group consists of 12 representatives from the scientific community, most of whom are experts in the development of and/or investigation (both basic and clinical) of radiosensitizers and/or radioprotectors. In addition, there are basic scientists who provide guidance in the pharmacological aspects of sensitizers and protectors. The Working Group held meetings in San Diego, California, on January 13, 1981, in conjunction with the Semi-Annual RTOG meeting in that city and on September 16, 1981, in Key Biscayne, Florida.

B. Radiosensitizer/Radioprotector Analog Committee

This intramural committee held meetings on January 31, May 12, and September 15, 1981, to review progress in the development of radiosensitizers and radioprotectors. Dr. David Davidson, Chief, Department of Parasitology, Walter Reed Army Institute of Research, attends these meetings to effect liaison between the Department of Defense and NCI. The committee is composed of 18 members who are experts in radiation biology, radiotherapy, drug development, and/or closely related fields. This group serves as an advisory body to the Chief of the Radiotherapy Development Branch, CTEP, in formulating and conducting the efforts to identify radiation sensitizer and radiation protector compounds using in vivo and in vitro screening systems. The final evaluation of potential radiosensitizer and radioprotector compounds will be made in clinical trials.

C. Interagency Radiation Research Committee

Dr. Pistenma is alternate to this committee for Dr. DeVita and participated in the meetings of the committee as well as in the preparation of a Federal Strategy for Study of the Biological Effects of Ionizing Radiation.

D. Subcommittee to Coordinate Radiation Activities

Dr. Pistenma is the NIH representative to this DHHS committee and attended its meetings throughout the year.

E. Biological Effects of Low-Level Radiation (BELLRAD) Task Group

This group reorganized in 1980 and developed recommendations for the organization of radiation activities in the National Cancer Institute. These recommendations are undergoing study by the Director and senior staff. Dr. David Pistenma is Co-Chairman.

V. Workshops

The Radiotherapy Development Branch has brought together basic, prethera-peutic, and clinical investigators in workshops in selected areas of research to facilitate the transfer of information in all directions in the research logic pathway. During the past year workshops and working group meetings were as follows:

November 13-14, 1980	Brain Tumor Study Group Semi-Annual Meeting	Bethesda
November 17-18, 1980	Workshop on the Treatment of Prostatic Carcinoma with Radiotherapy	Bethesda
December 9, 1980	Workshop on the Role of Radiotherapy in the Treatment of Ovarian Carcinoma	Bethesda
January 13, 1981	RS/RP Working Group	San Diego
January 22, 1981	Brain Tumor Study Group Neuroradiologists Meeting	Bethesda
February 19, 1981	Brain Tumor Study Group Protocol Design Committee	Washington, DC
March 16-17, 1981	Workshop on Time-Dose Relationships in Clinical Radiotherapy	Bethesda
May 8, 1981	Charged Particle Workshop	Bethesda
May 14-15, 1981	Neutron Therapy Workshop	Bethesda
May 18, 1981	Brain Tumor Study Group Protocol Design Committee	Washington, DC
September 1, 1981	Workshop on Physician Education in Radiotherapy	Bethesda

VI. Publications

1. Gilbert, E.H., Pistenma, D.A., and Mahoney, F.J.: Hyperthermia--research supported by the national cancer institute. J Microwave Power, In Press.

2. Pistenma, D.A.: Particle Beam Radiation Therapy. In DeVita, V.T., Hellman, S., and Rosenberg, S.A. (Eds.): Principles and Practice of Oncology. Philadelphia, J. B. Lippincott Company, In Press.

3. Pistenma, D.A.: The national cancer institute radiation development program. Cancer Treat Rev 7:225-234, 1980.

4. Walker, M.D. and Strike, T.A.: Misonidazole peripheral neuropathy--its relationship to plasma concentration and other drugs. Cancer Clin Trials 3:105-109, 1980.

5. Walker, M.D. and Strike T.A.: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303:1323-1329, 1980.

VII. Presentations

1. Pistenma, D.A.: "Economics of Research in Radiation Oncology." Presented to the American Society of Therapeutic Radiologists, Dallas, TX, October 1980.

2. Pistenma, D.A.: "Treatment of Prostatic Cancer by Teletherapy (Course #616)." Presented to the Radiological Society of North America, Inc., Dallas, TX, November 1980.

3. Pistenma, D.A.: "Brain Tumor Study Group Data." Presented at the Radiation Therapy Oncology Group Semi-Annual Meeting, San Diego, CA, January 1981.

4. Gilbert, E.H.: "The Issue of Shared Responsibility." Presented at the Symposium of The Foundation of Thanatology: Psychosocial Aspects of Radiation Oncology, New York, NY, January 1981.

5. Pistenma, D.A.: "Recent Advances in the Treatment of Prostatic Carcinoma." Presented at the Continuing Medical Education Conference at Holy Cross Hospital, Silver Spring, MD, March 1981.

6. Pistenma, D.A.: "NCI Research Programs in Hyperthermia." Presented to the Bureau of Radiological Health, Washington, DC, April 1981.

7. Pistenma, D.A.: "Research Opportunities in Radiotherapy." Presented to the Pan American Health Organization, Washington, DC, April 1981.

8. Mahoney, F.J.: "Radiology Funding in the NCI." Presented to the Radiation Research Society, Minneapolis, MN, June 1981.

VIII. New Initiatives

Plans for new initiatives in radiotherapy in the coming year include the following:

A. Hyperthermia--Technical Support for Clinical Evaluation of Equipment ($74,000)

The Division of Electronic Products, BRH, will provide technical support to the Radiotherapy Development Branch for work related to the performance of tasks under the contract program "Phase I Evaluation of Equipment for Hyperthermia Treatment of Cancer" as follows: (1) provide advice in the selection of heat generating and thermometry equipment to be evaluated; (2) perform sufficient measurements on all types of heat generating equipment (ultrasound, radiofrequency, or microwave) which are selected for study to fully characterize and calibrate the equipment in terms of heat generation, leakage radiation, thermal fields, energy requirements, and reproducibility; (3) perform measurements to calibrate thermometry equipment to be used in the project; and (4) provide consultation in the evaluation of reports prepared by contractors.

B. Quality Assurance Program for Clinical Hyperthermia ($200,000)

Interest in clinical applications of hyperthermia alone, with radiotherapy, and with chemotherapeutic agents has increased markedly and chaotically in the past few years. The physical and physiological challenges of heat generation and thermometry are enormous. It is essential that a mechanism similar to the Radiological Physics Center be established for quality assurance in Phase I/II as well as Phase III hyperthermia studies if this adjuvant modality is to be evaluated expeditiously. This contract will build upon the other two hyperthermia contracts.

C. Technical Improvements in Low LET Radiotherapy ($300,000)

Advances in tumor delineation with CT scanning, tomographic radionuclide scans, and ultrasound have not been exploited systematically by the radiotherapy community even though private industry has invested considerable effort in this area. A focused evaluation of the capability of improving photon and electron beam radiation dose distributions with presently available imaging and computerized treatment planning systems is a high priority. This project is complementary to the particle beam treatment planning contract and will facilitate assessment of tumor control by low LET radiotherapy as well as normal tissue tolerance for tumors in various parts of the body.

RADIOTHERAPY DEVELOPMENT BRANCH

GRANTS	FY 1980
Individual Investigator Grants (R01, R23, etc.)	$ 16,597
Program Project Grants (P01)	21,708
CREGS	1,300
RTOG (including RPC)	3,617
GRANT TOTAL COSTS	$43,222
CONTRACTS	
Radiosensitizer Development	$ 247
Clinical Neutron Research	1,833
Photoradiotherapy (RPMI)	50
Brain Tumor Study Group	709
Support (WSA)	6
CONTRACT TOTAL COSTS	$ 2,845
OVERALL TOTAL COSTS	$46,067

†Dollars are thousands

BIOLOGICS EVALUATION BRANCH

Introduction

The Biologics Evaluation Branch was started as the fourth Branch of the Cancer Therapy Evaluation Program in October 1980 to serve as the administrative entity to implement clinical trials of the Biological Response Modifiers Program. The Branch has been developed to be analogous to the Investigational Drug Branch, with one section devoted to IND development in relations with the Bureau of Biologics of the Food and Drug Administration, and the other section devoted to the administration of Phase I/II clinical trials for biological response modifiers. Dr. Don S. Poster has been the physician in charge of the Investigational New Drug Development Section, and Dr. Salvador Bruno has been the physician in charge of the Clinical Trials Development Section. Dr. Macdonald is currently the Acting Branch Chief for the Branch while recruitment is underway for a full-time permanent Branch Chief. The initial tasks undertaken by the Branch have gone well, with clinical trials in interferon initiated in seven institutions, clinical trials in thymosins initiated in five institutions, and clinical trials in a non-specific immunomodulator, MVE_2, being underway in two institutions. It is planned that clinical trials of other biological response modifiers will soon proceed.

Investigational New Drug Development Section

The Investigational New Drug Development Section is responsible for filing investigational new drug applications with the Food and Drug Administration (FDA), Bureau of Biologics, for agents which will be entering clinical trials under the sponsorship of the Biologics Evaluation Branch. This involves the preparation of a comprehensive document which provides details on every aspect of a new agent's chemistry, biochemistry, manufacturing, toxicity and results of any prior human studies. In addition, the IND contains the specific clinical protocols which will be used to test the agent in either a Phase I or II setting.

Following the preparation of this document, it is submitted to the Food and Drug Administration, Bureau of Biologics, which by law must review and approve the submission before any human trials may begin. Preparing the IND involves close cooperation with a number of other programs and Branches within the NCI who must supply some of the necessary and highly specialized information called for.

Following approval each IND must be maintained, in that new protocols and changes or additions to existing studies must be formally submitted to the FDA in accordance with federal regulations. Since a major amount of time is devoted to meeting FDA requirements, a close liaison is maintained by the Section head and the FDA, Bureau of Biologics, in order to effect the orderly and smooth transmission of the required information. Additionally, the Section maintains a close relationship with a number of private pharmaceutical organizations who may be involved with us in the development of new agents.

Since the inception of the Investigational New Drug Development Section five IND's have been filed and are now being maintained (see Table 1).

Other responsibilities of this Section include participation in the overall program of the Biologics Evaluation Branch. This involves protocol review, contract administration, liaison with non-NCI investigators and participation in the Biological Response Modifiers Program (BRMP) Decision Network Committee.

Clinical Trials Development Section

The Clinical Trials Development Section is responsible for the conduct of all extramural clinical research with biological response modifying agents through the contract mechanism. This involves close interaction with investigators under contract with the NCI, at every phase of the clinical trial. As sponsor of these studies, the Section head is responsible for the procurement and approval of all clinical study protocols as well as their modification at any time. Following procurement each protocol is extensively reviewed to ensure that it meets both the scientific and regulatory standard of the program. Following approval the protocol is submitted to the Food and Drug Administration (FDA) for inclusion in the investigational agent's Investigational New Drug (IND) file.

Once a clinical trial is started, close supervision is exercised by Section personnel. The principal investigator is responsible for frequent periodic progress reports which are reviewed by the Branch. Furthermore, should any unexpected toxic effect occur secondary to the use of the investigational agent, Section personnel are responsible for investigating it and reporting it to the FDA through the Investigational New Drug Development Section. At periodic and frequent intervals, the Section sponsors meetings of investigators doing control studies. Close scrutiny of the results reported leads to new ideas and approaches for future clinical trials.

Another important responsibility of this Section is contract administration. As project officer for 24 contracts, the Section head is responsible for the overall supervision and conduct of contracts studies within the Branch. Table 2 lists the contracts currently under this Section's supervision.

An ongoing and close liaison is maintained by the Section and all other divisions of the Biological Response Modifiers Programs in order that new agents of interest can be expediently brought to clinical trials.

Recent publications by members of the Branch are appended.

Table 1

IND's Filed Through the Biologics Evaluation Branch, by Drug

I. Human Leukocyte Interferon - Meloy

A. A Phase I Trial of Single Agent Human Leukocyte Interferon for Inoperable Extensive Cancer
Protocol Number NCOG OC01X
T. Merigan, Stanford University
Filed January 28, 1981

B. Phase I and Phase II Studies of Human Leukocyte Interferon (HuLeIF)
Protocol Number B81-164
J. Neefe, Georgetown University
Filed February 24, 1981

II. Human Leukocyte Interferon - Warner-Lambert

A. Metastatic Breast Cancer Treated with Human Leukocyte Interferon
Protocol Number B81-348
S. Grundberg, Sidney Farber Cancer Institute
Filed May 8, 1981

III. Human Lymphoblastoid Interferon

A. A Phase I Study of Human Lymphoblastoid Interferon
Protocol Number B81-242
J. Laszlo, Duke University
Filed March 26, 1981

B. A Phase I Study of Human Lymphoblastoid Interferon
Protocol Number B81-266
G. Sarna, UCLA
Filed April 6, 1981

IV. Thymosin $Alpha_1$

A. Phase I Trial: Thymosin Fraction 5 and Thymosin $Alpha_1$
Protocol Number
I. Royston and R. Dillman, UCLA
Filed December 15, 1980

B. Clinical Protocol for PhaseI-II Evaluation of Thymosin Fraction 5 and Thymosin $Alpha_1$
Protocol Number
P. Wright, Fred Hutchinson Cancer Research Center
Filed December 15, 1980

V. Thymosin Fraction 5

A. Phase I Trial: Thymosin Fraction 5 and Thymosin Alpha$_1$
Protocol Number
I. Royston and R. Dillman, UCLA
Filed December 15, 1980

B. Clinical Protocol for Phase I-II Evaluation of Thymosin Fraction 5 and Thymosin Alpha$_1$
Protocol Number
P. Wright, Fred Hutchinson Cancer Research Center
Filed December 15, 1980

C. A Phase I Trial of Thymosin Fraction 5
Protocol Number
W. Wara, University of California-San Francisco
Filed December 15, 1980

Table 2

Contracts

I. Immunology Contracts (General)

Albany Medical Center (NO1-CB5-3940)

This contract was recently transferred from DCBD to the Biologics Evaluation Branch. It consists of a clinical trial designed to evaluate intrapleural BCG with or without cutaneous BCG in patients with lung cancer. The study is close to completion. Final data are expected within one or two years.

Health Research, Inc. (Rosewell Park Memorial Institute (NO1-CB6-4007)

This contract has recently been transferred from DCBD. It consists of the clinical assessment of tumor associated antigen effectiveness in patients with resectable lung cancer. Patients are no longer being accrued but currently are being followed to determine the value of antigen treatment.

Memorial Sloan-Kettering Cancer Institute (NO1-CB5-3815)

This contract was recently transferred to the Biologics Evaluation Branch from DCBD. The purpose is to evaluate the adjuvant effect of levamisole in head and neck cancer. The study is in the fifth year of funding. It will be completed within the next year.

Memorial Sloan-Kettering Cancer Institute (NO1-CB5-3970)

This contract was transferred from DCBD. It consists of the evaluation of immunotherapeutic agents for acute and chronic toxicity, and their effects on the immune system. Agents included are: intravenous BCG,

POLY IC-LC, and endotoxin. The study is in its fifth year and results should be available within one year.

Memorial Sloan-Kettering Cancer Institute (NO1-CB7-4146)

Iravesical and percutaneous BCG are being evaluated for their efficacy in patients with recurrent superficial bladder cancer. Preliminary results suggest an advantage for BCG treatment. Continuation of this study is planned.

Mount Sinai School of Medicine (NO1-CB4-3879)

The goal of this contract is to evaluate the usefulness of neuromini-dose-treated allogenic AML cells in acute leukemia. Preliminary data suggest that immunotherapy may offer an advantage over chemotherapy alone. Continuation of this contract is planned through June 1984.

University of California (NO1-CB1-5525)

The purpose of this study is to determine whether BCG therapy alone or BCG combined with tumor cell vaccine will decrease the recurrence rate or prolong survival in melanoma patients with metastases of the regional L-nodes. To date, BCG appears to have some positive effect. This study is planned for continuance.

University of California at Los Angeles (NO1-CB0-4344)

Adjuvant chemoimmunotherapy is being compared to adjuvant chemotherapy alone in preventing disease recurrence in patients with highly malignant skeletal or soft tissue sarcomas. Currently, there is a trend in favor of the combined approach. This study will continue to 1983.

University of Texas (NO1-CB3-3888)

The purpose of this contract is to develop immunologic agents for cancer treatment. Included are Phase I and II studies with MER, C-Parvum, BCG, and thymosin. Linear followup of treated patients is continuing. This contract is scheduled to terminate in January 1982.

Yale University (NO1-CB7-4191)

This contract has recently been transferred from DCBD. It consists of the evaluation of intratumor given BCG prior to surgical resection in patients with lung cancer. The trial is in its fifth year. It is expected to be completed in one year.

II. Interferon Contracts

Duke University Cancer Center (NO1-CM0-7436)

This Phase I/II clinical trial utilizing human lymphoblastoid inter-

feron has been recently activated. It is estimated that the Phase I and II portions of the task will be completed in less than two years.

Georgetown University (NO1-CB0-7437)

This study is a Phase I and II evaluation of human leukocyte interferon. Currently patients are being accrued at an adequate rate and laboratory studies to define the role of interferon on the immune system are also being performed.

Memorial Sloan-Kettering Cancer Institute (NO1-CM0-7435-2)

This contract is for Phase I and II clinical trials utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in May 1981, at which time patient accrual will start.

Northern California Cancer Project (NO1-CM0-7443-2)

This study is a Phase I and II evaluation of human leukocyte interferon. Currently patients are being accrued at an adequate rate and laboratory studies to define the role of interferon on the immune system are also being performed.

Sidney Farber Cancer Center (NO1-CB0-7433)

This is a Phase I and II evaluation of human leukocyte interferon. The IND has been filed with the Food and Drug Administration. It is estimated that patients will be entered in the study during May 1981.

University of California at Los Angeles (NO1-CM0-7341)

This is a Phase I and II clinical trial utilizing human lymphoblastoid interferon that has been recently activated. It is estimated that the Phase I and II portions of task will be completed in less than two years.

University of Wisconsin (NO1-CM0-7434)

This contract is for Phase I and II clinical trials utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in May 1981, at which time patient accrual will start.

III. MVE_2 Contracts

Ohio State University (NO1-CM0-7442)

This is a Phase I evaluation of the pyran copolymer MVE_2. Preliminary results are being produced. The study should be completed in 1981.

Vanderbilt University (NO1-CMO-7438)

This contract is a Phase I evaluation of the pyran copolymer MVE_2. Preliminary results are being produced. The study should be completed in 1981.

IV. Thymosin Contracts

Fred Hutchinson Cancer Center (NO1-CMO-7445)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

George Washington University (NO1-CMO-7446)

This is a Phase I evaluation of thymosin alpha-1. This study is in an early stage and preliminary data are being collected.

Memorial Sloan-Kettering Cancer Institute (NO1-CMO-7435-1)

This contract is for a Phase I clinical study of thymosin fraction 5. The trial has only recently begun. It is expected to include 20-30 patients and should be completed within one year.

Northern California Cancer Project (NO1-CMO-7446-1)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

University of California at San Diego (NO1-CMO-7444)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 - September 30, 1981

The Clinical Oncology Program of the National Cancer Institute conducts clinical and laboratory investigations into the etiology, diagnosis, and treatment of human malignancies. The program is composed of seven branches, each of which carries out an independent laboratory research effort, and conducts clinical studies either independently or in collaboration with the other branches of the program. During the past year significant advances have been made in the treatment of several malignancies, including ovarian cancer, soft tissue sarcomas, acute leukemia of childhood, and small cell carcinoma of the lung. In addition, significant research advances have occurred in the development of monoclonal antibodies to small cell carcinoma of the lung, in the clarification of mechanisms of hormone resistance in human breast cancer, in the cloning and proliferation of cytotoxic human T-cells, in the characterization of important new metabolites of methotrexate, and in understanding the mechanisms of malignant transformation induced by RNA and DNA tumor viruses in mice. These accomplishments will be described in summary form in the following abstract, and in greater detail in the accompanying report.

Program Accomplishments

Biometric Research Branch: Dr. Richard Simon, Chief

The Biometric Research Branch continues to support the ongoing research programs of the Clinical Oncology Program. A senior statistician has been assigned to each clinical branch and is intimately involved in the planning and execution of all clinical trials. A new system of computerized data storage and retrieval for clinical studies (the CAPRI system) has been instituted. This Branch has also supported the extramural treatment evaluation program and new preclinical *in vitro* screening experiments, using the human tumor stem cell assay.

Clinical Pharmacology Branch: Dr. Charles E. Myers, Chief

The formation of polyglutamate derivatives of methotrexate by human breast cancer cells has been described. These derivatives become the predominant form of drug found after extended periods of drug exposure, replace methotrexate in the binding to dihydrofolate reductase, are retained selectively in the cell in the absence of extracellular drug, and prolong the inhibition of thymidylate synthesis resulting from methotrexate treatment. These compounds were the subject of a very fruitful meeting on Folate and Antifolate Polyglutamates held under the sponsorship of the Clinical Oncology Program at Airlie House, Warrenton, Va., May 20-23, 1981, at which time 50 scientists gathered to asess the importance of polyglutamation in the physiologic and pharmacologic actions of the pteridine derivatives. Asssessment of the importance of this process will be considerably facilitated by the development of HPLC methodologies for separation and quantitation of polyglutamate metabolites of methotrexate by Jolivet and colleagues of the Clinical Pharmacology Branch (Biochem. Pharmacol. in press). Clinical pharmacologic studies on the usefulness of test doses of methotrexate have been initiated in order to provide a rational basis for dose selection in patients at high risk of having altered pharmacokinetics. Other clinical pharmacologic studies conducted by this branch include (1) intraperitoneal pharmacokinetics of adriamycin, misonidazole, 5-fluorouracil, (2) intravenous pharmacokinetics of bromodeoxyuridine and adriamycin, and (3) oral cis-13-retinoic acid. A comprehensive model for 5-FU disposition in man has been developed by Dr. Jerry Collins of the Biomedical Engineering and Instrumentation Branch in conjunction with the Clinical Pharmacology Branch staff, based on our studies of intraperitoneal and intravenous 5-FU.

Productive studies continue on the mechanism of free radical damage and defense related to injury by adriamycin and other radical-producing agents. In particular, the role of glutathione conjugation in protection against radical injury has been demonstrated for adriamycin and for the model pulmonary toxin, 4-ipomeanol. A new, Se-independent glutathione perioxidase has been isolated and partially purified from mitochondrial membrane.

Medicine Branch: Dr. Robert C. Young, Chief

A broad range of clinical trials continue. The most important results include:

1) A new platinum containing combination for advanced ovarian cancer, called Chex-up has produced an overall response rate of 75% and complete remissions, as clinically staged, in 41% of patients. Twenty per cent of patients are confirmed to be free of disease by repeat laparotomy, and all CR's remain alive at 2 years. Results appear to indicate long-term disease free survival of at least 10% of patients in this trial.

2) A ten year follow-up of patients with advanced Hodgkin's disease treated with MOPP indicate that 80% of patients achieve CR, that 73% of these CR's are alive at 10 years, with a significant plateau in relapse frequency at 2 years after the end of MOPP. A defeat in T cell function persists in Hodgkin's patient despite cure of their disease. This defect is manifested as an increased sensitivity to normal monocyte suppressor cells and to suppressor T cells.

3) An increased complete remission rate of 64% in patients with advanced diffuse large cell lymphoma has been obtained using Pro-MACE-MOPP alternating cycle therapy. This is the first NCI study to show a prolongation of median survival past 1 year in this disease, since earlier curative regimens produced less than 50% long-term disease free survival.

4) The tendency of nodular lymphomas to evolve to diffuse patterns of histology, with a concomittant change in the pace of disease, has been documented in a retrospective study of 500 patients with non-Hodgkin's lymphoma. This finding greatly enhances our understanding of, and treatment approach toward, the nodular lymphomas.

5) Improved response rates have been obtained in patients with metastatic breast cancer treated with combination chemotherapy enhanced by hormonal synchronization, estrogen and tamoxifen.

Significant laboratory accomplishments include:

1) Discovery of a consistent chromosomal abnormality (deletion 3p) in 16 cell lines derived from patients with small cell carcinoma of the lung.

2) Isolation and partial characterization of hormone-resistant mutants of human breast cancer, some of which contain defective estrogen receptor proteins.

3) Demonstration that the immune response of T cells is determined by the genotype of antigen presenting cells, and recognition of a specific surface marker present on some, but not all antigen presenting cells. This marker becomes lost when antigen-presenting cells become tissue bound.

NCI-VA Medical Oncology Branch - Dr. John D. Minna, Chief

During the past year, significant progress has been made in the establishment of continuous cell lines of small cell carcinoma of the lung from patient biopsy or surgical specimens. Approximately, 20 continuous lines have been established, a unique chromosomal deletion (3p) has been found in these lines, and monoclonal antibodies have been produced which are specific for these cell lines, with little cross-reactivity for normal lymphoid or malignant cells. The possible uses of these antibodies for diagnosis or treatment of these tumors is being explored.

The reliable cloning of tumor specimens from patients with small cell carcinoma has made possible in depth studies of drug sensitivity patterns in 40 patients. As has been seen in other diseases, small cell tumors resistant to drugs in vitro do not respond to the same agents in vivo, while those which are sensitive to drugs in vitro have a 3/7 response rate in vivo. It is expected that the availability of these specimens will allow a broad range of pharmacologic studies aimed at establishing the mechanisms of resistance to common drugs, as well as allowing the selection of drug therapies tailored to the sensitivity patterns of individual tumors.

Our interest in cutaneous T-cell tumors has continued. Therapy with electron beam irradiation plus intravenous nitrogen mustard has produced 10/14 complete remissions, 8 of whom remain in remission. We have identified VP-16 as an active agent in mycosis fungoides. Monoclonal antibodies have been developed to cutaneous T-cell tumor cell lines and will be employed for treatment in the coming year. One such cell line has yielded a highly interesting retrovirus, which is being characterized by Dr. Gallo's laboratory.

Pediatric Oncology Branch: Dr. Arthur Levine, Chief

The current acute lymphocytic leukemia (ALL) treatment regimen in the Pediatric Oncology Branch tests whether the likelihood of long-term CNS damage can be lessened by eliminating cranial irradiation from the treatment of leukemia. The trial compares (by a random, weighted allocation) conventional "prophylaxis" of meningeal leukemia (intrathecal methotrexate plus cranial irradiation) with high-dose, protracted intravenous methotrexate infusions (33.6 g/M^2 over 24 hours) as the sole "prophylactic" approach. Therapy in both arms of the study is otherwise identical. At this time, 76 patients have been entered on study, of whom 75 achieved complete remission. Among 24 patients receiving cranial irradiation plus intrathecal methotrexate, there have been 2 CNS relapses and 5 bone marrow relapses. Among 52 patients receiving the high-dose protracted methotrexate infusions, there have been 3 CNS relapses and 1 marrow relapse. The 2-sided p values are 0.33 for bad prognosis and 0.046 for good prognosis patients (both in favor of high-dose infusion). The improvement in patients receiving infusions is primarily related to freedom from systemic relapse, an unanticipated result. While the study is ongoing, it appears that results in the experimental arm of the current protocol are the best yet achieved in the management of this disease.

The current Pediatric Oncology Branch treatment regimen in non-Hodgkin's lymphomas of young patients demonstrates a significant improvement in the proportion of patients remaining free of disease as compared with results on earlier protocols. This regimen is administered to all young patients with diffuse lymphomas, and is novel in that high-dose methotrexate infusion is given ten days following treatment with CHOP; there is no delay for neutropenia. Durable survival will be 70%, and the results are as encouraging for lymphoblastic lymphomas as for Burkitt's lymphomas.

The current treatment regimen in the Pediatric Oncology Branch for patients presenting at diagnosis with metastatic Ewing's sarcoma includes treatment with intensive combination chemotherapy, total body irradiation, and radiation to bulk disease. Stem cell reconstitution is accomplished with autologous marrow transplantation. This regimen is yielding a better result than any previous NCI protocol, with 30% of patients now surviving in what had previously been an almost uniformly fatal setting.

In studies on the clonogenic human tumor stem cell assay, it was found that by modifying the medium employed in the assay and by using tritiated thymidine incorporation as a measure of cell growth, it is possible to significantly improve the plating efficiency for most human tumor cells without increasing contamination with normal stromal elements. Moreover, these innovations permit one to determine chemosensitivity at 5 days subsequent to plating with an accuracy equal to that previously attained only at three weeks. These findings should greatly enhance the clinical applicability of the assay.

Hamster cells transformed in vitro by SV40 are highly oncogenic in adult syngeneic and allogeneic hamsters, but the same cells transformed by adenovirus (Ad) 2 form tumors only in newborn or thymectomized hamsters. Somatic cell hybrids were generated from Ad 2 and SV40-transformed hamster cells and it was found that hybrids which express both Ad 2 and SV40 antigens induce tumors only in newborn animals. Thus, the Ad function which induces tumor rejection (possibly a surface membrane antigen) is dominant over the SV40 function in hybrid cells which contain both transforming genomes. This new system is thus a powerful tool for separating transformation from oncogenicity, and may make it possible to determine how very subtle differences in viral-encoded proteins influence whether tumor rejection will or will not occur.

In a Rhesus monkey model for the study of CNS pharmacokinetics, the influence of body position on ventricular CSF methotrexate concentration was studied following intralumbar administration. Maintenance in the flat or Trendelenberg position for one hour following intralumbar administration resulted in a significantly greater ventricular CSF methotrexate level than the immediate upright position. Since there is much clinical variation in the administration of intrathecal methotrexate, these results have important implications for techniques which may or may not achieve effective tumoricidal CNS drug levels.

A phase I trial of a new agent, ICRF-187, was conducted in children and it was found that the maximally tolerated pediatric dose of this drug was almost three times greater than had been observed in adults. As a result, the literature was reviewed on all cancer drugs which have had comparable Phase I trials in adults and children, and it was found that for 16/20 agents, the maximally tolerated dose in children was significantly higher than had been observed in adults. For a majority of these agents, dose-limiting toxicity was myelosuppression, and it may be that children have a higher number of bone marrow stem cells per unit surface area than do adults and are thus able to tolerate higher drug doses (and/or that the metabolism of these drugs differs between children and adults).

It may also be that the dose required to achieve a tumoricidal effect differs between children and adults. These results indicate that all new chemotherapeutic agents should be independently tested in children as well as in adults.

Radiation Oncology Branch: Dr. Eli Glatstein, Chief

A residency training program in radiation oncology has been established in cooperation with Walter Reed and the National Naval Medical Center. This program will be vital to the development of permanent senior staff for the Radiation Oncology Branch, which continue to have difficulty in recruiting and retaining high quality senior staff. Nonetheless, despite a staff of only three senior radiotherapists, very productive clinical studies continue in collaboration with the other clinical branches in the area of soft tissue sarcoma, the lymphomas, childhood sarcomas, lung and breast cancer. Programs in intra-operative radiation of pancreatic carcinoma and radiosensitizer studies using misonidazole and bromodeoxyuridine have been initiated. Seven of 8 patients with inoperable chondro- or osteosarcomas have responded to a combination of x-ray and misonidazole, a surprising result in a radio-resistant tumor. Computer technology has been developed to interface whole body CT scanning and irradiation field planning and dosimetry, a crucial step in the use of high dose therapy for bulky trunkal sarcomas or carcinomas, and breast cancer patients. A randomized trial comparing lumpectomy and breast irradiation with modified radical mastectomy has been initiated and has accrued approximately 50 patients in 18 months.

We look forward to the opening of new radiotherapy facilities in our newly constructed wing in the next fiscal year.

Surgery Branch: Dr. Steven Rosenberg, Chief

The following significant developments have occurred in clinical studies of the Surgery Branch during the past year:

1) A randomized clinical trial has shown significant improvement in disease-free survival for patients with soft tissue sarcoma who receive adjuvant chemotherapy with adriamycin, cytoxan, and methotrexate, as compared to control patients not receiving chemotherapy.

2) A randomized trial has shown that in most clinical situations (limited size tumors), soft tissue sarcomas of the extremity can be prevented from locally recurring by limited limb-sparing surgery and radiotherapy. Results of this approach equal those of amputation.

3) A randomized clinical trial has shown that cytoreductive surgery does not improve the disease-free survival or the response to subsequent chemotherapy in patients with bulky testicular carcinoma.

4) A clinical trial has been initiated using activated autologous T lymphocytes expanded in culture using T-cell growth factor.

Significant laboratory accomplishments include:

1) Demonstration that cytotoxic T-cells are capable of curing mice bearing syngeneic lymphoid malignancy. Cytotoxic cells were obtained by expansion of T-cell populations in vitro using T-cell growth factor.

2) Development of technique for cloning and expanding the progeny of single lymphoid cells in culture.

3) Development of techniques for depleting bone marrow of T-cells, a method which may allow grafting of allogeneic marrow.

4) Development of a method for isolated perfusion of a single lung with high concentrations of chemotherapeutic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06308-10 BR

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biometric Research Branch

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Simon	Chief, Biometric Research Branch	COP, DCT, NCI
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COOPERATING UNITS (if any) Clinical Oncology Program, DCT, NCI; Developmental Therapeutics Program, DCT, NCI; Cancer Therapy Evaluation Program, DCT, NCI; Baltimore Cancer Research Program, DCT, NCI; Immunology Branch, DCBD, NCI; Laboratory of Pathology, DCBD,NCI; Dermatology Br., DCBD,NCI; Dr. D. Von Hoff, Univ. of Texas.

LAB/BRANCH
Biometric Research Branch

SECTION

INSTITUTE AND LOCATION
NCI, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.75	5.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The branch is the statistical component of the Division of Cancer Treatment. The branch designs, conducts and analyzes intramural and national clinical trials of experimental treatments, and conducts studies to identify important prognostic and treatment selection factors. Collaborations with the Developmental Therapeutics Program are directed to evaluating and improving methodology for pre-clinical drug screening and synthesis. The branch collaborates with the Cancer Therapy Evaluation Program in the planning review and coordination of national clinical therapeutic research in oncology. The branch designs and maintains computerized data collection systems and develops new biometric methods. A list of current major activities follows.

1. Design of Clinical Trials

1.1 The Biometric Research Branch (BRB) is organized with a designated coordinating statistician for each Clinical Oncology Program (COP) branch and for the Baltimore Cancer Research Program (BCRP). Consequently a member of the BRB participates in the planning and drafting of new intramural protocols. In addition, a member of the branch serves on the Clinical Research Panel to review all intramural clinical trials.

1.2 The branch reviews major clinical protocols submitted to the Division of Cancer Treatment (DCT) by cooperative groups and contractors. This is a major activity for ensuring the quality of supported clinical research. Approximately 100 protocols are reviewed per year. In some cases substantial problems are found (e.g. bone marrow transplantation group) and the BRB interacts with the Cancer Therapy Evaluation Program (CTEP) and extramural organizations to develop scientifically sound and ethical designs. A member of the branch serves on the Clinical Oncology Review Committee to evaluate all contract and inter-agency clinical studies supported by the division.

1.3 The branch participates in the development of national and inter-group studies. In the past year this has included new studies of soft tissue sarcoma, neutron radiotherapy, acute myelocytic leukemia, cervical cancer chemoprevention, and multi-national studies of the Pan-American Health Organization.

2. Ongoing Multi-Institution Studies

The branch serves as statistical center for multi-institution clinical trials of ovarian cancer, head and neck cancer and acute leukemia in children. Data collection and study monitoring is continuing.

The branch has collaborated in the conduct of an international study of histological classification systems for lymphomas. Approximately 1100 cases of carefully staged modernly treated lymphoma have been classified according to six major histologic systems by a panel of expert pathologists. This data has been transferred to the BRB and manuscripts are being written concerning the evaluation of classification systems, staging systems and other major findings.

The branch is completing the analyses of multi-institution studies done under the Diet Nutrition and Cancer Program.

3. Statistical Analyses

The branch conducts statistical analyses of the results of clinical and laboratory studies and actively participates in data collection and interim monitoring for clinical studies. In the past year, major analyses have included the following.

3.1 Therapeutic Clinical Studies
(1) Evaluation of adjuvant chemotherapy versus no adjuvant and amputation versus resection plus radiotherapy for patients with non-metastatic soft tissue sarcoma. (2) Evaluation of debulking surgery for patients with non-resectable testicular cancer. (3) Evaluation of total parenteral nutrition for patients with advanced diffuse lymphoma and for pediatric sarcoma patients. (4) Evaluation of CHex-UP chemotherapy for advanced ovarian cancer. (5) Evaluation of the scopolamine transcutaneous patch for the control of chemotherapy induced emesis.

(6) Evaluation of Promace-MOPP chemotherapy for advanced diffuse histiocytic and mixed lymphomas. (7) Evaluation of immunotherapies or methyl-CCNU for the treatment of stages I and II melanoma. (8) Evaluation of chest and mediastinal radiation for the treatment of limited disease small cell lung cancer. (9) Evaluation of prophylactic CNS irradiation for the treatment of Ewing's sarcoma. (10) Phase I study of intravenous misonidazole. Prediction of peripheral neuropathy based on total dose and pharmacokinetic parameters. (11) Evaluation of prophylactic bactrim plus erythromycin for preventing infection in granulocytopenic patients with cancer. (12) Evaluation of the duration of empiric antibiotics and of amphotericin for febrile granulocytopenic cancer patients. (13) Evaluation of a very intensive chemotherapy program for the treatment of patients with diffuse undifferentiated lymphomas. (14) Evaluation of four chemotherapeutic regimens for the treatment of advanced breast cancer. (15) Evaluation of two chemotherapy regimens (CVP versus CVPA) for patients with poorly differentiated and mixed non-Hodgkin's lymphomas. (16) Evaluation of a new induction regimen (methotrexate, vincristine, asparaginase and dexamethasone) for adult ALL. (17) Evaluation of bone marrow transplantation for remission maintenance of AML. (18) Evaluation of HLA matching for the platelet transfusion support of leukemic patients. (19) Evaluation of the sensitivity and specificity of four invasive methods of lung biopsy. (20) Evaluation of the sensitivity and specificity of three non-invasive methods for detecting liver metastases. (21) Evaluation of the incidence of second cancers, and its relationship to therapy, for patients with non-Hodgkin's lymphoma. (22) Evaluation of ovarian function of women treated with MOPP for Hodgkin's disease. (23) Evaluation of doses and timing of therapy on the outcome of patients with Hodgkin's disease receiving MOPP. (24) Evaluation of the effects of whole body hyperthermia on adriamycin and cyclophosphamide pharmacokinetics in sarcoma patients. In addition to the above completed studies, the branch collaborates in numerous ongoing clinical trials for which interim analyses are performed.

3.2 Clinical Studies of Prognostic Factors and Tumor Biology

(1) Evaluation of prognostic factors for patients with advanced diffuse non-Hodgkin's lymphomas. (2) Evaluation of prognostic factors for patients with advanced nodular mixed lymphoma. (3) Evaluation of the relationships between initial liver function, acute adriamycin toxicity and response to therapy for patients with ANLL. (4) Evaluation of factors predicting response to pre-operative chemotherapy in patients with resectable head and neck cancer. (5) Evaluation of prognostic factors for patients with non-resectable hepatocellular carcinoma. (6) Evaluation of the relationships between pretreatment delayed hypersensitivity skin reactions to disease extent, performance status and survival for patients with small cell lung cancer. (7) Evaluation of four histological classification systems for lymph nodes of patients with mycosis fungoides or Sezary syndrome. (8) Evaluation of sequential alterations in plasma lipids and lipoproteins in patients with leukemia or lymphoma. (9) Evaluation of the prognostic value of the histological distinction between Burkitt's lymphoma and undifferentiated non-Burkitt's type for American patients. (10) Evaluation of the prognostic significance of hyperleukocytosis for patients with ANLL. (11) Evaluation of non-invasive procedures for predicting the development of radiation pericarditis. (12) Evaluation of the relationship between gastrointestinal toxicity and response to therapy for ANLL patients receiving cytosine arabinoside and an anthracycline. (13) Evaluation of factors for predicting the development of serious infections in pediatric cancer

patients. (14) Evaluation of the usefulness of surveillance cultures for the identification of infectious sites in pediatric cancer patients at high risk for developing serious infections. (15) Evaluation of the incidence and prognostic significance of histologic progession in nodular lymphomas. (16) Evaluation of the possible etiologic role of aryl hydrocarbon hydroxylase in acute leukemia.

3.3 Laboratory and Pre-Clinical Studies

(A) Drug Screening

The BRB has continued its extensive involvement in the eveluation of the clonogenic assay for drug development. Members of the branch served on the technical evaluation and source selection committees to select four participating institutions from the 16 bidders. We have participated in the specification of the data collection system and the design of the study protocol. A member of the branch is co-project officer and is active in monitoring early results concerning reproducibility and findings with established drugs. The BRB also collaborates with the Drug Evaluation Branch on projects related to in-vivo drug screening. In the past year we have designed experiments to identify important sources of variability in the subrenal capsule mammary tumor model. These experiments are being conducted by screening contractors with new data reported to NCI for our analysis.

(B) Other Laboratory Investigations

(1) Determination of optimal conditions for generating and expanding cytotoxic lymphoid cells in-vitro directed against syngeneic lymphoma tumors in mice. (2) Evaluation of a new simple single step assay for quantitating the proportion of a lymphocyte population having B cell surface markers and the proportion having T cell markers. (3) Evaluation of the effect of mitogens, theophyllin and T cells on the growth of transformed lymphocytes. (4) Evaluation of delivery of adriamycin in rats by encapsulation in liposomes. (5) Evaluation of responses in mixed lymphocyte reactions to two types of simultaneous stimulator cells.

4. Monitoring of Extramural Clinical Research

In addition to the protocol review activities of the BRB, the branch actively collaborates with the CTEP in monitoring the quality of supported clinical research. BRB staff attends selected cooperative group meetings and performs site visits to group statistical centers. Specific recommendations are prepared to resolve identified problems and determine how costs can be reduced. The BRB is also participating in the design of an information system that will better permit the CTEP to monitor ongoing group studies.

5. Clinical Data Base System

The branch has completed the development and testing of a generalized file management system for clinical trials. The system is currently being implemented for all intramural protocols.

6. Development of Statistical Methodology

(1) Introduction of composite randomization designs for clinical trials. These new designs were developed by the BRB and permit cooperative group studies to be augmented by the participation of community physicians. The community physicians themselves are randomized rather than their individual patients. Cancer and

Leukemia Group B is planning to use this design.

(2) Development of clinical trial designs incorporating early termination criteria when initial results do not appear promising for the new treatment. Such early termination decisions are common, but good statistical criteria have not previously been published.

(3) Development of simple methods for calculating confidence intervals for a percentile (e.g. median) of a survival distribution based on censored data.

(4) Development of new statistical methods for evaluating how relative therapeutic efficacy varies among patient subsets.

(5) Study of clinical research strategies for improving treatment; the role of consecutive historical control groups and moderate-size studies.

(6) Determination of required sample sizes for comparing more than two treatments with a temporal endpoint (survival, duration of remission).

(7) Representation of survival curves for two or more treatments adjusted for a comparable mix of patients.

(8) Evaluation of factors causing "worsening survival curve syndrome" with accumulating data.

(9) Study of sample re-use methods for estimating error rates of classification rules and for validating identified prognostic factors.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06402-11-CP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Tumor Growth Kinetics and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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5	3	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computer modeling studies of drug scheduling studies have been carried out. A kinetic compartment model has been developed for the Sezary syndrome. In vitro studies of combinations of hydroxyurea and adriamycin, and cytosine arabinoside and adriamycin were performed. The lethality of vincristine in sarcoma 180 in vitro was studied. Flow cytometry studies are being carried out in human lymphoma, myeloma, and various other solid tumors in man.

1. Theoretical Studies

1.1. Computer modeling studies

1.1.1. The mammalian cell population kinematic simulator (MACKS)

Simulation of the CxT effects of drugs such as Hydroxyurea and cytosine arabinoside have been carried out. Simulated flow cytometry studies, autoradiographic data, and cloning studies are in general agreement with observed data. Major conclusions to date are that the recovery of drug-inhibited cells and the recruitment of drug-spared cells are two separate processes which have similar time courses in experimental systems but different time courses in man. The available data would suggest that the optimum recruitment-dependent drug scheduling interval in man may be between 8 and 21 days rather than during the first 48-72 hours after initial treatment. ment.

2. Experimental studies

2.1. Drug studies in sarcoma 180 in vitro

2.1.1. Sangivamycin studies

Studies of the effects of sangivamycin were completed, submitted, and published this year.

2.1.2. Adriamycin combination studies

Studies of the mechanisms underlying the schedule dependent synergism between HU TADR and ara-C TADR were undertaken. Serial ^{3}HTdR incorporation studies showed no schedule dependent diferences in two days cells. ^{3}HUR incorporation into RNA was inhibited to a greater degree with a split dose schedule than when the drugs were given together. However, further studies have suggested that ^{3}HUR incorporation into RNA may be inhibited by multiple medium changes. This effect is being examined more closely, to determine if it might account for the decreased incorporation seen in the drug scheduling studies.

Schedule dependent synergism between ara-C and HU was compared in log phase and plateau phase cells. Plateau phase cells exhibited synergism comparable in degree to log phase cells, but the optimum timing occured later than in log phase cells. These studies are continuing.

2.1.3. Vincristine studies

In several published studies with synchronized cells vincristine has been reported to be maximally effective in late S and early G_2, rather than in mitosis. We have studied this

phenomenon in detail in sarcoma 180 in vitro. HU treated cells were exposed to VCR or a second dose of HU at intervals. Maximum VCR effect occured later than the maximum effect of a second dose HU, indicating a period of maximum susceptibility that corresponded to late S and early G_2. It may be that this is the "S" phase for tubulin. This line of investigation has been pursued in two ways:

a) VCR scheduling studies are being carried out in mitotically selected cells to rule out the possibility of HU-VCR synergism, and b) antibodies are in the process of being raised the tubulin for use in flow cytometry studies of tubulin-content distributions and the effects of VCR on them.

2.2 Studies of cell RNA content by flow cytometry

There have been recent reports of the use of pyronin Y as a quantitative fluorescent measure of intracellular RNA in flow cytometry studies. Preliminary studies in our laboratory confirm the value of pyronin Y as an RNA stain and have demonstrated interesting population size dependent RNA distribution patterns in sarcoma 180. Our studies indicated that this stain can be used with ethanol fixed cells, which may permit retrospective studies of stored lymphoma samples.

2.3 Combined Kinetic-immunologic studies

Experience was gained during the past several months with combined propidium iodide-FITC staining of normal lymphocytes, in anticipation of DNA-surface marker studies in mouse B cell lymphoma lines and human lymphomas.

3. Clinical studies

3.1 Flow cytometry - clinical studies

3.1.1. Non-Hodgkin's lymphoma-collaborative study with Western lymphoma group

A manuscript describing dual parameter flow cytometry studies in sixty seven cases of human lymphoma has been published in THE JOURNAL OF CLINICAL INVESTIGATION. During the past year we have performed dual parameter studies on an additional sixty to seventy cases sent to us by the Western group. Long term clinical followup is available on 56 of the older cases and this study is being prepared for publication.

Major findings to date include:

1. Aneuploidy in untreated patients implied no greater susceptibility or resistance than diploidy.

2. Aneuploid subpopulations had higher S fractions than their diploid counterparts, and

3. Low S fractions implied a favorable prognosis reflecting predominantly the indolent natural history of the small B cell lymphomas.

3.1.2. Non Hodgkins lymphoma-collaborative study with Medicine Branch, DCT.

Approximately seventy cases have been studied by flow cytometry to date. Of these, approximately forty have been processed for comparative autoradiography. Combined DNA-grain count analyses are being done on these cases.

3.1.3. Non-Hodgkins lymphoma-collaborative studies with BCRP

Studies have been initiated with Dr. Richard Leavitt of BCRP to study combined DNA-volume-surface markers in in the lymphomas. During the past several months, we have been concerned predominantly and methodologic problems, and awaiting the installation of power for multiple laser studies.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06512-05-CP

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October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our efforts this year have been devoted primarily to the continuation and expansion of projects described in detail in our FY 1980 Annual Report. In the area of analytical pharmacology, we are investigating (in collaboration with Surgery Branch) drug pharmacokinetics in a new isolated in situ pulmonary perfusion protocol. This protocol is being developed for treatment of metastatic sarcomas in lung. Several lines of investigation are ongoing in the area of drug-induced pulmonary injury. Long-term toxicity and/or carcinogenicity studies centered primarily upon the lung-toxic furans and the nitrosoureas (eg BCNU) are in progress. The roles of glutathione in drug induced tissue injury continues to be a major interest. The role of the drug-metabolizing enzymes in the pathogenesis of chemical-induced injury to extrahepatic target cells, such as pulmonary Clara cells and renal proximal tubular cells, continues to be a major area of endeavor.

I. Objectives of Research; Methods Employed:

A. Objectives

The primary goals of the Molecular Toxicology Section are: 1) to elucidate fundamental chemico-biologic interactions underlying target organ and target cell cytotoxicities and carcinogenicities by chemical agents, and 2) to provide consultation and expertise in the development and application of analytical methodologies required by clinical research programs of the Clinical Oncology Program, DCT, NCI.

B. Methods

A wide variety of instrumentation and techniques are employed in these investigations. Metabolic and dispositional studies utilize traditional methodologies and instrumental techniques including GC-mass spectrometry and high-pressure liquid chromatography. Toxicologic studies employ biochemical measures of cellular alterations, as well as morphologic measures such as high-resolution light microscopy, electron microscopy, and autoradiography.

II. Research Program Description: Specific Projects and Subprojects; Background; Accomplishments; Ongoing Activities; Future Plans:

A. Analytical and Clinical Pharmacology

Our recent efforts in this area have centered primarily upon the study of antitumor drug kinetics in an isolated in situ pulmonary perfusion protocol, in collaboration with the NCI Surgery Branch (Drs. Shull and Johnston). This protocol is being developed in dogs with the view toward possible applications to treatment of metastatic sarcomas in lungs of human patients. Initial studies have centered upon adriamycin. This drug has proved to be extremely toxic to lung tissue in this protocol; we are investigating the mechanism of this toxic effect with the hope of developing strategies to prevent or ameliorate lung damage but maintaining the desired antitumor effect.

B. Molecular Toxicology

1. Biochemical mechanisms in drug-induced pulmonary disease.

Experimental investigations in this area are continuing along several avenues, including: 1) in vitro studies of the metabolic activation of 4-ipomeanol in reconstituted systems containing purified components, including pulmonary cytochrome(s) P-450, NADPH-cytochrome P-450 reductase, and cytochrome b_5 (these studies are being done in collaboration with Dr. J. Bend and Dr. R. Philpot, NIEHS), 2) studies of the metabolic bases for the striking species-, age-, and sex-related differences in the in vivo target organ alkyla-

tion and cytotoxicity by 4-ipomeanol in laboratory animals, 3) studies of the metabolic and pharmacokinetic bases for alterations, by metabolic inducers and inhibitors, in the target organ specificity of metabolically activated alkylating agents, 4) studies of the metabolic activation (and cellular specificity thereof) of 4-ipomeanol in systems containing intact pulmonary cells (these systems include lung slices, isolated whole lungs, and partially resolved lung cell fractions; some of these studies are being pursued collaboratively with Dr. J. Bend, NIEHS), and 5) continuing metabolic, histopathologic, and autoradiographic studies with lung-toxic furans, including 3-methylfuran and perilla ketone (both of which occur naturally in the environment). Studies with other lung-toxic chemicals, including carbon tetrachloride and nitrofurantoin, have been described previously, and now are essentially completed. In continuing investigations, we are attempting to determine if any of our mechanistic models for chemical-induced lung injury can account for the pulmonary toxicities of clinically important anticancer drugs such as BCNU, procarbazine, and cyclophosphamide. In particular, we have recently been successful in developing an experimental model in rats for BCNU-induced pulmonary fibrosis; we are currently studying the mechanism of this effect and its possible influence by exogenous and endogenous sulfhydryl compounds.

2. Physiology, pharmacology, and toxicology of the pulmonary Clara cell.

In ongoing investigations, we are continuing to explore the vulnerability of the Clara cell to cytotoxic and/or carcinogenic chemicals that require oxidative metabolic activation. These studies involve detailed histopathologic, autoradiographic, and metabolic investigations with a variety of pulmonary cytotoxins and carcinogens. We also are utilizing the selective destruction of pulmonary Clara cells with agents such as 4-ipomeanol as an approach to the study of the biochemical and physiologic role of the Clara cell in normal lung function.

3. Biochemical mechanisms in drug-induced renal disease.

We are investigating the possible role of metabolism, and the possible protective role of GSH or other sulfhydryl compounds, in renal injury by methyl-CCNU, and are considering the possible modulatory roles of age- and sex-related differences in their metabolism, and how this may affect susceptibility to their renal toxicity.

4. Long-term toxicity and/or carcinogenicity studies.

Because of the possible importance of 4-ipomeanol, 3-methylfuran, and several other related furans as naturally occurring toxicants, carcinogenicity studies of these compounds have been undertaken. (These studies are being pursued in collaboration with

Drs. James and Elizabeth Miller, McArdle Laboratory for Cancer Research, Madison, Wisconsin.)

In addition, long-term inhalation toxicology studies are underway with 3-methylfuran (studies in collaboration with Dr. H. Witschi, Oak Ridge National Laboratory, Oak Ridge, Tennessee).

5. Glutathione (GSH): Cellular homeostasis; role as protective factor in cytotoxicity by alkylating agents and reactive metabolites; formation of conjugates with reactive metabolites; and role of GSH transferases.

We have developed an HPLC method for the isolation of glutathione adducts formed with direct alkylating agents or with reactive drug metabolites. Structural studies, presently underway on such conjugates, should help elucidate the chemical identity of the akylating metabolite(s) responsible for the pulmonary alkylation and toxicity by 4-ipomeanol in vivo. The possible role of pulmonary and hepatic GSH-transferase enzymes in promoting the detoxification of 4-ipomeanol also is being investigated. The possible general applicability of the GSH conjugate assay method to the analysis of adducts formed from a wide variety of alkylating agents and metabolites is being examined.

III. Significance to Biomedical Research and the Program of the Institute:

The potential utility of existing cancer chemotherapeutic agents is frequently hindered by dose-limiting toxicities to target organs such as the gastrointestinal tract, bone marrow, liver, kidney, and lung. Moreover, many antitumor agents in current use are also, in themselves, potent carcinogens. Thus, as the longer-term survival of patients on antitumor drug therapy becomes a reality, the problems of drug-induced secondary cancer paradoxically will assume greater significance.

The elucidation of molecular mechanisms whereby cytotoxic and/or carcinogenic chemicals exert their biologic effects hopefully will contribute to the improved usage of existing chemotherapeutic agents, and will help provode a more rational basis for the design of new drugs with optimal tumoricidal activity but minimal toxicity to normal tissues.

IV. Publications and Other Activities:

A. Articles

Dutcher, J.S., Jones, R.B., and Boyd, M.R.: A sensitive and specific assay for pentamethylmelamine in plasma: application to preliminary pharmacokinetic studies. Cancer Treat. Rep. 64: 99-104, 1980.

Boyd, M.R.: Effects of inducers and inhibitors on extrahepatic drug metabolism and toxicity. In Environmental Chemicals, Enzyme Function and Human Disease (Ciba Fdn. Symposium No. 76), Excerpta Medica, Amsterdam, 1980, pp. 43-66.

Boyd, M.R.: Biochemical mechanisms of chemical-induced lung injury: Roles of metabolic activation. CRC Crit. Rev. Toxicol. 7: 103-176, 1980.

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Boyd, M.R.: Metabolic activation of pulmonary toxins. In Mechanisms in Respiratory Toxicology, H. Witschi and P. Nettesheim (Eds.), CRC Uniscience Press, West Palm Beach, 1981, in press.

Boyd, M.R. and Dutcher, J.S.: Convenient methods for the preparation of [5-^{14}C]-4-ipomeanol and [^{3}H(G)-4-ipomeanol of high specific radioactivity. J. Labeled Cpds. and Radiopharmaceut, 1981, in press.

Dutcher, J.S. and Boyd, M.R.: Organ specificity in toxic action: Biochemical aspects. In The Pesticide Chemist and Modern Toxicology, Bandal, S.K., et al (Eds.), American Chemical Society, Washington, D.C., 1981, in press.

Boyd, M.R.: Toxicity mediated by reactive metabolites of furans. In Biological Reactive Intermediates, Vol. 2, Snyder, R., et al (Eds.), Plenum Press, New York, 1981, in press.

Boyd, M.R.: Pulmonary toxicity of carbon tetrachloride. In Industrial and Environmental Xenobiotics: Biotransformation and Pharmacokinetics, Gut, I. (Ed.), Springer-Verlag, New York, 1981, in press.

Boyd, M.R.: Mechanisms of pulmonary toxicity. In Chemical Indices and Mechanisms of Organ-Directed Toxicity. Pergamon Press, Ltd., Oxford, U.K., 1981, in press.

B. Invited Presentions at National and International Symposia

Interagency Task Force on Environmental Cancer, Heart, and Lung Disease, Workshop on "Exposure, Metabolism and Mechanisms of Toxicity", January 27-30, 1981, Rockville, MD

International Symposium on "Chemical Indices and Mechanisms of Organ-Directed Toxicity", March 4-7, 1981, Barcelona, Spain.

Symposium on "Nonrespiratory Metabolic Functions of the Lung", Annual Meeting of the Federation of American Societies for Experimental Biology, April 12-17, 1981, Atlanta, Georgia.

Symposium on "Biological Kinetics of Chemically Reactive Metabolites", November 1-6, 1981, Sarasota Florida.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06513-05-CP

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bruce A. Chabner, M.D., Chief	CPB, DCT, NCI
	Jacques Jolivet, M.D., Visiting Fellow	CPB, DCT, NCI
	Peter Ellims, M.D., Ph.D., Visiting Fellow	CPB, DCT, NCI
	Chi-Ming Liang, Ph.D., Visiting Fellow	CPB, DCT, NCI
	Richard Schilsky, M.D., Clinical Associate	CPB, DCT, NCI
	Marc Lippman, M.D., Senior Investigator	MB, DCT, NCI
	James Drake, Biologist	CPB, DCT, NCI
	Brenda Bailey, Biologist	CPB, DCT, NCI
	Robert Do, Student Scientist	University of Maryland
	Lawrence Tamarkin, Ph.D.	NICHHD, NIH

COOPERATING UNITS (if any)

Pediatric Oncology Branch, Surgery Branch, Lab. of Chemical Pharmacology, Medicine Branch, Veterans Administration Oncology Branch, Lab. of Medicinal Chemistry and Biology

LAB/BRANCH

Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	3.5	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. We have shown that methotrexate polyglutamates (with one to four additional glutamates) are formed in a dose-dependent and time dependent manner, and their formation is associated with prolonged inhibition of DNA synthesis in human tumor cells. 2. We have examined the interaction of Pala and 5-FU with respect to their synergistic effects on thymidylate synthetase. 3. Studies have been completed on the kinetics, substrate specificity, and allosteric regulation of dCMP hydrolyase from human spleen and from leukemic leukocytes. 4. We have pursued investigations of the utility of test doses of methotrexate as predictors for the pharmacokinetics of high dose MTX in patients with altered renal function. 5. We have confirmed that melatonin decreases the incidence of DMBA induced breast cancer in prepubertal rats, that it partially corrects the increased rate in pinealectomized animals, and that these effects may be mediated by a drop in prolactin levels.

PHS-6040

1. We have shown that methotrexate polyglutamates (with one to four additional glutamates) are formed in a dose-dependent and time dependent manner, and their formation is associated with prolonged inhibition of DNA synthesis in human tumor cells. The MDA breast cell line does not form MTX G's at 2μm, a dose which causes brisk formation of MTX G's in two other breast cell lines; in the MDA line DNA synthesis recovers rapidly after removal of free drug while prolonged inhibition is seen in the lines which form polyglutamates. We are pursuing the possibility that MTXG formation is an important determinant of response to MTX and perhaps responsible for selective toxicity toward human tumor cells.

2. We have examined the interaction of PALA and 5 FU with respect to their synergistic effects on thymidylate synthetase. We have shown that pretreatment of Sarcoma 180 cells with PALA, an inhibitor of de novo pyrimidine synthesis, blunts the increase in dUMP and augments formation of FdUMP. Both actions increase the effectiveness of FdUMP inhibition of thymidylate synthetase as determined by direct assay of enzyme activity or by measurement of the terniary inhibited complex. A phase I-II study of PALA-FU has been completed in man, and has shown modest activity in malignant melanoma (1 partial response in 15 patients).

3. Studies have been completed on the kinetics, substrate specificity, and allosteric regulation of dCMP hydrolyase from human spleen and from leukemic leukocytes. This enzyme is is an important catalyst in the formation of the dUMP pool which in turn determines the thymidylate pool.

4. We have pursued investigations of the utility of test doses of methotrexate as predictors of the pharmacokinetics of high dose MTX in patient with altered renal function. Initial results in approximately 10 patients suggest a general correlation between test dose and high dose drug clearance rates in individual patients. It is hoped that test dose studies will allow dose adjustment and greater safety of use of high dose MTX.

5. We have confirmed that melatonin decreases the incidence of DMBA induced breast cancer in prepubertal rats, that it partially corrects the increased rate in pinealectomized animals, and that these effects may be mediated by a drop in prolactin levels. We have initiated a study of pineal hormone levels (melatonin) in patients with breast cancer.

Publications

Erlichman, C., Donehower, R.C., and Chabner, B.A.: The practical benefits of pharmacokinetics in the use of antineoplastic agents. Cancer Chemother. Pharmacol. 4: 139-145, 1980

Donehower, R.C., Allegra, J.C., Lippman, M.E., and Chabner, B.A.: Combined effects of methotrexate and 5-fluoropyrimidine on human breast cancer cells in serum-free culture. Europ. J. Cancer 16: 655-661, 1980.

Schilsky, R.L., Bailey, B.D., and Chabner, B.A.: Methotrexate polyglutamate synthesis by cultured human breast cancer cells. Proc. Nat. Acad. Sci. USA, 77: 2919-2922, 1980.

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Chabner, B.A.: Nucleoside and base analogs. In Cancer Chemotherapy, S.T. Crooke (Ed.), Academic Press, in press.

Karle, J.M., Anderson, L.W., Erlichman, C., et al: Serum uridine levels in patients receiving N-(phosphonacetyl)-L-aspartate. Cancer Res., in press.

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Cowan, K., Myers, C.E., and Chabner, B.A.: Drug monitoring of antineoplastic agents. In Richen, A., and Marks, V. (Eds.): The Therapeutic Drug Monitoring. London, Churchill-Livingstone, 1980, in press.

Chabner, B.A. and Myers, C.E.: Clinical pharmacology. In DeVita, V.T., Rosenberg, S., and Hellman, S. (Eds.): Principles and Practice of Oncology. Philadelphia, J.B. Lippincott, Co., in press.

Hande, K.R., and Chabner, B.A.: Pharmacology and biochemistry of high dose methotrexate. In Block, J. (Ed.): High-Dose Methotrexate Therapy of Clinical Cancer. CRC Press, in press.

Jones, R.B., Collins, J.M., Brooks, A.E., Hubbard, S.M., Balow, J.B., Brennan, M.F., Dedrick, R.L., Chabner, B.A., Young, R.C., DeVita, V.T., and Myers, C.E.: High volume intraperitoneal chemotherapy with methotrexate. Cancer Res., in press

Hande, K.R., and Chabner, B.A., B.A.: Pharmacology and biochemistry of high dose methotrexate. In High-Dose Methotrexate Therapy of Clinical Cancer, J. Block (Ed.), CRC Press, in press.

Hande, K.R., Stoller, R.G., Drake, J.C., Myers, C.E., Rosenberg, S.A., and Chabner, B.A.: The value of monitoring plasma methotrexate concentrations during high-dose infusions. Europ. J. Cancer, in press.

Ellims, P., Kao, A., and Chabner, B.A.: Deoxycytidylate deaminase: Purification and some properties of the enzyme isolated from human spleen. J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06514-03-CP

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacology and Clinical Application of Intraperitoneal Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles E. Myers, M.D., Head, Biochemical Pharmacology Section, CPB, DCT, NCI

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R. L. Dedrick, Ph.D., Biomed Eng. & Instrum. Branch, DRS, NIH
J. M. Collins, Ph.D., Biomed. Eng. & Instrum. Branch, DRS, NIH
P. H. Sugarbaker, M.D., Surgery Branch, DCT, NCI
Robert Ozols, M.D., Senior Investigator, Medicine Branch, DCT, NCI
Luca Gianni, M.D., Visiting Fellow, CPB, DCT, NCI
Raymond Greene, Pharmacist, CPB, DCT, NCI

COOPERATING UNITS (if any)

Medicine Branch, DCT, NCI; Biomedical Engineering & Instrumentation Branch, DRS, NIH; Surgery Branch, DCT, NCI; Diagnostic Radiology Department, CC, NIH

LAB/BRANCH

Clinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4	1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This laboratory has been involved in trying to improve the therapeutic efficacy of antineoplastic agents by administering them intraperitoneally via semi-permanent dialysis catheters to patients with tumor confined to the intra-abdominal space.

We have finished clinical trials of methotrexate and 5-FU and are currently conducting phase I trials of adriamycin and phase II trials of i.p. 5-FU. Pharmacologic studies are being conducted to define the transport of these drugs across the human peritoneum, measure the possible first-pass hepatic clearance and levels of hepatic perfusion, and correlate drug levels to clinical efficacy and toxicity.

1. I.P. Methotrexate

With the assistance of Drs. Brennan and Balow, we have administered increasing doses of i.p. methotrexate with peripheral leucovorin rescue to five patients. Limiting toxicity consisted of apparent chemical peritonitis. There were no tumor responses. A ratio between peritoneal fluid and plasma drug levels of 25 was achieved.

2. I.P. 5-FU

With the assistance of Drs. Sugarbaker, Brennan, Collins, and Dedrick, we have conducted phase I trials of 5-FU i.p. in ten patients. Dialysate concentrations ranged from 5 M to 5 mM and was administered for eight consecutive 4-hour exchanges every two weeks. Limiting toxicity was the same as systemically administered 5-FU. A very steep dose toxicity relationship was observed. There were two objective responses, both in prior treated ovarian patients. Peritoneal drug levels fell by a first order mechanism with a t of 1.6 hours. At 4 hours, a ratio between peritoneal fluid and plasma drug levels of 298 was achieved.

Currently, the phase II trial is being conducted. Special concurrent investigations include 1) correlation of drug response to in vitro cell culture sensitivity assay in collaboration with Dr. Ozols, 2) correlation of drug levels to indocyanine green clearance, and 3) correlation of drug levels and toxicity with bone marrow FdUMP levels.

3. I.P. Adriamycin

Preclinical studies have been conducted by Drs. Ozols, Myers, and Young, and Ms. Karen Grotzinger to establish a model and rationale for i.p. adriamycin, a murine ovarian tumor model. Drs. Ozols and Myers with the assistance of Drs. Sugarbaker and Brennan are conducting phase I studies. Three patients have been treated with 5 mg to 60 mg per 2-liter dialysate. Fever and hyperamylasemia in several patients may indicate local toxicity. In two patients there was a significant decrease in ascites volume subsequent to drug administration. Preliminary measurements indicate the peritoneal fluid to plasma drug ratio achieved to be at least 200. Dr. Weltz worked on adapting an HPLC assay to analysis of these samples so that both parent compound and metabolites can be measured and correlations made separately to clinical efficacy and toxicity.

4. I.P. Misonidazole

In conjunction with the Radiation Therapy Branch, we are studying the pharmacology of misonidazole intraperitoneally. We hope by this means to deliver the radiation sensitizer to the hypoxic areas of ovarian tumor.

Publications

Murinson, D.C., Anderson, T., Schwartz, H.S., Myers, C.E., and Chabner, B.A.: Competitive binding radioassay for 5-fluorodeoxyuridine-5' monophosphate in tissues. Cancer Res. 39: 2471-2475, 1979.

Jones, R., Buckpitt, A., Londer, G., Myers, C., Chabner, B., and Boyd, M.: Potential chemical applications of a new method for quantitation of plasma levels of 5 FU and 5-FUdR. Bulletin du Cancer 66: 67-74, 1979.

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Ozols, R.F., Locker, G.Y., Doroshow, J.H., Grotzinger, K.R., Myers, C.E., and Young, R.C.: Adriamycin treatment of ovarian cancer: Pharmacokinetics, tissue penetration and rationale for intraperitoneal administration. Cancer Res. 39: 3209-3214, 1979.

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Dunnick, N.R., Jones, R.B., Doppman, J.L., Speyer, J.L., and Myers, C.E.: Intraperitoneal contrast infusion for assessment of intraperitoneal metastases and fluid dynamics. Am. J. Radiol. 133: 221-223, 1979.

Speyer, J.L., Collins, J.M., Dedrick, R.L., Brennan, M.R., Londer, H., DeVita, V.T., and Myers, C.E.: Phase I and pharmacologic studies of intraperitoneal 5-FU. Cancer Res. 40: 567-572, 1980.

Speyer, J.L. and Myers, C.E.: The use of peritoneal dialysis in delivery of chemotherapy in intraperitoneal malignancies. In Recent Results in Cancer Research, G. Mathe and F. Muggia (Eds.), Springer-Verlag, in press.

Collins, J.M., Dedrick, R.L., King, F.G., Speyer, J.L., and Myers, C.E.: Nonlinear pharmacokinetic models for 5-fluorouracil in man. Clin. Pharm. Therap., in press.

Speyer, J.L. and Myers, C.E.: Intraperitoneal chemotherapy of ovarian cancer. In Recent Advances in Clinical Oncology. Whitehouse and Williams (Eds.), Churchill-Livingstone, Edinburgh, in press.

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Young, R.C., Myers, C.E., Ozols, R.G., Hogan, W.M.: Current Concepts in Cancer: Ovary chemotherapy in advanced disease Jr. Rad. Oncol. Biol. Phys. in press.

Speyer, J.L., Sugarbaker, P.H., Collins, J.M., Dedrick, R.L., Klecker, R.W., Myers, C.E.: Portal levels and hepatic clearance of 5FU after intraperitoneal administration. Cancer Res 41: 1916-1922, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06515-02-CP

PERIOD COVERED October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biologic Response Modification: Free Radical Defenses and Their Modification by Nutrition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Elizabeth Travis, Ph.D. ROB, DCT, NCI
James Schwade, Ph.D. ROB, DCT, NCI
Stephen Rosenberg, M.D., Ph.D., Chief, Surgery Branch, DCT, NCI
Victor Ferrans, M.D., Chief, Ultrastructure, NHLBI
Robert Bonow, M.D. NHLBI

COOPERATING UNITS (if any)

Radiation Oncology Branch and Surgery Branch, DCT, NCI; Ultrastructure, National Heart, Lung and Blood Institute.

LAB/BRANCH

Clinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.9	PROFESSIONAL: 4.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our work with adriamycin cardiac toxicity has led us to re-examine the role of endogenous free radical defenses in moderating free radical damage. Since free radical damage is a process which plays a role in such diverse phenomena as aging, ionizing radiation damage, oxygen toxicity, carcinogenesis, and drug toxicity, we have tried to structure our investigation in such a way so as to yield results pertinent to this broad range of problems rather than to focus narrowly on anthracycline cardiac toxicity.

I. Free Radical Defense Systems

The existing defenses against free radical attack have not been adequately described. We have, therefore, begun to examine these defenses in greater detail. In the process, we have found a relationship between selenium-dependent glutathione peroxidase and vitamin E which suggests that they interact positively. In addition, Dr. Katki discovered a new peroxidase bound to mitochondrial membrane and nuclear envelope which may play an important role in maintaining mitochondrial function and in protecting DNA from free radical damage. This new enzyme is of interest because it is membrane-bound, is not affected by selenium, and will utilize both hydrogen peroxide and lipid peroxides. We plan to examine the physiologic role of this enzyme further and to extend our examination of free radical defenses to other enzyme functions.

II. Role of Membrane Tocopherol

Vitamin E is a known *in vitro* free radical scavenger. *In vivo* it is found almost exclusively in cell membranes. In that setting it is not clear whether it operates as a radical scavenger or via other membrane-stabilizing mechanisms. In order to examine this process more thoroughly, Dr. Breed has developed an HPLC assay for tocopherol and one of its oxidation, tocopherol quinone.

These assays exceed by far the sensitivity of any previous technique, and we have been able to establish that baseline tocopherol quinone levels in cardiac tissue are 600-800 femtomoles per gram wet weight of tissue. This level increases to 1,600 femtomoles a few hours after DEM, suggesting that thiols are critical for the maintenance of vitamin E in the membrane.

What is most confusing is that supralethal radiation or adriamycin do not alter this pool. These results suggest minimal direct involvement of tocopherol in acute free radical attack. We intend to pursue this question further as it is of major theoretical importance.

III. Role of Glutathione in Radiation Damage

Although reduced glutathione is known to be a major defense against free radical attack, its role in radiation effects has really not been examined carefully. In collaboration with the radiation biologists under Dr. Travis, we hope to examine the role of glutathione in some detail. To that end, we have established assays for reduced glutathione, total thiols, and glutathione peroxidase, the major enzyme which utilizes glutathione to protect against free radical attack.

In addition, we have established mouse models where we can selectively delete each of the free radical defense enzymes either genetically or via diet. This includes superoxide dismutase, catalase, and glutathione peroxidase. These animals will allow us to test the role of glutathione in the presence and absence of each or all of the radical defenses.

Publications

Doroshow, J., Locker, G., and Myers, C.: Experimental animal models of adriamycin cardiotoxicity. Cancer Treat. Rep. 63: 855-860, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03403-16 M

PERIOD COVERED: October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert C. Young	Chief	M	NCI
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	Charles Myers	Chief	CP	NCI
	Richard Fisher	Sr Investigator	M	NCI
	Marc Lippman	Sr Investigator	M	NCI
	Dan Longo	Sr Investigator	M	NCI
	Robert Ozols	Sr Investigator	M	NCI
	Jacqueline Whang-Peng	Sr Investigator	M	NCI
	Vincent DeVita	Director		NCI
	Richard Simon	Chief	BR	NCI
	Steven Rosenberg	Chief	S	NCI
	Eli Glatstein	Chief	RO	NCI
	Elaine Jaffe	Sr Investigator	LP	NCI

COOPERATING UNITS (if any) Radiation Oncology Branch, NCI; Clinical Pharmacology Branch NCI; Biometrics Research Branch,NCI; Surgery Branch,NCI; Immunology Branch, NCI; Laboratory of Molecular Pharmacology, Environmental Epidemiology Branch, NCI.

LAB/BRANCH: Medicine Branch

SECTION:

INSTITUTE AND LOCATION: Clinical Oncology Program, NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
26	19.5	7.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medicine Branch is a major clinical facility of the NCI. Its activities are divided between clinical therapeutic trials in cancer patients and related laboratory research. Clinical trials of cancer treatment are currently underway in breast cancer, ovarian cancer, Hodgkin's disease, non-Hodgkin's lymphomas, testicular tumors, soft tissue sarcomas, cervical carcinoma, and brain tumors. Programs in melanoma and pancreatic carcinoma have been closed and the information published. Phase I-II clinical trials have been completed this year on the following new experimental agents or combinations: AMSA, PALA-5-FU, chlorozotocin, pentamethylmelamine. Phase II trials continue on AZQ, 13-cis-retinoic acid,intraperitoneal chemotherapy of adriamycin and AMSA by infusion. New Phase I studies include Interferon (recombinent DNA produced) and Aclacinomycin A. Additional summaries of clinical studies are summarized under reports entitled "Clinical Program in Breast Carcinoma." Laboratory researach of the branch is summarized under reports entitled "Clinical Pharma-

Other (cont'd):	Susan Hubbard	Chief,	SI	NCI
	Audrey Barlock	Chemo Res Nurse	M	NCI
	Jane Cassidy	Chemo Res Nurse	M	NCI
	First and Second Year Associates		M	NCI

Summary of Work (Continued):

cology of Antineoplastic Agents, Kinetics of Cellular Proliferation, Cytogenetic Studies, Immunologic Aspects of Cancer, Mechanisms of Hormone Dependence of Human Malignancy, and Genetic Regulation of the Immune Response."

Major Accomplishments in 1980-1981

General:

In 1980 the Medicine Branch staff published 78 papers, articles, or book chapters and in 1981 the Medicine Branch staff has published or has in press 93 publications. This is the largest number of scientific publications in the history of the Branch. Details of the clinical laboratory studies will be reviewed in the subsequent sections.

Ovarian Carcinoma: Established:

1) The CHex-UP combination (cyclophosphamide, hexamethylmelamine, 5-fluorouracil and cis-platinum)for advanced epithelial ovarian cancer has been reported this year. Fifty-one patients have been entered on study. Overall response rate is 74.5% with 41% complete remissions. At restaging 20% of all patients are pathologically free of disease and none of these patients have died with a median followup of 2 years. Analysis of survival by response is highly statistically significant and the beneficial results cannot be explained by histologic grade or extent of disease. The randomized portion of the study testing intraperitoneal 5-FU therapy vs no additional therapy in patients in pathologic CR continues.

2) The activity of the Hexa-CAF combination in advanced ovarian cancer continues to be confirmed by other investigators (Mendiola et al) and is now being compared in the prospective trial with CAP (cyclophosphamide, adriamycin, platinum) at the Princess Margaret Hospital, Toronto, Canada. The median survival of 29 months seen the Medicine Branch Hexa-CAF trial, remains the longest yet reported for advanced epithelial ovarian cancer.

3) Phase I & II trials of 5-FU and methotrexate have been completed and published and will be reviewed under sections of the Clinical Pharmacology Branch Annual Report. Essentially large volume intraperitoneal chemotherapy with these agents is feasible and produced a 25-300 fold excess of drug within the peritoneal space compared to plasma concentrations. In the adjuvant 5-FU study in patients rendered disease-free with intensive induction chemotherapy, 2 patients without intraperitoneal therapy have have relapsed at 8 and 17 months. One patient in the I.P. 5-FU group has relapsed at 24 months. The phase II I.P. adriamycin trial continues. Six patients have been entered at doses from 10-60 mg/2 liters. Two patients

thus far had objective regression of disease.

4) Extensive experience with the human ovarian cancer clonogenic cell assay has been completed. Over 120 patients have been studied. Approximately 80% of samples from ascites, pleural fluid and peritoneal washings can be successfully cloned, and about 40% have sufficient cells for drug testing. Clinical correlation with assay findings exceeds 90% for inactive agents and exceeds 64% for those deemed active in the assay. A dose response relationship between adriamycin concentrations and ovarian cancer cells has been demonstrated which gives direct rationale to the intraperitoneal use of the drug in selected patients.

5) Over 100 patients with "early" ovarian carcinoma have now had staging laparotomy prior to definative therapy at Ovarian Cancer Study Group institutions. Prior to referral only 25% of patients had a surgical incision which was adequate to evaluate the entire abdomen. Thirty two percent of patients referred, apparently free of disease, had residual disease identified by careful staging. This study has established the need for careful surgical evaluation in "early" ovarian cancer and should alter significantly the future management of such patients.

6) Approximately 126 patients have now been randomized to the Ovarian Tumor Study Group/GOG study on early ovarian cancer initiated by the Medicine Branch. Preliminary results from the Stage Ia & Ib study suggest that there will be few relapses in carefully staged patients regardless of initial adjuvant therapy.

Ovarian Carcinoma: Published:

The use of percutaneous aspirations of retroperitoneal lymph nodes in ovarian cancer; that the defective monocyte killing in patients with ovarian cancer can be restored after cis-platinum therapy; histologic grade has prognostic importance in response to chemotherapy in advanced disease;the use of peritoneoscopy in the management of ovarian cancer; reviews of the staging and treatment of ovarian cancer; strategies for effective management of early ovarian cancer; comprehensive reviews of the chemotherapy of gynecologic malignancies.

Hodgkin's Disease: Established:

1) The lack of influence of drug dose or timing on response to MOPP in previously untreated advanced Hodgkin's disease. Previous investigations had suggested that dose or dose rate might be an important influence on ultimate response and/or survival. Review of 156 patients in the MOPP study revealed that nearly all of our patients received over 75% of the projected dose of chemotherapy and there were no effects of nitrogen mustard or procarbazine dose or timing on treatment outcome.

2) The long-term effect of MOPP therapy on ovarian function of women cured of Hodgkin's disease. Twenty-seven women were studied. Persistent amenorrhea occurred in 46% and was age related. Ovarian failure is often gradual in onset but to date children born to women treated with MOPP

appear entirely normal.

3) That T cells from untreated Hodgkin's disease patients have increased sensitivities to normal monocyte suppressor cells and this is not reversed by indomethacin nor is it caused by plasma factors. Increased sensitivity of T cells to monocyte suppressor cells is detected in long-term survivors of Hodgkin's disease but not in diffuse lymphoma. T cell proliferation is also more sensitive to the suppressor T cell. Con A induced suppressor cells from Hodgkin's disease patients exhibit normal regulatory control of the proliferative responses of normal T cells but show enhanced effect on T cells from Hodgkin's disease patients.

Hodgkin's Disease: Published:

1) The 10 year followup of 198 patients with advanced Hodgkin's disease was published this year. Complete remission rate is 80%. Sixty-eight percent of the complete remitters remain continuously disease free at 10 years. Overall survival for those achieving remission is 82% at 5 years and 73% at 10 years. These results show that advanced Hodgkin's disease is curable with combination chemotherapy.

2) A persistent defect in T cell function occurs in Hodgkin's disease patients who have been cured of their disease. This defect appears intrinsic to the disease and is not seen in patients with diffuse histiocytic lymphoma treated with similar chemotherapy. The defect appears to be related to abnormal responses to normal suppression T cells in patients with Hodgkin's disease.

3) That prolonged disease free survival can be achieved in Hodgkin's disease following MOPP reinduction after first relapse. Median survival of all patients so treated exceeds 5 years from the time of first relapse.

Non-Hodgkin's Lymphoma: Established:

1) The importance of histologic conversion on the relapse frequency, response to therapy, and subsequent survival of patients with non-Hodgkin's lymphoma. Nodular lymphomas which convert to diffuse histologies have a worse prognosis and shorter survival unless induced into a complete remission by aggressive chemotherapy. These data have important implications on the designs of new trials in nodular lymphomas.

2) The activity of ProMACE-MOPP induction therapy in advanced diffuse large cell lymphoma. The overall objective response rate to this regimen is 93%. Pathologically documented complete remissions have been seen in 64% of patients and the median survival of the group has not yet been reached but will exceed 30 months. This is the longest thus far reported in this disease. Relapse after achieving a complete remission is very uncommon.

3) The successful use of a lipid soluble contrast material to enhance the resolution of lymphomatous lesions in the liver and spleen using computed tomography.

4) The lack of benefit of total parenteral nutrition (TPN) as an adjuvant to the induction chemotherapy of diffuse lymphoma. This prospective randomized study revealed that TPN did not enhance the response rate, nor did it improve the tolerance to chemotherapy. Furthermore, it was associated with significant complications, particularly venous thrombosis and local infections.

Non-Hodgkin's Lymphoma: Published:

1) Comprehensive review of 473 patients with non-Hodgkin's lymphoma treated at the NCI over a 22 year period has resulted thus far in 7 publications. Highlights of these comprehensive studies include: the recognition of the frequency of histologic conversion in the "good prognosis" lymphomas; the aggressive nature of nodular histiocytic lymphoma; comparisons of alternative histologic classifications; the staging of non-Hodgkin's lymphomas; and prognostic factors in non-Hodgkin's lymphoma.

2) The characterization of the natural history of divergent histologies which present at initial diagnosis.

3) Description of the central nervous system complications of non-Hodgkin's lymphoma and the characterization of the population at high risk for CNS lymphoma. Successfully defined the population requiring prophylactic therapy.

4) Gallium Scanning in non-Hodgkin's lymphoma: comprehensive review of our 10 year experience staging the lymphomas shows that routine use of the gallium scan in staging is not cost effective and uncommonly is positive when other studies are not.

Breast Carcinoma:

Details of the clinical programs on breast cancer may be found with section entitled Clinical Program in Breast Cancer. Highlights of this program include:

1) A prospective randomized study of chemotherapy $\pm$ hormonal therapy to induce tumor cell synchrony now has 75 patients on study. Response rate and duration of remission are better for the group receiving hormonal synchronization and particularly for ER+ patients.

2) A prospective randomized trial of hormonal therapy has been completed this year with 112 evaluable patients. Patients receiving a combination of tamoxifen + halotestin had a higher response rate and remission duration than those receiving tamoxifen alone.

Melanoma: Established:

Our long term effort in melanoma has been reduced over the past several years because of severe bed constraints presently effecting the Medicine Branch. However, several long-term studies continue to be maintained.

1) The prospective trial randomizing poor prognosis Stage I and II melanoma patients to MeCCNU, BCG, and BCG + tumor cell vaccine has been closed to new accrual. Preliminary analysis suggests a somewhat longer disease free interval for patients treated with MeCCNU or with BCG + tumor cell vaccine. However, survival is not different for any of the 4 groups. The followup continues.

Melanoma: Published:

1) A comprehensive review of the presence of steroid receptors in malignant melanoma and their potential role in response to hormonal therapy was published this year as was a review of the endocrine aspects of malignant melanoma.

Testis Tumors: Established

1) The lack of benefit associated with aggressive surgical cytoreduction prior to the use of combination chemotherapy in advanced testicular tumor. This is the first prospective clinical trial in any malignancy which tests the potential benefit of surgical cytoreduction prior to curative chemotherapy. In this disease, pre-chemotherapy cytoreduction is of no benefit.

2) The successful cloning of human testicular cancer in the soft agar clonogenic assay. Special staining with flourescent labeled HCG and alfa feto protein antibodies has established the true origin of these clones. Studies screening new agents against non-seminomatous testicular cancer are now underway.

Testis Tumors: Published:

1) The lack of benefit of cytoreductive surgery prior to aggressive chemotherapy in the treatment of non-seminomatous testicular cancer.

2) Correlation of computerized tomography and serum tumor markers in metastatic retroperitoneal testicular cancer.

3) Reviews of the role of chemotherapy in the management of bladder cancer and other genitourinary neoplasms. Hypomagnesemia and renal magnesium wasting in patients receiving cis-platinum.

Sarcomas: Published:

1) The apparent lack of improvement in survival in osteogenic sarcoma from high dose methotrexate but the improved survival related to repeated surgical resection.

2) An anaplastic sarcoma arising in a benign chrondroblatoma.

Pancreatic Carcinoma:

The Medicine Branch program on pancreatic carcinoma has been phased out because of the severe restrictions on the available inpatient beds. Never-

theless, considerable information generated from these clinical trials has been published this year. These include comprehensive reviews of the management of pancreatic cancer; the role of peritoneoscopy in the staging of pancreatic cancer; a prospective evaluation of tumor associated polypeptide hormones in pancreatic adenocarcinoma; and lymphography in surgically unresectible adenocarcinoma of the exocrine pancreas.

Other major accomplishments in 1980-1981 will be found in the individual project title reports for the Medicine Branch.

Z01 CM 03404-10 M
Z01 CM 06119-12 M
Z01 CM 06702-06 M
Z01 CM 06700-08 M
Z01 CM 06708-02 M
Z01 CM 06709-01 M

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1. DeVita, V.T., Simon, R.M., Hubbard, S.M., Young, R.C., Berard C.W., Moxley, H.J., Frei, E. III, Carbone, P.P. and Canellos, G.P.: On the curability of advanced Hodgkin's disease with chemotherapy: long-term follow-up of MOPP treated patients at the NCI and the influence of disease variables on prognosis. Ann. Int. Med. 92: 587-595, 1980.

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BREAST CARCINOMA

See Section entitled "Clinical Program in Breast Carcinoma"

MELANOMA

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SARCOMAS

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MISCELLANEOUS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03404-10 M

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunologic Aspects of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard Fisher	Sr Investigator	M	NCI
	Bruce Silver	Clinical Associate	M	NCI
	Zdenko Krizan	Visiting Fellow	M	NCI
	Frieda Bostick-Bruton	Technician	M	NCI
	Dean Mann	Sr Investigator	E	NCI
	Elaine Jaffe	Sr Investigator	LP	NCI

COOPERATING UNITS (if any)

Epidemiology Branch, DCCP, NCI; Laboratory of Pathology, DCBD, NCI

LAB/BRANCH

Medicine Branch

SECTION

INSTITUTE AND LOCATION

Clinical Oncology Program, NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent studies in this laboratory have demonstrated that long-term survivors with Hodgkin's disease have significant abnormalities of immune function unassociated with the presence of active disease. Assays of non-specific immunity are abnormal in patients with Hodgkin's disease cured by MOPP chemotherapy. These abnormalities cannot be attributed to the chemotherapy since they are not present in patients with diffuse histiocytic lymphoma cured by similar chemotherapy regimens. T lymphocytes in both untreated and cured patients with Hodgkin's disease have increased sensitivity to suppression by normal immune regulatory cells. This has been shown for both a macrophage suppressor cell and T cell suppressor induced by concanavalin A. Both suppressor systems operate independently of prostaglandins. Studies have demonstrated the presence of suppressor B cells that regulate the proliferative responses of human T cells. Patients with Hodgkin's disease have

markedly enhanced T cell responses after inactivation of their suppressor B cells. Studies are being conducted to determine whether these patients have increased sensitivity to the suppressor B cells. A new serologic specificity closely linked to the HLA-Dr locus has been observed in cured Hodgkin's patients. Studies are in progress to define the genetics of these immune abnormalities by studying families of affected patients. Lymphocytes from patients with non-Hodgkin's B cell lymphomas are being studies to determine their ability to proliferate, differentiate, and produce antibody. A lack of effective T helper cells has been demonstrated. Lymphocytes from non-Hodgkin's T cell lymphomas are being characterized. Although many have a T helper cell phenotype defined by monoclonal antibodies, they are functionally inactive. Both B and T cell non-Hodgkin's lymphomas can respond clinically to treatment with an anti-lymphocyte serum. A murine model of ovarian cancer has been studied. The inability to generate cellular immunity against the tumor has been explained by the tumor's lack of H-2 antigens. Successful immunotherapy has been shown to be dependent on the agent utilized, dose, route, and schedule. Synergism between chemotherapy and immunotherapy is observed. These studies provide a basis for a Phase I human ovarian immunotherapy trial.

Publications:

1. Fisher, R.I., Mandell, G.L., Bostick, F., McMenamin, M.G., and Anderson, T.: Chlorozotocin, an antitumor agent lacking bone marrow toxicity at therapeutic doses: Effects on lymphocyte subpopulations in mice. Clin. Exp. Immunol. 39: 416-425, 1980.

2. Fisher, R.I., DeVita, V.T., Bostick, F., Vanhaelen, C., Howser, D.M., Hubbard, S.M., and Young, R.C.: Persistent immunologic abnormalities in long term survivors of advanced Hodgkin's disease. Ann. Intern. Med. 92: 595-599, 1980.

3. Fisher, R.I., Vanhaelen, C., Bostick, F.: Increased sensitivity to normal adherent suppressor cells in advanced Hodgkin's disease. Blood 57: 830-835, 1981.

4. Vanhaelen, C.P.J., and Fisher, R.I.: Increased sensitivity of lymphocytes from patients with Hodgkin's disease to concanavalin A-induced suppressor cells. J. Immunol. (in press).

5. Vanhaelen, C., and Fisher, R.I.: Requirements for successful immunotherapy and chemo-immunotherapy of a murine model of ovarian cancer. Cancer Res. 41: 980-983, 1981.

6. Vanhaelen, C.P.J., Fisher, R.I., Appella, E.,and Ramanathan, L.: Lack of histocompatability antigens on a murine ovarian teratocarcinoma. Cancer Res. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06119-12 M

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cytogenetic Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacqueline Whang-Peng	Sr Investigator	MB	NCI
Other:	Turid Knutsen	Med Technologist	MB	NCI
	Elaine Lee	Chemist	MB	NCI
	Paul Bunn	Sr Investigator	VAMO	NCI
	Eli Glatstein	Chief	RO	NCI
	Sam Broder	Sr Investigator	I	NCI
	Ian Magrath	Sr Investigator	PO	NCI
	Susan Seiber	Sr Investigator	LCHP	NCI
	Nobuyuki Shitara	Sr Investigator	SN	NINCDS
	Chien-Song Kao	Visiting Fellow	MB	NCI
	Jose Costa	Chief	SP	CC
	Leonard Zwelling	Sr Investigator	LMP	NCI
	Stuart Bentley	Expert Consultant	LP	NCI

COOPERATING UNITS (if any)

Lab Immunology, NCI; Pediatric Oncology Br, NCI; Lab Chem Pharm, ET, NCI; Clin Pathology, ET, NCI: Radiation Oncology Br, NCI; VA-MOP; Surg Neurology Br, NINCDS; Surg Path Post-Morten Service, CC: Lab Path, NCI

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology

INSTITUTE AND LOCATION

Clinical Oncology Program, NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The areas of investigation:

1. Cytogenetic studies of human neoplastic, hematological, and congenital disease.
2. In vitro cytogenetic studies of direct tumor material, tissue culture lines, and colony cultures derived or established from patients with Burkitt's lymphoma, small cell carcinoma of lung, and cancer of the brain, ovary, or testis.
3. Early detection of secondary leukemia in patients with non-Hodgkin's lymphoma, CLL, small cell carcinoma of lung using chromosomal abnormalities as markers.
4. Detection of sister chromatid exchanges in vivo and in vitro following exposure to ultrasound.

5. Observation of chromosomal abnormalities in patients with cutaneous T-cell lymphomas; studies performed on peripheral blood, bone mrrow, lymph node, and tissue culture lines.
6. Collaborative study of the cytogenetics of various mitotic inhibitors such as AMSA and other Phase I drugs, using both in vitro and in vivo techniques.
7. Study of steroid protection of spermatogenesis in chemotherapeutic trials using a mouse model.
8. Study of the origin of the hematopoietic microenvironment in continuous culture of mouse bone mrarow using cytogenetic markers.
9. Study using high resolution chromosome banding to determine whether the 8;14 translocation observed in Burkitt's lymphoma is a simple or reciprocal translocation.

Projects Completed in Past Year:

1. Cytogenetic study of lung cancer: 16 small cell and 5 non-small cell tissue culture lines were studied. The results showed a specific chromosomal abnormality (deletion 3p) in all the small cell lines: all lines involved chromosome bands 3p (14-23). This specific abnormality was not seen in any of the non-small cell lung cancer lines.

2. Study of the origins of marrow-derived adherent cells in continuous marrow cultures in mice: The use of the T6 cytogenetic marker showed that the miciroenvironment in such cultures is of stromal origin.

3. Studies of chromosome aberrations and sister chromatid exchanges in families with ataxia telangiectasia: These studies were conducted to more fully understand the mechanisms of genetic instability, and to correlate chromosome abnormalities with the early detection of malignant transformation.

4. Preliminary study of the chromosomal effects of ultrasound: Ultrasound applied to in vitro lymphocyte cultures at frequencies from 1.0-5.0 MHz and 10-200 volts produced no detectable increase in chromosome damage.

5. Cytogenetic study of the in vivo and in vitro effects of the drug AMSA: The results of in vitro studies showed an increase in sister chromatid exchanges with increasing drug concentration (lowest effctive dose was 0.025 ug/ml) and increase in chromosome breakage from 0.005 to 100% at a dose of 0.25%. The in vivo studies are ongoing.

Publications:

1. Seiber, S.M., Botkin, C.C., Soong, P., Lee, E.C., Whang-Peng, J: Embryotoxicity in mice of phosphonacetyl-L-aspartic acid (PALA), a new antitumor agent. I. Emryolethal, teratogenic, and cytogenetic effects. Teratology 22: 311-320, 1980.

2. Lees, D.E., Kim, Y.D., Bull, J.M., Whang-Peng, J., Schuette, W., Smith, R., Macnamara, T.E.: Anesthetic management of whole-body hyperthermia for the treatment of cancer. Anesthesiology 52: 418-428, 1980.

3. Bunn, P.S., Whang-Peng, J., Carney, D.N., Schlam, M.L., Knutsen, T., Gazdar, A.F.: DNA content analysis by flow cytometry and cytogenetic analysis in mycosis fungoides and Sezary sundrome. J. Clin. Invest. 65: 1440-1448, 1980.

4. Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Serial cytogenetic studies of non-endemic Burkitt's lymphoma cell lines. JNCI 65: 891-895, 1980.

5. Douglass, E.C., Poplack, D.G., Whang-peng, J.: Involvement of chromosome 22 in neuroblastoma. Cancer Genet. Cytogenet. 2: 287-291, 1980.

6. Whang-Peng, J., Knutsen, T.: Chromosomal abnormalities in CML. In Chronic Granulocytic Leukaemia. Shaw, M.T. (Ed.), W.B. Saunders, Co., in press.

7. Shuette, W.H., Lees, D.E., Bull, J.M., Kim, Y.D., Whang-Peng, J., Smith, R., Tipton, H.W.: Regulation of core body temperature during whole body hyperthermia by oesophageal temperature feedback. Med. Biol. Eng. Comput. 18: 299-302, 1980.

8. Lees, D.E., Kim, Y.D., Schuette, W., Bull, J., and Whang-Peng, J.: Causes of induced hyperthermia. Anesthesiology 50: 69-50, 1979 (letter).

9. Schuette, W.H., Lees, D.E., Bull, J.M., Tipton, H., Kim, Y.D., Whang-Peng, J., Smith, R., and Bynum, G.D.: Feedback control of esophageal temperature during whole body hyperthermia. In Advances in Bioengineering. Eberhart, R.G. and Burstein, A.H. (Eds.), New York, American Society of Mechanical Engineers, 1979, pp. 109-110.

10. Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Cytogenetic studies in non-African Burkitt's lymphoma. Blood 55: 148-155, 1980.

11. Eil, C., Douglass, E.C., Rosenberg, S.M., Kano-Sueoka, T.: Receptor characteristics of the rat mammary carcinoma cell line 64-24. Cancer Res. 41: 42-48, 1981.

12. Whang-Peng, J., Lee, E., Knutsen, T., and Solanki, D.: Dicentric isochromosome for the long arm of chromosome 17 dic i (17q) in a patient with chronic myelogenous leukemia. Cancer Genet. Cytogenet., in press.

13. Whang-Peng, J., Lees, D.E., Schueete, W.H., Smith, R., Bull, J.M., DeVita, V.T.: Erythrocyte osmotic fragility in patients receiving hyperthermia with and witout chemotherapy. Cancer Treatment, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06700-08 M

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Clinical Program in Breast Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marc E. Lippman	Senior Investigator	M	NCI
Other:	Jane Cassidy	Nurse	M	NCI
	Margaret Wesley	Biostatistician	BR	NCI
	Allan Lichter	Senior Investigator	RO	NCI
	Ernest DeMoss	Senior Investigator	S	NCI
	Sandra Levy	Senior Investigator	DCCR	NCI
	David Danforth	Guest Worker	M	NCI

COOPERATING UNITS (if any)

Biometrics Research Branch, NCI; Radiation Oncology Branch, NCI; Surgery Branch, NCI

LAB/BRANCH
Medicine Branch and Division of Cancer Control and Rehabilitation

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
Clinical Oncology Program, NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3 1/2	2 1/2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at breast cancer. Clinical trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal regimens are underway. Biochemical and hormonal receptor studies are undertaken and coordinated by the Medical Breast Cancer Section. Clinical studies consist of a major chemotherapy trial aimed at synchronizing human breast cancer cells with hormonal agents for more successful cell cycle phase specific chemotherapy; a hormonal therapy trial aimed at prospectively evaluating the usefulness of steroid receptors for estrogens, androgens and progestins in human breast cancer; an advanced disease hormonal therapy trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol, and several phase II trials including 13 Cis Retinoic acid and Aclacinomycin. A trial for stage IV NED patients is about to be initiated. In addition there is an endocrine and chemotherapy program for male breast cancer. A cooperative

trial between the Surgery, Radiation and Medicine Branches is underway comparing excisional biopsy plus definitive radiotherapy to simple mastectomy in clinical Stage I and II breast cancer. All patients have axillary dissections; A-C chemotherapy is given to all N+ patients.

Finally, a prospective psychological study aimed at discovering whether or not patients' emotional responses to their disease influences outcome is underway.

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Recurrent disease trials.

1. MB-160, a randomized trial of chemotherapy ± hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has approximately 75 patients on study and preliminary analysis suggests ER+ patients may benefit from synchronization. Response rate and duration currently favor the synchronization arm.

2. MB-132, a randomized trial of tamoxifen ± halotestin. This trial is completed with 112 evaluable patients. Tamoxifen plus halotestin is superior in response rate and duration. Both regimens are substantially less effective in heavily pre-treated patients but the combination is superior in all prognostic categories.

3. A new randomized primary endocrine trial comparing tamoxifen plus fluoxymesterone to tamoxifen plus danazol has been initiated.

4. A Phase II trial of 13 cis retinoic acid in breast, ovary, testicular cancers and melanoma is underway. No responses in 18 BC patient have been seen.

5. A psychological study of how patient attitudes influence survival is ongoing.

6. A protocol for sequential endocrine approaches to male breast cancer with concurrent receptor analyses is ongoing.

7. A randomized trial of radical RT versus simple mastectomy is underway with 50+ patients on study.

II. Ancillary Studies

A. Steroid Binding Proteins (SBP)

SBP are being prospectively evaluated in all breast cancer samples.

This includes analyses for androgen, estrogen, glucocorticoid and progestin receptors. In addition, analyses are being performed on melanoma, ovary, colon, male breast and hematologic malignancies. Studies of retinoic acid and retinol binding proteins in breast cancer are also being carried out. Several current publications resulting from these data are listed below.

III. Extramural Activities

A. National Surgical Adjuvant Breast Project

Dr. Lippman is on the Endocrine Committee of the National Surgical Adjuvant Breast Project.

B. Outside Teaching Responsibilities

Dr. Lippman is Associate Clinical Professor of Medicine and Pharmacology at the USUHS Medical School.

Publications:

1. Allegra, J.C., Barlock, A., Huff, K.K., and Lippman, M.E.: Changes in multiple or sequential estrogen receptor determinations in breast cancer. Cancer 45: 792-794, 1980.

2. Lippman, M.E.: Interpreting steroid receptor assays. Western J. Med. 130: 450-451, 1979.

3. Lippman, M.E., Do, H.M.T., and Hochberg, R.: Specific estrogen receptor binding and biologic effects of 16-iodestradiol on human breast cancer cells. Cancer Research (In press).

4. Lacroix, A. and Lippman, M.E.: Binding of retinoids to human breast cancer cell lines and their effects on cell growth. J. Clin. Invest. 65: 586-591, 1980.

5. Mercer, W.D., Wahl, T.M., Carlson, C.A., Wahl, D.A., Lippman, M.E., Lezotte, D., and Teague, P.O.: The use of immunocytochemical techniques for the detection of steroid hormones in breast cancer cells. Cancer 12: 2859-2868, 1980.

6. Tormey, D.C., Bull, J.M., and Lippman, M.E.: L-Phenylalanine mustard, adriamycin, and vincristine in advanced breast cancer - A pilot study. Cancer Treat. Rep. 64: 1015-1016, 1980.

7. Cowan, K. and Lippman, M.E.: Recent progress in breast cancer management: Combined modality (adjuvant) therapy. Arch. Int. Med. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06702-06 M

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Mechanisms of Hormone Dependence of Human Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marc E. Lippman	Senior Investigator	M	NCI
Other:	Kenneth Cowan	Clinical Associate	M	NCI
	Kay Seibert	Clinical Associate	M	NCI
	David Danforth	Guest Worker	M	NCI
	Susan Scholl	Visiting Fellow	M	NCI
	Diane Bronzert	Technician	M	NCI
	Karen Huff	Technician	M	NCI
	Susan Aitken	Technician	M	NCI
	Jeffrey Schlom	Senior Investigator	LCM	NCI

COOPERATING UNITS (if any)
Laboratory of Biochemistry, NCI; Endocrinology Branch, NICHD; Clinical Pharmacology Branch, NCI

LAB/BRANCH
Medicine Branch

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
Clinical Oncology Program, NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10	10	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings.

A. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer and male breast cancer.

B. Assays for specific gene products (-lactalbumin, casein, fatty acid sythetase, thymidine kinase) Thymidylate synthetase, aspartate transcarbamylase dihydrofolate reductase have been developed and we are studying the effects of steroid hormones on the activities and synthesis of these proteins.

C. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells, including their regulation by mitogens such as phytohemagglutinin. In addition we are studying glucocorticoid receptors in Burkitt's Lymphoma, hairy cell leukemia, ANLL and the lymphomas.

D. We are studying intracellular pharmakokinetics of estrogen and antiestrogen metabolism and efflux from human breast cancer cells using perfusion systems. These studies have led to new insights into hormone receptor interactions with the genome. Specifically, we have discovered that intranuclear estrogen receptors are changed over time to a less easily extractable form associated with the onset of steroid induced effects.

E. We are studying the detailed regulation of DNA synthesis in human breast cancer cells and as such have developed ways for accurately quantifying total DNA synthesis together with the scavenger and denovo pathways of pyrimidine biosynthesis.

F. We have developed a soft agar cloning technique which has permitted the development of clones of antiestrogen resistant variant (putative mutant) cell lines derived from hormone dependent wild typed cells. These variant cells are currently being analyzed biochemically and via somatic cell hybridization.

G. We have developed methotrexate and PALA resistant cell lines from human breast cancer cells. Pathways of resistance are under investigation. Gene reduplication has been demonstrated by molecular hybridization studies with cloned DNA.

H. We have developed assays for all of the enzymes in the denovo pyrimidine biosynthetic pathway and their hormonal regulation is under investigation.

I. The effects of pineal function and its secetion melatonin are being explored as modulators of breast cell growth in vivo and in vitro. Melaton stimulates estrogen receptors in uterus and breast cancer cells.

J. We are studying the interactions of novel ligands with human estrogen receptors. The goal is to develop better assays and evaluate cytotoxics linked to the hormone moiety.

K. We are developing a new rapid assay for steroid receptors using HPLC.

Publications

1. Strobl, J.S. and Lippman, M.E.: Long term nuclear retention of estradiol by human breast cancer cells in tissue culture. Cancer Research 39: 3319-3327, 1979.

2. Weisenthal, L.M., Von Hoff, D.D., and Lippman, M.E.: A comparison of cell counting, colony counting, and ^{32}P orthophosphate labeling in the determination of drug-induced cytotoxicity. Cancer Research (in press).

3. Weisenthal, L.M., Ruddon, R.W., Von Hoff, D.D. and Lippman, M.E.: Dye exclusion techniques in the determination of drug-induced cytotoxicity. Cancer Research (in press).

4. Lacroix, A., Anderson, G.D.L. and Lippman, M.E.: Retinoids and cultured human fibroblasts: Effects on cell growth and presence of cellular retinoic acid binding protein. Exp. Cell Res. (in press).

5. Nawata, H., Chong, M., Bronzert, D., and Lippman, M.E.: Estradiol independent growth of a subline of MCF-7 human breast cancer cell in culture. JBC (in press).

6. Nawata, H., Bronzert, D. and Lippman, M.E.: Isolation and characterization of a tamoxifen resistant cell line derived from MCF-7 human breast cells. J. Biol. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06708-02 M

PERIOD COVERED
October 1, 1980 to September, 1981

TITLE OF PROJECT (80 characters or less)
1) Genetic Regulation of the Immune Response
2) The Biology and Treatment of Malignant Lymphomas
3) Control of Chemotherapy-induced Emesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dan L. Longo	Sr. Investigator	M	NCI
Other:	Ronald Schwartz	Sr. Investigator	LI	NIAID
	Laurie Glimcher	Research Assoc.	LI	NIAID
	Patricia Dobson	Technologist	M	NCI

COOPERATING UNITS (if any)
Laboratory of Immunology, NIAID

LAB/BRANCH

SECTION
Medicine Branch

INSTITUTE AND LOCATION
Clinical Oncology Program, NCI, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Examination of expression of immune response genes in B cells.
2. Study of the autologous mixed lymphocyte reaction, the stimulating and responding cells in this response, and its physiological significance.
3. Analysis of T cell recognition of foreign antigen and self Ia molecules.
4. Identification of the heterogeneity of antigen presenting cells.
5. Clinical trial of MOPP vs MOPP-SCAB in advanced Hodgkin's disease.
6. Effects of treatment on the natural history of nodular mixed lymphoma.
7. Review of the "good prognosis" lymphomas.
8. Clinical trial of scopolamine for chemotherapy-induced emesis.
9. Review of mechanisms of vomiting and its treatment.
10. Review of the natural history and treatment of ovarian cancer.

Selected Highlights of Work Completed This Year:

I. What the T cell sees: We have shown that type A T cells maturing in a type B environment recognize foreign antigen only in association with type B (not type A) MHC products. Furthermore, the interaction between the type A T cells and type B antigen presenting cells is inhibited by antisera directed against the antigen presenting cell but not by antisera acting on the T cell. (Longo & Schwartz, PNAS 78:514, 1981).

II. We have shown that, in contrast to the effects of the developmental milieu on T cell recognition, genetic nonresponder B cells maturing in a responder environment fail to become responders. Thus, the immune response gene phenotype of the B cell, like that of the antigen presenting cell, is genetically determined. (Tse, Mond, Longo, J.Immunol. in press).

III. The autologous mixed lymphocyte reaction (AMLR) has been shown to be absent in certain autoimmune diseases. We examined the nature of the responding and stimulating cell populations and found that the AMLR represents the recognition of self Ia antigens in the absence of foreign antigen by T cells. There are 2 T cells involved, one of which is MHC-restricted and the other is not. The T cell acquires the capacity to respond in the AMLR during development in the thymus. (Glimcher, Longo, Green, Schwartz, J.Exp. Med. in press).

IV. We have found that the cells which process and present antigen to T cells are not homogeneous but differ in the expression of a particular surface marker (Lyb 4) depending upon whether they are tissue-bound or not. Since all antigen presenting cells (APC) originate in the bone marrow, the suggestion is that Lyb 4 is a differentiation marker lost when APC become tissue-bound. (Longo, Ahmed, Dobson, Kirkland, Schwartz, Fed.Proc. 40:959, 1981).

Publications:

1. Longo, D.L. and Schwartz, R.H.: Inhibition of antigen-induced proliferation of T cells from radiation-induced bone marrow chimeras by a monoclonal antibody directed against an Ia determinate on the antigen presenting cell. Proc. Natl. Acad. Sci. USA, 78: 514-519, 1981.

2. Longo, D.L., Matis, L.A., Schwartz, R.H.: Insights into immune response gene function from experiments with chimeric animals. CRC Crit. Rev. Immunol., 2: 311-338, 1981

3. Longo, D.L., Young, R.C., DeVita, V.T.: What is so good about the "good prognosis" lymphomas? Recent Advances in Clinical Oncology, ed. C.J. Williams, in press.

4. Longo, D.L. and Paul, W.E.: Immune response genes and Ia antigens: the relationships between them and their role in lymphocyte interactions. In Transplantation Antigens, P. Parkham, J.L. Strominger, eds., in press.

5. Seigel, L.J., Longo, D.L.: Control of chemotherapy-induced emesis. Ann. Int. Med., in press.

6. Rubinstein, D.B. and Longo, D.L.: Peripheral destruction of platelets in chronic lymphocytic leukemia: recognition, prognosis, and therapeutic implication. Amer. J. Med. in press.

7. Tse, H.Y., Mond, J.J., Longo, D.L.: B lymphocyte immune response gene phenotype is genetically determined. J. Immunol. in press.

8. Glimcher, L.H., Longo, D.L., Green, I., Schwartz, R.H.: Syngeneic mixed lymphocyte reaction: studies on the nature of the stimulating and responding cells. J. Exp. Med. in press.

9. Longo, D., Ahmed, A., Dobson, P., Kirkland, T., Schwartz, R.: Lyb 4 distinguishes recirculating from tissue-bound antigen-presenting cells. Fed. Proc. 40: 959, 1981.

10. Wiernik, P.H., Longo, D., Duffey, P.L., Young, R.C., DeVita, V.T.: MOPP vs MOPP alternating with streptozotocin, CCNU, adriamycin, and bleomycin for advanced Hodgkin's disease. Proc. Amer. Assoc. Cancer Res. 22: 159, 1981.

11. Longo, D., Howser, D., Wesley, M., Anderson, T., Young, R.: Randomized double-blind trial of scopolamine vs placebo administered by transcutaneous patch for the control of chemotherapy-induced emesis. Proc. Am. Assoc. Cancer Res. 22: 161, 1981

12. Longo, D., Hubbard, S., Wesley, M., Jaffe, E., Chabner, B., DeVita, V., Young, R.: Prolonged initial remission in patients with nodular mixed lymphoma. Proc. Amer. Soc. Clin. Oncol. 22: 521, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06709-01 M

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanisms of Drug Resistance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert F. Ozols	Sr. Investigator	M	NCI
Other:	Robert C. Young	Chief	M	NCI
	Charles E. Myers	Sr. Investigator	CP	NCI
	W. Michael Hogan	Visiting Assoc.	M	NCI
	Jan Keizer	Visiting Fellow	M	NCI
	Brenda Foster	Clinical Assoc.	M	NCI
	Karen Grotzinger	Med Technologist	M	NCI
	Wilma McCoy	Med Technologist	M	NCI

COOPERATING UNITS (if any)

LAB/BRANCH Clinical Pharmacology Branch, NCI

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the in vitro patterns of sensitivity of human tumors, particularly ovarian and testicular cancer, to antineoplastic agents and the mechanisms of resistance to antineoplastic drugs. Drug sensitivity studies are preformed using: 1) a clonogenic assay in fresh human tumor specimens, 2) established tissue culture lines of human tumors, and 3) in selected murine neoplasms. These studies have provided a rationale for new thereapeutic approaches in ovarian cancer and have aided in the selection of chemotherapy for individual patients. The mechanisms for drug resistance are being examined at the cellular level in fresh human tumor cell suspensions and in established cell lines.

Ongoing Studies:

1. Ovarian Cancer. We have grown ovarian cancer colonies from malignant effusions from 75 patients with ovarian cancer. We have demonstrated that dose response relationships to adriamycin are dependent in part upon the nature of the patient's previous chemotherapy. These results have provided, in part, a rationale for a Phase I trial of intraperitoneal adriamycin.

We have also been studying the conditions required to establish long term tissue culture lines of ovarian carcinomas.

2. Testicular Cancer. We have demonstrated that single cell suspensions of tumor cells obtained from metastatic testicular cancer can produce colonies in the clonogenic assay. These colonies have been stained for human chorionic gonadotropin and alpha fetoprotein.

3. Drug Resistance Studies. We have demonstrated that amphotericin B is unable to increase the sensitivity of human ovarian cancer cells to either adriamycin or melphalan. In addition, amphotericin B is unable to overcome the resistance in vivo to melphalan of mice inoculated with melphalan resistant L1210 cells. Studies are currently in progress correlating in vitro patterns of drug resistance with the amount of drug uptake and efflux as well with the ability of tumor cells to metabolize the antineoplastic agents.

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October 1, 1980 to September 30, 1981

ANNUAL REPORT OF THE NCI-VA MEDICAL ONCOLOGY BRANCH OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE AND THE WASHINGTON, DC VETERANS ADMINISTRATION MEDICAL CENTER

1.0 Summary

1.1 Description

The NCI-VA Medical Oncology Branch is an intramural branch of the COP/DCT/NCI/NIH, a section of the Medical Service and a section of the Research and Education (R & E) Service of the Washington, D.C. Veterans Administration Medical Center (WVAMC) and thus is designated as a Program by the WVAMC and the Veterans Administration Central Office (VACO). The primary goals of the NCI-VA Medical Oncology Branch are: investigation of patients with malignant disease in clinical therapeutic trials; laboratory investigations of tumor cell biology. Secondary aims are to provide a service function to the WVAMC for management of patients with malignant disease and training of fellows in clinical oncology and investigation.

1.2 Administration

The NCI-VA Medical Oncology Branch is a cooperative program between the COP/DCT/NCI/NIH and the VA via an interagency agreement. The NCI provides for a transfer of funds and several senior staff personnel and administrators from the DCT/NCI to VACO and WVAMC. The WVAMC provides inpatient ward (2CN), office, outpatient clinic, and research laboratory space, billets to run the program, clinical laboratory, radiology and pharmacy services as well as subspecialty medical and surgical consultations for patients. Both veteran and nonveteran patients are entered into clinical protocols approved by clinical review committees of the WVAMC and the NCI. A yearly contract is signed between the VACO and the COP/DCT covering the interag ncy agreement and project plan. The NCI reimburses the WVAMC for non-veteran patients through fixed daily inpatient and outpatient charges and pays an overhead charge for administrative and other support to the WVAMC. The WVAMC provides for some financial coverage for patient care.

1.3 Clinical Resources

All clinical and ward supporting work for protocol and non-protocol patients is performed by four senior staff oncologists, one infectious disease specialist, two pathologists, five first year, five second and third year clinical associates, two rotating interns, one pharmacist, three research nurses, 22 full time and part time nurses, two medical technologists, three ward and outpatient clinic secretaries and one medical DMT. WVAMC provides part time use of a dietitian, food service workers, social worker, housekeeping aid and ward supply. The inpatient unit has 30 beds and an outpatient treatment

area. The outpatient clinic runs three half days a week in the WVAMC outpatient area, and five days a week in the inpatient (2CN) ward area. A satellite ambulatory care program (SACP) with five rooms in the United Inn, Bethesda, Md. runs as part of the COP/DCT outpatient facility contract with NCI funding. A driver and a van (funded by the NCI transferred funds) transports patients to and from the SACP and the main NIH campus for various procedures. Radiotherapy is primarily handled by the Radiation Oncology Branch (ROB/DCT/NCI) at the NIH campus. Anti-neoplastic drugs are provided by the NCI. All transportation costs for protocol patients are met by the NCI transferred funds. The statistical design, monitoring, and analysis of the clinical trials are carried out by the Biometry Branch/NCI (Dr. Robert Makuch). The completion of the CAPRI system for automated data collection and analysis by the Biometry Branch will allow computerization of the clinical research data.

1.4 Clinical Patient Load

The NCI-VA Medical Oncology Branch assumes complete primary care responsibility (including inpatient and outpatient) for all patients on its protocols and in addition, the primary care for veteran patients deemed likely by the NCI-VA M.O.B. Chief to have significant benefit from complex chemotherapy treatment regimens. This amounts to approximately 300 patients per year. The program also provides for consultative services for all patients with malignant disease as requested in the inpatient and outpatient areas (approximately 40 consultations per month). The program provides for outpatient chemotherapy and care for WVAMC veterans requiring non-protocol chemotherapy (approximately 100 patients in FY 1981). The inpatient ward (2CN) has had approximately 80% occupancy, and there are approximately 240 outpatient visits per month. Nonveteran inpatient occupancy has been approximately 30% of the total, and nonveteran outpatient visits approximately 20% of the total. A number of the veteran patients come for protocol treatment from outside the WVAMC area and the WVAMC hospital district on NCI transferred funds.

The senior staff provides 6 months of inpatient attending coverage on the general medical service, two months of attending coverage on the Infectious Disease Section consultation service; provides consultation when asked by the WVAMC tumor registry; participates in the WVAMC Cancer Committee and weekly Medical-Surgical Chest Conferences; provides weekly consult rounds conference for patients seen in consultation, and multiple individual conferences for the WVAMC through the general hospital education program. Also, the NCI-VA pathologists provided additional opinions on histologic material to the hospital.

1.5 Oncology Fellowship Training Program

As part of the oncology fellowship training program of the Clinical Oncology Program (COP/DCT/NCI/NIH the NCI-VA Medical Oncology Branch provides for clinical training in the subspecialty of Medical Oncology for the Clinical Associates assigned to it (as special positions designated by the VACO) or to the Clinical Associate rotating from the Clinical Center, NIH. In addition, it provides general medical and oncology training to twelve interns from the Medical Service, WVAMC, George Washington University and Georgetown University

that rotate through the 2CN inpatient ward.

1.6 Laboratory Research Facilities and Staffing

The laboratory research program in tumor cell biology is carried on in 3000 square feet of wet lab space. The program has the use of common research space of the WVAMC R & E Service including instrument storage and animal rooms totaling an additional 1500 square feet of space. There are four senior investigators, two post doctoral fellows, four clinical associates, and 10 technicians working in this area.

1.7 Move to the National Naval Medical Center

The interagency agreement between the NCI and the VACO expires on October 1, 1981. Dr. Martin Cohen has been named the new Chief of the Oncology Section at the Washington VAMC effective this date. The VACO and the WVAMC will continue to have a subspeciality 30 bed ward (2CN) for the care of cancer patients as part of this section. Dr. Stephen Krasnow of the NCI-VA Medical Oncology Branch will be one of the new senior staff members, and Dr. Cohen has recruited Dr. Mark Citron from Detroit as another senior staff member. Dr. Byron Fossieck will remain as the infectious disease specialist with the section. Dr. Cohen plans to devote the new Oncology section's effort to the study of combined modality therapy in a variety of malignant diseases. Dr. Cohen and Dr. Minna have discussed the possibility of continuing collaboration on clinical protocols on a protocol by protocol basis. The senior staff of the new NCI-Navy Oncology Branch will include Drs. Minna (Chief), Ihde (Deputy Chief for Clinical Affairs, Chief Clinical Studies Section), Gazdar (Deputy Chief for Laboratory Affairs), and Drs. Bunn (Chief, Cell Kinetics Section), Carney, and Matthews all of the former NCI-VA Medical Oncology Branch as Senior Investigators. Because of the increased load, types, and diversity of patients at the NNMC considerable effort will be made to develop collaborative clinical programs with the other clinical branches of the COP/DCT.

The clinical responsibilities of the new NCI-Navy Medical Oncology Branch will begin at the National Naval Medical Center July 1, 1981. The current limited interagency agreement between the NCI and the NNMC will be expanded to fully replace the old NCI-VA interagency agreement as of October 1, 1981 (FY 82). The current NCI-Navy interagency agreement provides for the reimbursement to the NNMC for a research nurse, clinic secretary, chemotherapy drugs, and staff travel for the entry of patients onto ECOG protocols. With the starting up of the NCI-Navy M.O.B. the emphasis will be on the entry of NNMC oncology patients onto NCI-Navy Medical Oncology Branch or other NCI clinical protocols.

The following administrative steps are in process: 1.) a draft agreement was prepared by Dr. Minna (NCI) and Dr. Pasquale (NNMC) covering the operations of the NCI-Navy Branch and is being reviewed by the NNMC after the NCI and the NNMC have agreed to the draft proposals in principle; 2.) the NCI-VA protocols have been submitted to the NNMC for review; 3.) the final architectural plans for the rennovation of the "swing space" in building 1

("the tower") and for the final space in building 8 are completed and approved, monies for the design and rennovation have been transferred via an interagency agreement to the NNMC, and bids have been solicited for a firm, fixed price contract to be awarded in July, 1981. The rennovation should be completed by early 1982; 4.) Dr. Minna and Dr. Pasquale have met with the various services at the NNMC (including Medicine, Surgery, ENT, Laboratory, Radiology, Nursing, Pharmacy) in preparation for the move as well as having papers submitted to the Uniformed Services University for the Health Sciences for faculty positions. 5.) an Senior Attending schedule for providing Oncology-Hematology inpatient, outpatient and consultative coverage for the NNMC has been prepared; 6.) position descriptions were submitted to the NCI in preparation to transfer required personnel from VA billets in the NCI-VA Branch to NIH billets for work at the NCI-Navy Branch.

Because the laboratory rennovation at the NNMC will not be completed until January - April, 1982 Dr. Minna has asked permission to stay in the laboratory space at the WVAMC until the rennovation is completed. He and Dr. Levine (WVAMC ACOS for R and E) are working out the details of this arrangement which should be mutually beneficial to both the NCI and the WVAMC for a smooth and orderly transition.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03024-12 NCI-VAMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Other Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Senior Staff Oncologists, PI:

John D. Minna, M.D.	Chief	NCI-VA MOB NCI/WVAMC (USPHS)
Martin H. Cohen, M.D.	(Deputy Chief, Clin)	NCI-VA MOB NCI/WVAMC
Paul A. Bunn, M.D.		NCI-VA MOB NCI/WVAMC (USPHS)
Daniel C. Ihde, M.D.		NCI-VA MOB NCI/WVAMC (USPHS)
Desmond N. Carney, M.D.		NCI-VA MOB NCI/WVAMC

Associate Senior Staff Oncologist

Stanley E. Shackney, M.D.	CPB/DCT/NCI/NIH (USPHS)

(Continued)

COOPERATING UNITS (if any)

See attached sheets.

LAB/BRANCH
NCI-VA Medical Oncology Branch

SECTION
None

INSTITUTE AND LOCATION
Washington, D.C. VA Medical Center COP/DCT/NCI

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
55	15	40

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The NCI-VA Medical Oncology Branch studies new methods of evaluating and treating patients with malignant disease and provides general medical oncology consultations for the Washington Veterans Administration Medical Center, a 600 bed hospital. Clinical investigations are carried out in patients with small cell lung cancer and other types of lung cancer (epidermoid, large cell, and adenocarcinoma), mycosis fungoides and the Sezary syndrome, carcinoma of the prostate, multiple myeloma and other plasma cell dyscrasias, gastric carcinoma, and hepatocellular carcinoma. New Phase I agents, both chemotherapeutic and immunotherapeutic, are studied. Other interests involve general medical oncology and miscellaneous cancers. Within each disease category, investigations are centered in one or more of the following areas: 1) therapeutic trials and complications of treatment; 2) staging procedures, prognostic factors, and natural history; 3) clinical-cell biologic correlations; 4) clinicopathologic correlations; 5) infectious diseases; and 6) preparation of review articles. Some 20 oncology consultations per month are seen in the Washington VAMC and outpatient care provided for patients requiring chemotherapy who are not eligible for any protocol studies. Clinical Associates are trained in medical oncology and clinical investigation.

Senior Staff Other, PI:

Byron E. Fossieck, M.D.	Infectious Disease	NCI-VA MOB NCI/WVAMC
Adi F. Gazdar, M.D.	Deputy Chief (Lab) Pathologist	NCI-VA MOB NCI/WVAMC
Mary J. Matthews, M.D.	Pathologist	NCI-VA MOB NCI/WVAMC

Clinical Associates

Fourth Year

Peter Radice, M.D.	NCI-VA MOB NCI/WVAMC	(USPHS)	(MB)

Third Year

Paul G. Abrams, M.D.	NCI-VA MOB NCI/WVAMC		
Raul Alvarez, M.D.	NCI-VA MOB NCI/WVAMC	(USPHS)	(POB)
Robert Levenson, M.D.	NCI-VA MOB NCI/WVAMC	(USPHS)	(IDB/CTEP)

Second Year

Martin Earle, M.D.	NCI-VA MOB NCI/WVAMC
Steven Krasnow, M.D.	NCI-VA MOB NCI/WVAMC
Steven Rosen, M.D.	NCI-VA MOB NCI/WVAMC
Stephen Sherwin, M.D.	NCI-VA MOB NCI/WVAMC

First Year

Cameron Little, M.D.	NCI-VA MOB NCI/WVAMC
Robert Meister, M.D.	NCI-VA MOB NCI/WVAMC
Jeffrey Ochs, M.D.	NCI-VA MOB NCI/WVAMC
William Tester, M.D.	NCI-VA MOB NCI/WVAMC

Other Full Time NCI-VA MOB:

Anita Johnston-Early, RN	Clinical Research Nurse	NCI-VA MOB NCI/WVAMC
Joyce Eddy, RN	Clinical Research Nurse	NCI-VA MOB NCI/WVAMC
Rosemarie S. Cordes, RN	Clinical Research Nurse	NCI-VA MOB NCI/WVAMC

Rotating Medical Intern, M.D. WVAMC
Rotating Medical Intern, M.D. George Washington University

Cooperating Units

Washington DC VA Medical Center

R. Bates, M.D.	Nuclear Medicine Service
H. Benisimon, M.D.	Chief, Urology Service
B. Fischmann, M.D.	Chief, Dermatology Section
J. Guccion, M.D.	Laboratory Service
J. Hawley, M.D.	Neurology Service
G. Higgins, M.D.	Chief, Surgical Service
S. Lunzer, M.D.	Chief, Radiotherapy Section
R. Parker, M.D.	Chief, Infectious Disease Section
B. Sauerbrunn, M.D.	Chief, Nuclear Medicine Service
G. Schecter, M.D.	Chief, Hematology Section
L. Seeff, M.D.	Chief, GI-Hepatology Section

Cooperating Units

National Institutes of Health

R. Abrams, M.D.	Clinical Associate	ROB/POB/DCT/NCI
J. Aisner, M.D.	Senior Investigator	BCRP/DCT/NCI
M. Boyd, M.D.	Senior Investigator	CPB/DCT/NCI
S. Broder, M.D.	Senior Investigator	MET/DCBD/NCI
B. Chabner, M.D.	Assoc. Director For COP	DCT/NCI
A. Deisseroth, M.D.	Senior Investigator	POB/DCT/NCI
R. Donehower, M.D.	Clinical Associate	CPB/DCT/NCI
R. Dunnick, M.D.	Senior Staff Radiologist	DR/CC
R. Fisher, M.D.	Senior Investigator	MB/DCT/NCI
E. Glatstein, M.D.	Chief	ROB/DCT/NCI
E. Jaffe, M.D.	Senior Pathologist	LP/NCI
N. Javadpour, M.D.	Senior Staff Surgeon	SB/DCT/NCI
A. Lichter, M.D.	Senior Investigator	ROB/DCT/NCI
M. Lippman, M.D.	Senior Investigator	MB/DCT/NCI
R. Makuch, M.D.	Statistician	BRB/DCT/NCI
K. McIntire, M.D.	Senior Scientist	LID/DCBD/NCI
R. Newman, M.D.	Senior Staff Radiologist	DR/CC
R. Oldham, M.D.	Assoc. Director for BRMP	DCT/NCI
R. Ruddon, M.D.	Senior Investigator	Frederick CRC/NCI
R. Schilsky, M.D.	Clinical Associate	MB/DCT/NCI
J. Schwade, M.D.	Senior Investigator	ROB/DCT/NCI
T. Waldman, M.D.	Chief	MET/DCBD/NCI
J. Whang-Peng, M.D.	Senior Staff (Cytogenetics)	MB/DCT/NCI
R. Young, M.D.	Chief	MB/DCT/NCI
M. Zweig, M.D.	Asst. Chief	CC/DCT/NIH

Cooperating Units, Other:

F. Hirsch, M.D.	Finsen Institute, Copenhagen, Denmark
L. Napoli, M.D.	Radiologist, Providence Hospital
I. Royston, M.D.	University California, San Diego
R. Yesner, M.D.	Yale University

1.0 SMALL CELL LUNG CANCER (SCLC) AND OTHER TYPES OF LUNG CANCER (NSCLC) (EPIDERMOID, LARGE CELL, AND ADENOCARCINOMA)

1.1 Therapeutic Trials and Complications of Treatment

1.11 Treatment of Limited Stage SCLC (Collaboration: Radiation Oncology Branch). Twenty patients have been randomized to each arm of a prospective clinical trial evaluating whether the addition of irradiation of the primary chest tumor to intensive combination chemotherapy provides survival or other clinical benefit. Complete responses and severe non-hematologic toxicity occur more frequently on the combined modality arm. Although statistically significant differences in survival are yet to be achieved, actuarial projections suggest that 44% of combined modality and 16% of chemotherapy alone patients will survive beyond 3 years.

1.12 Treatment of Extensive Stage SCLC (Collaboration: Pediatric Oncology and Radiation Oncology Branches). Twenty-two patients have entered a study of conventional intensive combination chemotherapy for 12 weeks, followed by autologous bone marrow collection and late intensive combined modality treatment with multiple local field irradiation to all disease sites plus high doses of cyclophosphamide and VP-16 with reinfusion of autologous marrow. Only good performance status patients with negative bone marrow examination and a complete or partial response at 12 weeks receive the late intensive therapy. Five patients have thus far been given late intensive treatment in this ongoing study, with 1/3 who were in partial response converting to complete response and unmaintained remissions of 4 and 8 months in the 2 complete responders.

1.13 Phase II Trials of Single Agents in SCLC Patients Resistant to Combination Chemotherapy with or Without Radiotherapy (Collaboration: Baltimore Cancer Research Program). Three Phase II trials in these heavily pretreated patients have been initiated. Essentially no useful activity was observed with cisplatin (42) or vindesine (30), while the trial employing aziridinylbenzoquinone (AZQ) has begun only recently.

1.14 Thymosin Fraction V as an Adjunct During Induction Chemotherapy of SCLC (Collaboration: Surgery Branch). Further follow-up of approximately 60 patients randomized to receive one of two doses of thymosin twice weekly or no thymosin during 6-week induction chemotherapy reveals that a significant survival advantage is maintained for patients receiving 60 mg thymosin compared to those receiving no thymosin (25). The mechanism of this improvement is uncertain, and further trials should be encouraged.

1.15 Long Term Survivors with SCLC in NCI Trials (Collaboration: Radiation Oncology Branch). Of a series of 168 patients treated at the NCI between 1974 and 1977, a group of 20 (12%) 30+-month disease-free survivors who are potentially cured have been identified. A higher frequency of long-term survivors was seen in limited stage and chemotherapy plus chest irradition treated patients, but some patients with extensive stage and no chest irradiation also lived beyond 30 months. Further analysis, focusing on long-term complications, second malignancies, and possible late relapses, is continuing.

1.16 Complications of Intensive Therapy of SCLC (Collaboration: Radiation Oncology Branch, Medicine Branch, WVAMC Hematology Section). Neurologic evaluation of patients treated with combination chemotherapy plus prophylactic cranial irradiation has not revealed major deficits (20). Weekly administration of vincristine in association with VP-16 may lead to a higher than expected frequency of peripheral neuropathy (58). Erythroleukemia with chromosomal abnormalities or prolonged pancytopenia developed in 2/8 longterm survivors (4).

1.17 Adriamycin-Mitomycin C for Treatment of NSCLC. This trial has accrued 42 patients, 29 receiving mitomycin-C every 3 and 13 receiving the drug every 6 weeks. The mitomycin-C schedule was changed because of otherwise unexplained pulmonary toxicity occurring in 3 patients on the q 3 wk schedule. No such toxicity has been observed with less frequent administration of the drug, but the partial response rate has been 15% compared with 35% when mitomycin was given every 3 weeks, suggesting the more frequent schedule may have superior anti-tumor effects.

1.18 Comparison of Two Combination Chemotherapy Regimens in NSCLC (Collaboration: Working Party for Lung Cancer). A regimen of cyclophosphamide, methotrexate, and CCNU proved to have a higher response rate than adriamycin plus procarbazine in a randomized trial, but there were no survival differences (59).

1.2 Staging Procedures, Prognostic Factors, and Natural History.

1.21 Neurologic Sanctuary Sites and Complications (Collaboration: Radiation Oncology Branch, Baltimore Cancer Research Program, WVAMC Neurology Service, and Biometric Research Branch). The frequency, risk factors, clinical implications, and treatment of central nervous system metastases have been analyzed in a group of over 400 patients treated over the past 10 years. Prophylactic cranial irradiation was shown to significantly reduce both brain metastases and carcinomatous leptomeningitis (CLM) but did not significantly affect survival. Major reduction in CNS metastases occurred in patients with a complete response to treatment. CLM developed in 12% of all patients, and by actuarial estimate in 28% surviving 3 years. With increasing awareness, premortem diagnosis has been made much more frequently since 1977. The neuromyopathy associated with SCLC has been described in detail (31).

1.22 Prognostic Implications of Sites of Metastases in SCLC (Collaboration: Biometric Research Branch). Among 106 consecutive patients given intensive combination chemotherapy from 1973 to 1977, survival significantly worsened with increasing number of sites of distant metastases. Performance status was also more impaired with greater tumor burden. Patients with only a single site of distant metastases had survival that was not significantly different from patients with limited disease, while patients with liver or central nervous system metastases did especially poorly. Certain SCLC patients with extensive disease have real prospects for prolonged disease-free survival (38).

1.23 Peritoneoscopy in SCLC. 102 procedures in 94 patients have been performed over the past 2.5 years (3). Results of biopsies have been compared to biochemical, radiographic, and radionuclide tests in an ongoing analysis in

order to establish the best methods for diagnosis of hepatic metastases.

1.24 Abdominal Computed Tomography in SCLC (Collaboration: Diagnostic Radiology/Clinical Center). Employment of abdominal computed tomography (CT) at diagnosis and during therapy in 75 SCLC patients revealed that while CT could detect retroperitoneal disease not diagnosed by any other staging procedure, it only rarely changed the patient's stage (limited vs. extensive) or, during treatment, provided unique information that would have led to a change in therapy (35).

1.25 Radionuclide Bone Scans in SCLC (Collaboration: WVAMC Nuclear Medicine Service). Bone scan positivity in SCLC occurs more frequently with bone marrow involvement and increasing number of extensive disease sites. Patients with a positive scan plus other sites of extensive disease have inferior survival to other groups of patients. During therapy, 2/3 of patients with response proven by other methods have improving and 1/3 with progression proven by other methods have worsening scans, but the predictive value of a changing scan is 70% (44).

1.26 Smoking Abstinence Associated with Prolonged Survival in SCLC (Collaboration: Biometric Research Branch). Patients given combination chemotherapy who have stopped smoking prior to the diagnosis of SCLC survive significantly longer than those who continue to smoke. This observation cannot be explained by any association with other known prognostic factors. Patients who stop smoking at diagnosis live longer than those who continue to smoke, but this difference is less impressive (39).

1.27 Laboratory Parameters as a Substitute for Performance Status in Predicting Survival in SCLC (Collaboration: Biometric Research Branch). An extensive analysis has shown that the simple laboratory parameters of serum albumin and hemoglobin can provide most of the prognostic information that is contributed by performance status (which is difficult to determine retrospectively but is of major prognostic importance) in SCLC patients given combination chemotherapy (26).

1.28 Serum Markers for Tumor Burden in SCLC (Collaboration: Clinical Chemistry/Clinical Center, Biomarkers Program/Frederick CRC). Creatine kinase-BB (CK-BB) and neuron specific enolase (NSE) are produced in vitro by established SCLC cell lines. In serum from untreated patients, CK-BB is elevated in 28% and NSE in 64%; both are more often positive with increasing numbers of sites of extensive disease. During therapy, changes in CK-BB levels show an excellent correlation with clinical disease activity.

1.29 Red Blood Cell Deformability (RBCD) in SCLC Patients. RBCD, which if decreased might cause stasis in tumor capillaries and impede chemotherapy effectiveness, was measured serially in 51 SCLC patients during chemotherapy treatment (21). Patients with normal or increased RBCD prior to and during therapy survived significantly longer than patients with decreased values despite both groups' being equivalent with regard to other prognostic factors. Further study is warranted.

1.3 Clinical-Cell Biologic Correlations.

1.31 Soft Agarose Clonogenic and Other In Vitro Drug Sensitivity Assays in Lung Cancer Patients. In vitro tumor colony formation was observed in 80% of tumor-positive clinical specimens from 90 patients with lung cancer, but in the majority of these specimens low cloning efficiency prevented accurate determination of in vitro drug sensitivities. A total of 40 in vitro/in vivo drug sensitivity correlations were obtained: 33/33 with in vitro resistance were resistant in vivo, while 3/7 with in vitro sensitivity had a clinical response. Direct establishment of lung cancer cell lines may allow a much higher proportion of in vitro drug sensitivity data to be generated from clinical specimens. Protocols for initial treatment of patients with NSCLC and Phase II treatment of relapsed SCLC patients are being initiated.

1.32 Peripheral Blood Hematopoietic Colony Forming Units After Intensive Chemotherapy of SCLC (Collaboration: Radiation Oncology Branch). During hematologic recovery from intensive chemotherapy, 5- to 6-fold increases in absolute and relative numbers of buffy coat colony forming units (CFU_C) have been demonstrated (1). Calculations have shown that appropriately timed leukaphereses should produce sufficient CFU_C to allow autologous hematopoietic reconstitution.

1.33 Clonogenic Assay on Bronchial Washings from Lung Cancer Patients (Collaboration: Pediatric Oncology and Medicine Branches). Small numbers of tumor clones have been grown from lung cancer patients' bronchial washings, a repetitively available source of tumor material. However, contamination problems were substantial, and sufficient colonies to allow drug sensitivity testing were rarely if ever obtained (60).

1.4 Clinicopathologic Correlations.

1.41 Influence of SCLC Histologic Subtype on Presentation and Outcome of Therapy. SCLC occurs in two pathologic subtypes, lymphocyte-like or oat cell (45%) and the less well recognized (55%) intermediate subtype. We have shown that subtype has no influence on clinical presentation, response to therapy, or survival in patients given intensive combination chemotherapy (19). Subtypes may vary from site to site in an individual patient. This emphasizes the importance of review of diagnostic pathologic material by an experienced lung cancer pathologist prior to treatment decisions.

1.42 Mixed Small Cell-Large Cell Carcinoma of the Lung. Approximately 10% of SCLC patients at the NCI-VA MOB have elements of large cell carcinoma in their diagnostic biopsies. Review of 19 patients with this mixed histology established that these patients have lower response rates to combination chemotherapy and shorter survival than patients with "pure" SCLC, but occasional complete responses and long-term survival do occur, so aggressive therapy is appropriate.

1.43 Small Cell Carcinoma Presenting as an Extrapulmonary Neoplasm. 4% of a series of 203 consecutive patients with small cell carcinoma had no evidence of a pulmonary or mediastinal neoplasm by radiographic studies or fiberoptic

bronchoscopy. These 8 patients comprised 2 clinical groups: 4 had an obvious non-pulmonary primary site and 4 did not. Three of six evaluable patients responded to chemotherapy, and 2 others given adjuvant chemotherapy had prolonged survival. This study more firmly establishes that extrapulmonary small cell carcinoma can respond to chemotherapy and supports the use of aggressive chemotherapy in all patients with small cell carcinoma, with or without a primary lung tumor (43).

1.44 Observer Variability in Pathologic Diagnosis of SCLC and Other Lung Cancers (Collaboration: Finsen Institute, Yale University). Agreement between 3 pathologists was obtained regarding the diagnosis of SCLC from blinded lung cancer slides in 94% of cases, using the 1977 WHO Lung Cancer Classification criteria. However, there was only 54% unanimity regarding SCLC pathologic subtypes (50).

1.45 Alterations in SCLC Morphology In Vivo. Bone marrow biopsies were positive for SCLC in 26/173 patients undergoing pretreatment staging procedures. In sequential marrow biopsies performed during treatment, metastatic SCLC was replaced by mature squamous cell or adenocarcinoma in 3 cases. Tissue culture in one instance showed elements of both SCLC and squamous carcinoma in vitro. This study supports the pathologic plasticity of lung cancer.

1.46 Pathologic Consultations. Dr. Matthews serves as pathologic consultant for the Veterans Administration Surgical Oncology Group, the NCI Small Cell Carcinoma Registry of Long Term Survivors, the Eastern Cooperative Oncology Group, the NCI Lung Cancer Study Group, and the NCI SCLC Hyperalimentation Protocol. Numerous clinicopathologic correlations have been made from surgical specimens resected by members of the VASOG (56, 57). Tumor differentiation has no effect on outcome in resected squamous or adenocarcinoma, if stage, tumor size, and performance status are taken into account.

1.5 Infectious Diseases.

1.51 Rectal Infections in SCLC. Approximately 5% of patients receiving chemotherapy for SCLC at the NCI-VA MOB have developed rectal infections, one of which was fatal. These infections were confined to patients undergoing intensive therapy. Clinical aspects and therapy have been analyzed.

1.52 Herpes Zoster in SCLC. A review of herpes zoster occurring in intensively treated SCLC patients at the NCI-VA MOB has been published (32).

1.6 Review Articles.

1.61 Review articles on the general characteristics and treatment of SCLC and other lung cancers (7, 12, 13, 23, 24, 33, 34, 45, 52, 62), staging and diagnosis of lung cancer (22, 37), cell kinetics of SCLC (55), and pathology of lung cancer (46-49) by NCI-VA MOB staff members have been published or are in press.

2.0 MYCOSIS FUNGOIDES AND THE SEZARY SYNDROME: CUTANEOUS T-CELL LYMPHOMAS (CTCL)

2.1 Therapeutic Trials.

2.11 Combined Total Body Electron Beam (EBRT) Plus Intravenous Nitrogen Mustard for Limited CTCL (Collaboration: Howard University Radiotherapy Dept., WVAMC Dermatology and Hematology Sections). Follow-up is continuing on 14 limited (confined to the skin) CTCL patients who received EBRT plus nitrogen mustard. Of 10 complete responders, one died in remission, one relapsed, and 8 remain in continuous response. The 4 partial responders received topical nitrogen mustard and have yet to progress. This study is closed to accrual.

2.12 Combined EBRT Plus Alternating Combination Chemotherapy for Advanced CTCL (Collaboration: Howard University Radiotherapy Dept., WVAMC Dermatology and Hematology Sections). Twenty-five patients with extracutaneous CTCL were treated in this closed protocol on which follow-up is continuing. Nine achieved a complete response, 2 only after the addition of topical nitrogen mustard. Three complete responders remain disease free, and 6/9 are still living. Only 3/15 partial responders are still alive. The single non-responder has died.

2.13 Randomized Trial of Conservative Versus Combined Modality Therapy for CTCL (Collaboration: Radiation Oncology Branch, WVAMC Dermatology and Hematology Sections). This protocol accrues patients with both limited and advanced CTCL and was designed to determine whether aggressive therapy with curative intent with EBRT plus combination chemotherapy in higher doses than used in our previous study is superior to standard sequential topical therapies. To date 36 patients have been entered, 19 to the aggressive and 17 to the conservative treatments. A minimum of 100 patients should be entered on this trial, and at present accrual rates this should require a total of 4 to 5 years as initially projected. It is too early to evaluate the results of this trial, but toxicities of therapy have been acceptable without treatment-related deaths. Thus far there have been 3 disease-related deaths.

2.14 Phase II Studies in CTCL Patients Failing Standard Treatment (Collaboration: Medicine Branch). Several Phase II studies are in progress. Seven patients have received antithymocyte globulin, with two early deaths and one case of serum sickness; 3/4 remaining patients had partial responses. This trial was terminated because of toxicities including fever and myelosuppression. Of 8 patients receiving VP-16, 5 partial responses have been observed with one case of severe myelosuppression. VP-16 appears to be an active agent in CTCL and patient accrual is continuing. Of 13 patients given high dose methotrexate with leucovorin rescue, 6 had partial responses, but the median duration was only 2 months.

2.15 Study of Monoclonal Antibodies in Therapy of CTCL (Collaboration: Univ. of California at San Diego). A study employing a murine pan-T-cell monoclonal antibody, T 101, for the treatment of advanced refractory CTCL will soon be initiated.

2.2 Staging Procedures and Natural History.

2.21 Staging Evaluation of CTCL Patients (Collaboration: Diagnostic Radiology/ Clinical Center, Medicine Branch, WVAMC Pathlogy Service, WVAMC Hematology and Dermatology Sections). We have shown that half of CTCL patients have evidence of extracutaneous disease at the time of diagnosis by light microscopic studies, and 88% have extracutaneous disease when blood and lymph node cytogenetics, T-cell cytology, and electron microscopy are added to light microscopy. Peripheral blood, lymph node, and visceral involvement are poor prognostic features (11).

2.22 Family Histories and Environmental Exposures in CTCL (Collaboration: WVAMC Dermatology). Detailed histories of family malignant and dermatologic diseases and of environmental exposures are taken in all CTCL patients entering our therapeutic trials.

2.23 Unusual Clinical Manifestations of CTCL. We have demonstrated that CTCL may involve the skeletal system with associated hypercalcemia (5). Leptomeningeal involvement has also been observed.

2.3 Clinical-Cell Biologic Correlations.

2.31 Cytogenetic Analysis of CTCL (Collaboration: Medicine Branch). Detailed karyotypic analysis of blood, lymph node, and marrow specimens from untreated and treated CTCL patients have been performed and analyzed.

2.32 Correlation of Cellular DNA Content and Prognosis in CTCL (Collaboration: Medicine Branch). Correlations of DNA content by flow cytometry in CTCL cells with karyotypic analysis and stage of disease and prognosis have been published (15). Aneuploidy by flow cytometry is associated with a poor prognosis.

2.33 Cell Kinetics in the Sezary Syndrome (Collaboration: Clinical Pharmacology Branch). Detailed cell kinetic analysis with tritiated thymidine infusions in CTCL patients has been published (9). In these patients with advanced CTCL, the major site of tumor cell production was both extracutaneous and extravascular.

2.34 Investigations of the Possible Viral Etiology of CTCL (Collaboration: Laboratory Tumor Cell Biology). Following the discovery of a type C retrovirus strain strain CR by the Laboratory of Tumor Cell Biology (LTCB) in one of the CTCL continuous cell lines started by our laboratory, a study of the sera of CTCL patients for the presence of antibodies against viral antigens was conducted in collaboration with the LTCB. Patient CR, as well as some family members, and some other patients with CTCL had high titers of antibody against CR viral components. These data suggest the horizontal transmission of a type C virus. This virus may be the etiology of some cases of CTCL. (63)

2.4 Clinicopathologic Correlations.

2.41 Skin Biopsies in CTCL. A review of all diagnostic and post-treatment skin biopsies in our CTCL patients is underway in order to determine any pathologic correlates with clinical presentation and response to and toxicity of therapy.

2.42 Lymph Node Involvement in CTCL (Collaboration: Lab. of Pathology/NCI). A pathologic review of lymph node biopsy material in CTCL has been initiated in order to determine parameters that are important for the identification of tumor involvement. Statistical forms are designed, and blinded reviews are being completed. Materials used include initial and sequential node biopsies from CTCL patients and patients with benign chronic dermatoses.

2.5 Infectious Diseases.

2.51 Septicemia in CTCL. A review of documented septicemias in CTCL patients has been completed (53). Staphylococcus was the most common organism to be isolated. Difficulties in determining the clinical significance of a positive blood culture in these patients are discussed.

2.6 Review Articles.

2.61 Review articles on the therapy (6, 8, 10, 14) and clinical manifestations (16) of CTCL by NCI-VA MOB staff have been published or are in press.

3.0 ADENOCARCINOMA OF THE PROSTATE

3.1 Therapeutic Trials.

3.11 Combination Chemotherapy as Initial Treatment for Stage D-2 Carcinoma of the Prostate (Collaboration: WVAMC Urology Service, Surgery Branch). Twenty-two patients with distant metastatic (bone with or without other sites) have been given cyclophosphamide, adriamycin, and cisplatin combination chemotherapy prior to any hormonal manipulation. Objective response rate to chemotherapy was 39% (median duration 9 months), with 83% of evaluable patients responding to subsequent hormonal manipulation. Only 3/22 patients have died; median survival will exceed 18 months. Follow-up with documentation of further tumor responses and progressions is continuing. Combined modality chemotherapy plus hormonal therapy should be prospectively compared to hormonal therapy followed by chemotherapy only at disease progression in newly diagnosed patients with Stage D-2 disease.

3.12 Combination Chemotherapy for Hormone-Resistant Stage D-2 Prostatic Cancer. Nine patients with hormone-resistant metastatic prostate cancer received the same combination chemotherapy regimen given to freshly diagnosed patients with a 22% response rate. Patient numbers are too small for any conclusions, but these results suggest the possibility that combination chemotherapy may be more effective in prostate cancer patients when given initially rather than after hormonal failure.

3.2 Staging Procedures.

3.21 Objective Documentation of Response to Systemic Therapy in Carcinoma of the Prostate (Collaboration: Diagnostic Radiology/Clinical Center, Clinical Chemistry/Clinical Center, WVAMC Nuclear Medicine Service). Objective documentation of response to treatment is an exceedingly difficult problem in prostatic cancer. Multiple staging procedures, including bone radionuclide scans and radiographs, assessment of regional lymph nodes by lymphangiography and computed tomography, biochemical measurement of acid phosphatase by enzymatic and radioimmunoassay methods and of creatine kinase-BB by radioimmunoassay, and a variety of more standard observations, have been performed prior to chemotherapy and every 4-6 months during treatment in 53 patients. The best parameters for detecting response appear to be computed tomography of nodes and/or lymphangiography, examination of the prostate, bone scan, carcinoembryonic antigen, and serum acid phosphatase, while bone scan and radiographs, computed tomography, weight, and performance status are most likely to worsen with tumor progression. Worsening bone scans and radiographs are highly non-specific, however, as these findings are rather frequently associated with an objective response in other sites and almost certainly reflect "bone healing." Unfortunately, there is no one good, simple test that can be used to document response in this disease. Such documentation is clearly necessary since most therapeutic trials in prostatic cancer do not provide such information and thus raise difficulties in interpretation.

3.3 Clinical-Cell Biologic Correlations.

3.31 Androgen Receptor Determinations from Prostatic Biopsies (Collaboration: Medicine Branch, WVAMC Urology Service). Androgen receptor assays in prostatic cancer would be of great use if they could identify patients who would be unlikely to respond to hormonal manipulation at the time of diagnosis of metastatic disease (as estrogen receptor determinations can do in carcinoma of the breast) or identify the relatively small number of patients who are able to respond to more than a single hormonal treatment. Percutaneous or transrectal needle biopsies are being submitted for androgen receptor analysis in patients on our therapeutic protocols. Difficulties in obtaining sufficient tissue for analysis have sometimes arisen. This study is continuing.

4.0 MULTIPLE MYELOMA AND OTHER PLASMA CELL DYSCRASIAS

4.1 Therapeutic Trials.

4.11 Cyclic Alternating Combination Chemotherapy for Multiple Myeloma (Collaboration: WVAMC Hematology Section). Patients with multiple myeloma and Waldenstrom's macroglobulinemia are treated with alternating combination chemotherapy regimens every 15 weeks for 1 year. Regimens include melphalan/prednisone, vincristine/cyclophosphamide/adriamycin/prednisone, and BCNU/melphalan/prednisone. Actuarial median survival of the 32 patients entered in this trial is 33 months. Reduction in M-protein levels reaches a plateau after 6 to 9 months in these patients, similar to results in single drug combination trials. After 1 year of therapy, treatment can be safely discontinued, but progressive disease will occur in a median of 9 to 12 months.

4.12 Phase I Trial of Total Body Irradiation (TBI) in Patients with Myeloma Failing Combination Chemotherapy (Collaboration: Radiation Oncology Branch). This trial was initiated on the basis of anecdotal reports suggesting efficacy of TBI in myeloma and the effectiveness of TBI in other B-cell malignancies. Two patients have been entered thus far and have received 50 rad per week. Neither responded and both had substantial marrow toxicity, although both were heavily pre-treated.

4.2 Clinical-Cell Biologic Correlations.

4.21 Correlation of DNA Content by Flow Cytometry (FCM) with Prognosis in Patients with Multiple Myeloma. Aneuploid peaks by FCM in myeloma cells of our patients have been demonstrated to occur more frequently with more advanced disease. They are associated with a worse prognosis.

4.3 Review Articles.

4.31 Review articles on clinical aspects and therapy (6, 17) and cell kinetics assessed by FCM (2) have been published by NCI-VA MOB staff.

5.0 CARCINOMA OF THE STOMACH

5.1 Therapeutic Trials.

5.11 Phase II Trial of ICRF-159 in Gastric Cancer. 21 patients have been entered on this study, including 10 who were previously untreated and 11 who failed combination chemotherapy. In our hands, ICRF-159 has very limited activity in this disease.

6.0 HEPATOCELLULAR CARCINOMA (HC)

6.1 Therapeutic Trials.

6.11 Adriamycin Plus Streptozotocin for HC (Collaboration: Laboratory of Immunodiagnosis/NCI). This study was designed on the basis of definite activity for adriamycin and probable activity for streptozotocin in HC and the ability to combine both at full doses without overlapping toxicities. Unfortunately, only 2/21 patients have had objective responses (2 other patients are too early to evaluate), results no better than what would be expected with adriamycin alone. Both responders had unresectable disease confined to the liver prior to chemotherapy and were subjected to repeat laparotomy after response; the tumor was resectable in one case. This trial will be terminated in the near future.

6.2 Staging Procedures and Prognostic Features.

6.21 Computed Tomography (CT) in HC (Collaboration: Diagnostic Radiology/Clinical Center). CT was performed before and during chemotherapy in 15 HC patients. CT did not detect tumor in any patient who did not also have a positive radionuclide liver scan. During treatment, however, the two tests were complementary in detecting tumor response and progression. In some

patients, CT was the first indicator of change in tumor status, while in others the radionuclide scan changed first (29).

6.22 Long Term Survivors in HC Patients on Adriamycin-Based Chemotherapy (Collaboration: Biometric Research Branch, WVAMC GI-Hepatology Section). Of 35 patients with HC treated with chemotherapy at the NCI-VA MOB since 1973, 11 (30%) lived more than one year from initiation of treatment, an unusually long survival in this disease with a median survival of 2-4 months in most reported series. These "long survivors," all of whom were dead by 3 years, fell into 2 groups. The first consisted of young caucasian patients without any of the usual associated features of HC of cirrhosis, elevated alpha-fetoprotein levels, and presence of hepatitis B markers. These patients had frequent extrahepatic metastases, an unusual fibrocollagenous stroma within their tumor biopsies, and rarely responded to chemotherapy. They seem to constitute a clinicopathologic entity with an indolent clinical course. The remaining "long survivors" had typical manifestations of HC but all were fully ambulatory with normal serum bilirubin levels, and most had objective responses to chemotherapy. Relatively prolonged survival does occur in some HC patients in therapeutic trials, and patient characteristics must be known before results can be interpreted.

6.3 Clinicopathologic Correlations.

6.31 Fibrocollagenous Tumor Stroma in Young HC Patients. Diagnostic pathologic material from 35 patients on HC chemotherapy protocols (see B.2. above) and autopsy material from 13 of these patients were reviewed. In 7 cases, 6 of whom were young caucasians, the tumor was composed of giant hepatocytes arranged in trabecular or lobular pattern, separated by broad collagenous bands. Microcysts and acidophilic hyaline bodies are conspicuous features. The collagenous stroma was identifed in both primary and metastatic sites. This pathologic appearance is similar to "fibrolamellar carcinoma" of Dr. Robert Peters, and identifies patients with indolent tumor.

7.0 PHASE I TRIALS OF NEW ANTICANCER AGENTS

7.1 Therapeutic Trials.

7.11 Phase I Trial of Pentamethylmelamine (PMM) (Collaboration: Medicine and Clinical Pharmacology Branches). PMM is a water-soluble analog of hexamethylmelamine which is suitable for intravenous administration. 34 patients were entered into a Phase I trial at 8 dose levels given IV once weekly. The dose-limiting toxicity was nausea and vomiting, and the maximum tolerated dose was 1000 mg/m^2. Pharmacokinetic studies were performed concurrently and defined a two-phase plasma disappearance curve. This study is accepted for publication (36).

7.12 Phase I-II Trial of Dichloromethotrexate (DCM) (Collaboration: Clinical Pharmacology Branch). 21 patients have been entered onto a Phase I-II trial of DCM given as a weekly 6-hour IV infusion, with dosage escalation every 2 weeks. DCM is of interest since it is an antifol not wholly dependent on

renal elimination and because of reports of superior efficacy to methotrexate in some murine tumors. The maximum tolerated dose was 400 mg/m^2, with escalations of 400 mg/m^2 every 2 weeks. Myelosuppression was the dose-limiting toxicity, and was severe in 4 cases. Partial responses were seen in 4 patients, including 3/7 with hepatocellular carcinoma. Plasma pharmacokinetics were followed by radioimmunoassay for methotrexate. This study is complete. Further trials of DCM in hepatocellular carcinoma appear warranted.

7.13 Phase I Study of Aziridinylbenzoquinone (AZQ) (Collaboration: Medicine Branch). AZQ is a rationally designed drug with good access to the central nervous system and excellent activity against some murine intracerebrally implanted tumors. The drug was studied on a Day 1 and 8 IV schedule every 28 days. 40 patients were entered. The maximum tolerated dose was 20 mg/m^2, with myelosuppression being the dose-limiting toxicity. A Phase II trial in primary and metastatic brain tumors has been initiated.

7.14 SR-270258 in Solid Tumor Patients. SR-270258 is an extract of human blood that has thymic hormone-like activity. It is produced by Syntex Research. Because of the previous NCI-VA MOB experience with thymosin Fraction V, a dose-finding study to determine a dose that would modulate some immune parameters was initiated. Three patients were entered; 2 had immediate progressive disease and 1 died suddenly at home of unknown cause. This study has been terminated because of accrual problems.

7.15 Phase I Study of Recombinant DNA-Produced Human Leukocyte Interferon (Collaboration: Biologic Response Modifiers Program/Frederick CRC, Medicine Branch, Pediatric Oncology Branch). This Phase I trial is being initiated. No patients have been accrued as yet.

8.0 GENERAL MEDICAL ONCOLOGY AND MISCELLANEOUS CANCERS

8.1 Therapeutic Trials.

8.11 Chemotherapy of Adult Wilms Tumor. A response to chemotherapy and a literature review of treatment of this rare tumor have been reported (41).

8.12 Methyl-CCNU With or Without Chlorpromazine and Caffeine in Malignant Melanoma (Collaboration: Eastern Cooperative Oncology Group). This randomized study was designed because of improved efficacy of MeCCNU with chlorpromazine and caffeine in some murine tumors. The adjunctive drugs were of no benefit in human melanoma (27).

8.13 Treatment of Carcinomatous Meningitis (Collaboration: Medicine, Clinical Pharmacology, and Radiation Oncology Branches). Patients are being entered on a randomized trial of radiotherapy plus intrathecal methotrexate alone or radiotherapy plus methotrexate and thio-TEPA for carcinomatous meningitis.

8.14 Venepuncture Techniques in Problem Cancer Patients. This nursing-oriented study is in press (40).

8.2 Prognostic Factors.

8.21 Prognostic Effect of Weight Loss Prior to Chemotherapy (Collaboration: Eastern Cooperative Oncology Group). Weight loss has adverse prognostic effects that are independent of stage of disease and performance status in some tumors (28).

8.3 Clinical-Cell Biologic Correlations.

8.31 Kinetic Perturbations Induced by Chemotherapy in the Marrow of a Patient with Neuroblastoma (Collaboration: Clinical Pharmacology Branch). This detailed study of a single patient has been published (54).

8.4 Infectious Diseases.

8.41 Staphylococcal Septicemia in Cancer Patients. This analysis of the clinical features of staphylococcal sepsis in cancer patients is in press (18).

8.5 Review Articles.

8.51 A detailed review of paraneoplastic syndromes in cancer is in press (51).

9.0 GENERAL MEDICAL ONCOLOGY CONSULTATION SERVICE

9.1 Consultation Service. The NCI-VA MOB provides oncology consultation service to the Washington VAMC, a 600-bed hospital. This year an average of 20 consultations per month have been seen by Clinical Associates and an attending physician. Approximately 35% of patients entered into our therapeutic protocols are identified from this source.

9.2 Oncology Outpatient Clinic. In addition to outpatient care of patients on our protocols, the NCI-VA MOB provides outpatient care with chemotherapy for Washington VAMC patients who are not eligible for protocol studies. Some 10-15 non-protocol clinic patients per week are seen. This experience, plus the consultation service, enlarges the clinical experience of Clinical Associates.

10.0 MEDICAL ONCOLOGY TRAINING PROGRAM

10.1 Training Program for NCI-VA Clinical Associates. Four first year, three second year, four third year, and one fourth year Clinical Associate, with appropriate division of responsibility and supervision by senior staff medical oncologists, participate in primary care of patients on NCI-VA MOB clinical protocols, medical oncology consultations for the WVAMC, outpatient care for non-protocol WVAMC cancer patients requiring chemotherapy, and the planning, execution, and preparation for publication of clinical studies on cancer patients. Clinical Associates from other Branches of the Clinical Oncology Program/DCT/NCI rotate through the NCI-VA MOB in 3-month blocks.

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PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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See Attached Sheet

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
17.5		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project uses a multidisciplinary approach to study tumor cell biology so as to understand the basic nature of human malignancy and to develop methods for the diagnosis and control of human cancer. Particular emphasis is placed on lung cancer and cutaneous T-cell lymphomas. Our major efforts are in the growth of human tumors in vitro and in the nude mouse to study the differentiation, cell kinetics, immunology, experimental therapy, biochemistry, growth factor requirements, tumor markers, and ectopic hormone secretion in these model systems. The human tumor colony forming and nude mouse xenograft assays are used to study tumor biology and to test tumor sensitivity in vitro. Another major area is the use of somatic cell hybrids to study tumor cell biology and cell genetics. These include production of monoclonal antibodies by hybridomas against tumor antigens and defined proteins, comparative gene mapping, human hormone production, and genes controlling expression of the malignant phenotype. Other areas studies include tumor blood flow, tumor cell kinetics, flow cytometric analysis of human tumors, and DNA content of tumor samples.

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3.1 Establishment of cell culture lines from human lung Cancer. (Drs. Gazdar and Carney; E. Russell, Chemist; V. Bertness, Medical Technologist; H. Simms, Biologist and A. Simmons, Bio-Lab Technician).

For the last four years, we have embarked on a program to establish well defined, continous, clonable cell lines from lung cancers. To date we have established over 30 SCCL cell lines, and about 8 non-SCCL lung cancer cultures. With experience, and the developmnt of new techniques, our success rate has steadily increased from about 20% to over 80%. The latter rate is impressive, especially because many of our samples consist of needle biopsies, aspirates, etc. containing relatively few cells. In addition, we carry and characterize a few lung cancer lines established by other investigators. The cell lines have maintained the cyto/histological features of the original tumors. In some cases, paired B lymphoblasoid or skin fibroblast lines have been started from the same patients. The cell lines are the tool which forms the basis of much of the laboratory research program, and have been used for numerous studies including making and testing monoclonal antibodies, determining the growth factor requirements of lung cancers, investigating clonal heterogeneity, identifying new biochemical markers, cytogenetic studies, etc. They have been distributed to many collaborating units elsewhere, as well as to other independent investigators.

3.101 Characterization of small cell carcinoma of the lung cell lines (Drs. Gazdar, Carney, Minna and Russell, Chemist, in collaboration with Dr. Baylin).

SCCL cell lines are unique among epithelial cells, as they consist of tight or loosely packed floating cell aggregates that do not display substrate adhesion. They are classical APUD cells, and express the full program of neuroendocrine cell biochemistry and morphology. Over 95% of the cell lines express high levels of L-dopa decarboxylase and have neurosecretrory granules. Very occasionally, cell lines established from tumors having the classical morphological appearance of SCCL lack APUD biochemistry and neurosecretory granules. Two of these cultures had cytogenetic evidence of SCCL lineage (see below). We have studied APUD characteristics in fresh tumors and compared them to the cell lines established from them. In all cases, the APUD features are more readily identifiable in the cell lines, but were present in the original tumors. There are many reasons for these findings, including sampling problems, a mixture of stromal cells, tumor necrosis, and cell cycle dependence on expression of markers. However, the important point is that the cell lines do mirror the findings in the tumors, and are not a subpopulation selected during culture. Two D gel analysis indicates that SCCL cell lines have large amounts of a high molecular weight membrane protein that is either not present or present in much smaller amounts in other lung cancers.

3.102 Biochemical Markers for SCCL. (Drs. Gazdar, Carney, and Minna, in collaboration with Drs. S. Baylin, P. Marangos, C. Dermody, C. Pert, T. Moody and M. Zweig).

SCCL cultures are classical APUD cells, expressing all of the properties common to these cells, as well as expressing certain unique features. In addition to L-dopa decarboxylase and neurosecretory granules, SCCL cultures

express high levels of the APUD cell marker neuron specific enolase. In addition, we have identified very high levels of creatine kinase and its BB isoenzyme in SCCL tumors and cultures. An examination of more than 60 lung cancers and cultures indicates that with 2 possible exceptions, CK-BB levels invariably distinguish SCCL from other lung cancers. Thus it is a more specific marker than L-dopa decarboxylase, and is retained when APUD markers are lost. We have determined that CK-BB is not an APUD cell characteristic, but is a specific feature of SCCL. Of interest, the only normal tissue expressing high levels of CK-BB is the brain, suggesting that SCCL has many differentiated properties that it shares with neurons. We have measured the levels of over 12 polypeptides in our SCCL cultures. Most are expressed, to some extent, in some of the cultures. The levels are usually low compared to the normal tissues elaborating these peptides. Some are also expressed in other forms of lung cancer. By contrast, bombesin levels in SCCL are invariably high, and are undetectable in other lung cancers. The levels in some of the cultures exceed those present in the brain and cord. Of interest, levels of substance P in the SCCL cultures are low and are expressed in only a few of the cultures. Because substance P levels in the brain are much higher than those of bombesin, and because the distribution of the peptides is remarkably similar, our studies indicate that the two peptides can be expressed discordantly in SCCL, and perhaps elsewhere. Bombesin levels are very high in the fetal and neonatal lung, and very low in the adult. Thus our studies suggest that SCCL is derived from 'bombesinergic' endocrine cells present predominantly in the fetal lung.

3.103 Morphological biological, and biochemical changes in long term SCCL cell lines (Drs. Gazdar, Carney, Bunn and E. Russell, Chemist, Sims, Biologist, in collaboration with Drs. Baylin, Mitchell, Kinsella).

We have previously described alterations in morphology of long term SCCL cultures to other forms of lung cancer, especially large cell anaplastic carcinoma. By studying a variety of markers in these cultures during and after morphological conversion, some generalizations can be made about the sequence in which they occur. Polypeptide hormone secretion (other than bombesin) are frequently lost, even when the cultures retain SCCL morphology and APUD cell features. Loss of characteristic SCCL morphology is accompanied by loss of APUD markers, including L-dopa decarboxylase and neurosecretory granules; although neuron specific enolase (NSE) levels drop they remain higher than other lung cancers. CK-BB levels remain high after conversion, and NSE and CK-BB are the best biochemical markers for cell lines of SCCL lineage. However, the only marker invariably identified in all cultures of SCCL lineage is the characteristic cytogenetic marker 3p-. We have found the large cell variants are resistant to many chemotherapy agents.

In collaboration with the NCI-ROB (Drs. Mitchell and Kinsella) we have been studying the radiation sensitivity of the lung cancer lines and comparing the small cell with the large cell variants. We have found the D_0 is the same in both, but the extrapolation ("Hit") number is 1 in the SCCL line but about 3 in the large cell variants. This suggests the expression of a radiation repair mechanism in these cells. Characterization of the mechanisms of chemo - and radiation therapy resistance form an important approach to cancer treatment.

3.104 Clonal Heterogeneity within SCCL tumors and cell lines. (Drs. Alvarez, Carney, Gazdar, Minna and Bunn).

We have established and partially characterized over 100 clones by soft agarose cloning techniques from 6 different SCCL cultures. In addition, one fresh SCCL tumor specimen was cloned directly in agarose, and the clones successfully propagated in liquid culture. These studies indicate that while many polypeptide hormones show considerable clonal variation, the APUD cell features and CK-BB are tightly retained, and it is difficult to obtain even a single clone lacking these features. While these markers are expressed in virtually all tumors and their clones, the percentage of cells expressing markers is very variable, and seldom, if ever, 100%. The reason for this cell to cell variation within both tumors and clones is being investigated.

We have studied one cell line, NCI-H128 in detail. This line is of particular interest, because the original tumor and parent cell line contained at least two aneuploid peaks. By cloning, we have isolated 12 clones whose DNA contents are similar to those of one or the other of the two aneuploid peaks. While within each subgroup there is little variation, clones of the two aneuploid groups vary in L-dopa decarboxylase, growth rate, clonal morphology, colony forming efficiency, expression of 'SCCL' antigen (as detected by monoclonal antibodies), and latent time to tumor induction.

3.105 Clonal heterogeneity documented by FCM and cytogenetics (Drs Bunn, Carney, Gazdar, Minna in collaboration with Dr Whang-Peng)

We have demonstrated the in vivo presence of multiple clones of an individual tumor by both FCM and modal chromosone number. There is excellent correlation between FCM and chromosome number. When multiple stem lines were present in the patient all had the same 3 p- marker indicating a monoclonal abnormality and confirm the importance of the 3 p- marker. Clones of individual tumors established in soft agar or by limiting dilution techniques have marked heterogenity with respect to monoclonal antibody binding as well as other markers as noted elsewhere in this report.

3.106 Cytogenetic Studies in Lung Cancer (Drs Bunn, Gazdar, Carney, Minna in collaboration with Dr. Whang-Peng.

We have shown that established cell lines of small cell lung cancer have a specific, non random chromosomal abnormality, a deletion of a portion of the short arm of chromosome #3 ($3p^-$). This abnormality was present in all cells from 16 established cell lines including several that were mixed small cell large cell carcinoma. This specific abnormality was not present in non-small cell lines. Some small cell lines contained double minute chromosomes and homologous staining regions which have been reported to be associated with drug resistance, especially to methotrexate. We are correlating these results with in vitro drug senstivity studies. We are evaluating fresh patient specimens for the presence of the 3 p^- abnormality. More non-small lines will be studied. Modal chromosome numbers were higher in non-small cell tumors. No specific abnormality was found in these 5 lines although all had some abnormality of chromosome # 1. We are studing fresh specimens from

untreated patients with repeats after therapy to determine whether therapy induced hematologic abnormalities can be detected early with cytogenetics.

3.107 Genetic analysis of human lung cancer cells (Drs. Minna, Oie, Alvarez, Cuttitta, Rosen).

We have been trying to isolate the genes coding for malignancy in human lung cancer cells. The cytogenetic results suggest some genes on human chromosone 3p may be involved. We have been using somatic cell fusion and gene transfer techniques to introduce genetic material from human lung cancer cells into rodent cells (both malignant and non-malignant). We are testing for one expression of the malignant phenotype (soft agar growth and nude mouse tumorigenicity), tumor associated antigens by monoclonal antibodies, differentiated functions (eg APUD properties) in the hybrid cells and tyrosine kinase ("SARC") activity. The identification of study of the expression of such genes is of fundamental importance to developing new approaches to the control of lung cancer.

3.2 In vitro progagation and characterization of cell lines from T-cell lymphomas (Drs Bunn, Gazdar, Carney, Krasnow, E. Russell, Chemist).

We have reported the establishment and characterzation of 2 malignant T-cell lines from patients with cutaneous T-cell lymphomas. We have been able to propagate malignant T-cells from several additional patients for short periods (1 week - 4 months) using mitogens or crude T-cell growth factor (TCGF). We are currently attempting to establish additional long term malignant T-cell lines using purified and lectin-containing TCGF. In addition, we are attemping to establish normal cytotoxic T-cells lines from peripheral blood, lymph nodes and/or tumors from CTCL patients using lectins and TCGF. Monoclonal antibodies and DNA content analysis are used to determine the phenotype of responding cells. We are studying the differential effects of crude and purified TCGF on benign versus malignant T-cells.

3.201 Isolation and characterization of retroviruses from CTCL patients and tumors. (Drs. Gazdar, Bunn, Minna, Carney, in collaboration wiht Dr.s Gallo, Ruscetti and Poiesz).

Dr. Gallo's laboratory isolated and characterized a retrovirus from one of our CTCL cell lines. A cell line established from the patient's lymph node by us, as well as cultured peripheral blood lymphocytes releases a virus shown to have biologic and immunologic properties different from other retroviruses. The cell line, HUT 102, grows in the absence of T-cell growth factor, and releases the virus constitutively. In contrast, peripheral blood lymphocytes require the growth factor for replication, and require chemical induction for virus release. Thus HUT 102 is a preferable source of the virus. Dr. Gallo's laboratory has isolated a similar virus from another CTCL patient. We are continuing to cooperate with Dr. Gallo, searching for similar viruses in other CTCL patients, as well as doing immunologic studies on sera of CTCL patients and their relatives. These studies have demonstrated the presence of antibodies against the HUT 102 virus in some CTCL patients.

3.202 Monoclonal antibody characterization of CTCL cells. (Drs. Bunn, Minna, in collaboration with Drs. Haynes and Metzgar).

In collaboration with Dr. Barton Haynes we have been phenotyping CTCL cells with a panel of hybridoma monoclonal antibodies. We have shown that CTCL cells have a characteristic phenotypic pattern of monoclonal antibody binding including pan mature T cell + (OKT1,3+; leu 1 +, T101 +; ASO +); helper/inducer + (OKT4 +; leu 3A +); helper subset 3A1 -; cytotoxic suppressor - (OKT 5,8; Leu 2A); early thymocyte - (OKT9, 10). We have demonstrated that our malignant T-cell lines continue to express the same antigen profile. We are currently evaluating other monoclonal antibodies, new patient samples and heterogeneity in individual CTCL patients.

3.203 Cytotoxicity and antigen modulation by monoclonal T-cell antibodies. (Drs Bunn, Krasnow, Cuttitta, and M. Schlam, Microbiologist).

We are studying the cytotoxicity and antigen modulation of a panel of T-cell monoclonal antibodies after in vitro and in vivo administration of the monoclonal antibodies. In collaboration with Dr. Ivor Royston we will be studying the effects of radiolabeled antibody in cytotoxicity as well. Antibody T101 will be used to treat patients and the in vitro and in vivo responses will be monitored in the laboratory.

3.204 Development of hybridoma monoclonal CTCL antibodies. (Drs. Bunn, Minna, Cuttitta, Rubinstein, Krasnow, H. Sims, Biologist).

We are currently engaged in the production of monoclonal antibodies against CTCL cells. Cells used for immunization are taken from cell lines HUT 102 or HUT 78, or directly from patients and injected into mice and rats. After appropriate boosting doses, animals are sacrificed and their spleens harvested and fused to mouse myeloma cells. The hybrids are grown in selective HAT media in microwells and the supernatants tested for antibody production against the same CTCL cells, HUT 102 cells, and human B lymphoblastoid cells. In addition we are collaborating with Dr. Barton Haynes, Duke Univ. and Richard Edelson, Columbia Univ. who are using our cells for immunizations. We are collaborating in the evaluation of their antibodies.

3.205 Cytogenetic analysis of CTCL cells (Drs. Bunn, Carney, Gazdar, in collaboration with Dr. Whang-Peng).

We have demonstrated that many cytogenetic abnormalities can be identified early in the course of patients with CTCL but that clonal abnormalities appear late. Clonal abnormalities are associated with a poor prognosis. We are continuing these studies in direct patient samples before and after therapy to determine if specific abnormalities are detected, whether cell lines have matching abnormalities, and whether cytogenetic analysis provides meaningful clinical information.

3.3 Studies of megakaryopoiesis (Dr. Bunn, M. Schlam, Microbiologist in collaboration with Dr. R. Levine).

We are continuing our analysis of megakaryocyte ploidy in humans and laboratory animals. We have developed techniques for identification of megakaryocytes in bone marrow by dual labeling of DNA content and antiplatelet antibodies.

3.4 Primary in vitro cloning of clinical specimens of human small cell lung cancer (SCCL). (Drs. Carney, Gazdar, Minna, Bunn, V. Bertness, Medical Technologist).

We have evaluated the in vitro cloning of fresh tumor containing SCCL specimens obtained from patients undergoing approved protocol staging procedures. 75/84 specimens demonstrated tumor cell colony formation in vitro with a median number of 22 colonies per 10^5 viable mononuclear cells plated (range 3-360/plate). The tumorigenic nature of the cultured colonies was confirmed by cytology examination, DNA content analysis by flow cytometry, electron microscopic examination, and by the ability of colonies to form tumors in athymic nude mice. While the vast majority of clinical specimens of SCCL containing tumor cells will form colonies in agarose, improvement in the colony forming efficiency is required to permit in vitro drug sensitivity studies in the majority of patients.

3.401 Use of short and long term in vitro culture of SCCL specimens to predict in vivo drug sensitivity. (Drs. Carney, Gazdar, Minna, and V. Bertness, Medical Technologist).

10 established (4-16 months) cell lines of SCCL were assayed in the clonigenic assay for drug sensitivity. 6 cell lines were from previously treated patients and 4 from patients who had not received prior chemotherapy. Drugs tested in the assay included methotrexate, vincristine, vindesine, BCNU, adriamycin, melphalan, VP16, 5-FU and cisplatin. In vitro sensitivity was observed only in those lines established from newly diagnosed patients, while lines from patients who had relapsed from prior therapy were resistant to those drugs which the patient had received. Short term culture of SCCL specimens gareatly amplify the number of specimens which can be tested against a large number of chemotherapeutic agents. These results suggest the in vitro sensitivity of the cell lines is correlated with the response in the patient. The use of short term culture of such "mini" cell lines will dramatically increase the number of clinical samples which can have drug sensitivity studies performed, and forms the basis for the prospective clinical trials using in vitro sensitivity to select treatment for individual patients.

3.402 In vitro cloning of clinical specimens of non-small cell lung cancer (Drs. Carney, Gazdar, Bunn and Minna, V. Bertness, Medical Technologist).

The in vitro cloning of fresh specimens of non-small cell lung cancer was evaluated in 16 specimens. 11 specimens (66%) formed tumor cell colonies in agarose (median/plate 44, range 12-720). The tumor origin of these colonies was confirmed by cytology, DNA content analysis and tumorigenicity. Colony

formation was poorest in solid tumor specimens. These data suggest that in vitro drug testing directly from patient samples may be possible, but only in a small number of patients with non-small cell lung cancer. Because of this we plan to use the establishment of short term culture of "mini" cell lines to perform drug sensitivity testing. We will use this information in prospective trials to select therapy for individual patients.

3.5 Identification of growth factor requirements for lung cancer cells in chemically defined media (Drs. Oie, Carney, Gazdar, Minna).

Previously we identified a completely defined medium ("HITES") that supported the growth of all SCCL lines tested. In addition, it is a selective medium for the growth of SCCL tumors but not other forms of lung tumors or stromal cells (see below). However, it became apparent that the HITES medium (RPMI-1640 medium supplemented with hydrocortisone, insulin, transferrin, selenium and B-estradiol) was not a complete medium, and lacked one or more components required for optimal growth of SCCL. We have continued to investigate this problem, and have determined that addition of medium conditioned by certain replicating SCCL cultures added to HITES medium greatly aids the replication and cloning efficiency of other SCCL tumors and cultures. We are currently attempting to identify the factor(s) present in conditioned medium. We are focussing on peptides and other substances present of such substances in the cultures may represent examples of autocrine secretion. In addition, we are attempting to identify the growth requirements of other forms of lung cancers, as, with rare exceptions, these do not replicate in HITES medium. We have evaluated the production of growth factors for SCCL by a cell line NCI-N592 which was established in serum-free HITES supplemented medium from a nude mouse tumor formed by agarose colonies of a fresh bone marrow specimen of a patient with SCCL. The factor(s) produced by this cell line increased the colony forming efficiency (CFE) in serumfree HITES medium of itself, but also that of 6/7 other SCCL cell lines. Colony formation was increased by 3-12 fold with the growth factor(s) and the number of cells in each colony was also dramatically increased. Studies are in progress to characterise and purify these growth factors.

3.501 Selective growth of tumor cells from clinical specimens of SCCL in serum-free; chemically - defined medium. (Drs. Carney, Bunn, Gazdar, Minna).

The ability of tumor cells in fresh clinical specimens obtained from patients with SCCL to grow in a chemical defined, serum-free medium was tested. The serum-free HITES medium had been designed on the basis of studies using established cell lines of SCCL. Successful culture of tumor cells was observed in 20/26 clinical specimens while only 16/26 specimens demonstrated tumor cell proliferation in serum-supplemented medium. The nature of the cultured cells was confirmed by many studies including DNA content analysis by flow cytometry, and by nude mouse tumorigenicity. Using this culture system, pure populations of rapidly dividing SCCL tumor cells were obtained 7-14 days after plating. By this time all normal cells had died. The use of this chemicallydefined, serum-free medium should be useful for the evaluation of growth factors in SCCL, for cytogenetic analysis of fresh clinical specimens, for establishing new cell lines of SCCL, and for in vitro drug testing. The selective nature of

the medium was shown by the failure of normal cells and the majority (13/14) of other human tumors (including 7 non-SCCL lung cancers) to grow in this medium.

3.6 Hormone receptors on the surface of lung cancer cells (S. Sherwin, J. Minna, A. Gazdar, in collaboration with G. Todaro).

A panel of lung cancer cell lines was studies for receptors for epidermal growth factor (EGF) and nerve growth factor (NGF) using labeled hormone binding assays. We have found that non-small cell lung cancer has receptors for EGF while small cell lung cancer does not. In contrast, some small cell lung cancers express low levels of NGF receptors while non-small cell cancer does not. In cells which have converted from the small cell to large cell phenotype NGF receptor levels increase, and in some cases EGF receptors are expressed. In addition, we have assayed lung cancer and mesothelioma cell lines for the production of a soft agar growth factor which will stimulate the clonal growth in agar of normal rat kidney cells. We have found all lung cancer and mesothelioma cells make such a factor (s) and have begun biochemical purification of the factor(s). Such factors may be responsible for the growth of lung cancer cells in vivo or create previously undescribed paraneoplastic syndromes in lung cancer and mesothelioma patients. These studies are continuing.

3.7 Preparation and characterization of monoclonal antibodies. (Drs. Minna, Rosen, Abrams, and Cuttitta).

A considerable effort is focused on the preparation and characterization of monoclonal antibodies using the hybrid cell fusion technique of Milstein. Monoclonal antibodies with specificity for human lung cancer cells have been prepared by screening for hybrids producing antibodies that react with several lung cancer lines (usually small cell lung cancer) but not with autologous B lymphoid or skin fibroblasts lines from the same patients. A panel of 50 such antibodies have been isolated, cloned, stabilized and are being characterized. These do not react with normal human lung and many other human tissues but in some cases do react with normal human kidney. The antibodies detect several different antigenic determinants. Some detect antigens only expressed on small cell lung cancer but not other histologic lung cancer types, while others react with small cell, adeno, and epidermoid lung cancer. Many react with human neuroblastoma, and several with human breast cancer. Thus, they may have general use for cancer diagnosis and treatment. The molecules these antibodies interact with are being characterized and, at present, most seem to be cell surface antigens, several are proteins, probably of low molecular weight, and some represent molecules that are required for the growth of tumor cells. Using these antibodies we are setting up assays for: early lung cancer diagnosis by screening for antigens in serum, urine and bronchial washings; use in staging studies particularly using the fluorscent activated cell sorter analysis of clinically obtained specimens. In addition, we are beginning collaborative studies to put toxins (eg, ricin), drugs (adriamycin), or radio-isotopes onto the monoclonal antibodies to try to develop specifically targeted anti-cancer therapies. These reagents are potentially of great clinical and biologic use.

As described elswhere, we have prepared other monoclonal antibodies including monoclonal antibodies against myoglobin and DNA polymerase alpha. Using the anti myoglobin monoclonal antibodies in collaboration with Dr. J. Brezsofsky it has been possible to: pinpoint the exact binding site of the antibody to this protein antigen, the first such demonstration known; show that monoclonal antibodies binding to different determinants on myoglobin share idiotypes, a feature of considerable importance in understanding the genetics of the immune response; potentially develop immunoassays for circulating myoglobin in human patients. The monoclonal antibodies to DNA polymerase alpha (prepared in collaboration with Dr. S. Wilson) should allow the determination of the subcellular localization, precursor characterization, and allow quantitation of this important synthetic and repair enzyme in clinically relevant states (such as correlating its level with radiation repair).

3.8 Production of characterization of monoclonal antibodies with specificity for human lung cancer cells. (Drs. Minna, Cuttitta, Rosen, Oie, Bunn, Krasnow, and Fedorka, Microbiologist).

Our primary efforts have been in the development of monoclonal antibodies with selectivity for human small cell carcinoma. Such antibodies were produced by immunizing BALB/c mice with an established line of human small cell lung cancer (NCI-H69) and fusing the mouse spleen cells with mouse myeloma line X63-Ag8.653. The resulting hybrid cells were initially screened by immunoautoradiography for production of antibodies that react with NCI-H69, and another small cell lung cancer line (NCI-H128) but not its autologous B-lymphoblastoid line (NCI-H128BL). Using this approach, we have been successful in isolating approximately 100, independently derived, hybrid clones which produce antibody with preferential binding for small cell carcinoma. Three of these hybrid clones, designated as 525A5, 534F8, and 538F12 have been extensively characterized and were shown to react with three of the major types of human lung cancer (small cell, adenocarcinoma, and squamous carcinoma). They did not react with bronchioloalveolar and large cell lung cancers, myeloma, lymphomas, leukemias, osteogenic sarcoma, mesothelioma, hypernephroma, malignant melanoma, SV40-transformed human fetal lung cells, skin fibroblast lines, human B-lymphoblastoid lines, human red blood cells and rodent cells. Interestingly, these antibodies also bound to 3/3 human neuroblastomas and 2/3 breast cancers but failed to react with mouse neuroblastoma and rat pheochromocytoma. The monoclonal antibodies reacted with human small cell lung cancer tumors obtained at autopsy, but had insignificant reactions with normal human lung, liver, spleen, and skeletal muscle from necropsies. We conclude that monoclonal antibodies have been generated which react with common antigenic determinants expressed on several human lung cancer types, neuroblastoma, and some breast cancers, but are not detectable by our current assays on a variety of other human tumors, or normal adult human tissue.

We are now in the process of using these reagents in immunocytology as possible diagnostic tools for human small cell carcinoma. A two front approach has been undertaken in the immunocytologic examination series, evaluation at the light microscopy level as well as by flow cytometry. Light microscopy offers a rapid means of diagnostic verification for SCLC using immunofluorescence

or peroxidase staining. Flow cytometry on the other hand can give some insight as to the heterogeneity of the given tumor type and the DNA distribution of the reactive cells.

Preliminary physiochemical characterization of the small cell antigen to the monoclonal antibody 534F8 has been completed. The antigen appears to be extremely resistant to proteases, organic solvents, oxidants, glycosidases, and high and low pHs. Initial molecular weight estimation of the triton X solublized antigen, as determined by SDS-PAGE, appears to be less than 30,000 daltons.

We have also generated rat X mouse monoclonal antibodies using the techniques described above displaying reactivity with small cell lung cancer lines and tissue sections, which do not react with autologous and other B lymphoblastoid cell lines. Extended testing has been performed against malignant cell lines and tissue sections of various cancer histologies, as well as normal necropsy specimens. These monoclonal antibodies are being evaluated as reagents for immunohistochemistry, immunofluorescence, immunoprecipitation, and cytotoxicity assays. They offer great potential as reagents capable of discerning tumor heterogeneity. Potential clinical applications include the screening of bronchial washings and bone marrow sections for the presence of small cell lung cancer, radioimmunodetection of metastatic lesions and detection of circulating small cell lung cancer related antigens and autologous antibodies. The potential therapeutic options that will be explored include targeting of therapy utilizing the selectivity of these reagents - this may be applied to systemic therapy as well as in vitro treatment of bone marrow specimens obtained for autologous transfusions.

We are studying the fraction of tumor cells binding various monoclonal antibodies as well as the intensity and specificity of binding. There is marked variation in positive cell lines from <10% positive cells to >90% positive cells. The intensity of binding also varies from weak to strongly positive. The best antibody or combination of antibodies for clinical use can be established by these techniques. In addition we are evaluating each antibody for cytotoxicty as well as antigen modulation. As noted elsewhere, we are also using monoclonal antibody findings for detection of malignant cells in patients.

3.9 Preparation and characterization of monoclonal antibodies against myoglobin. (Dr. Minna, in collaboration with Drs. J. Berzofsky and F. Gurd).

A panel of monoclonal antibodies was prepared against sperm whale myoglobin. Biochemical and immunologic characterization of these antibodies has revealed that 1) they bind to myoglobin with high affinity (10^9 mol); 2) they bind to different sites on the myoglobin molecule; 3) that some of them bind to human myoglobin and thus can be used in radioimmunoassays for detecting circulating myoglobin, a test that would be potentially useful in diagnosing myocardial infrarctions; 4) that different species vary in whether or not their myoglobins will bind the antibodies and thus they can be used to study the evolution of myoglobins; 5) that the antibodies do not bind to isolated peptide fragments and thus they recognize some conformational determinant on

the myoglobin molecule; 6) that by testing a different species of myoglobins, the precise binding sites of some of the determinants can be localized on the surface of the molecule, in some cases these represent the bringing together of determinants far apart on the molecule, the first such demonstration known; 7) analysis of idiotypes possessed by the antibodies show that antibodies which detect different determinants share idiotypes, suggesting mechanisms of genetic regulation of antibody binding specificity. This work will continue.

3.10 Preparation and characterization of monoclonal antibodies against DNA polymerase alpha (Dr. Minna, Fedorko, Microbiologist, in collaboration with S. Wilson).

Using calf DNA polymerase alpha purified to homogeneity, monoclonal antibodies have been prepared which bind to the DNA polymerase. A sensitive solid phase radioimmunoassay was established which can detect nanagram quantities of DNA polymerase alpha. These antibodies will be used to study the subunit structure and precursor of DNA polymerase through immunoprecipitation; used as an immunoabsobent to purify the polymerase; study the cellular localization, biology and regulation of the polymerase; used to facilitate isolation of message and recombinant DNA clones coding for the polymerase; test human tumor cells for DNA polymerase levels and DNA repair. It is likely that several of the human lung cancer lines which have developed drug and radiotherapy resistance have developed a new DNA repair mechanism and the most probable candidate is DNA polymerase alpha. These studies are continuing.

3.11 Establishment and characterization of monoclonal antibodies to rat islet cells. (Drs. Oie, Cuttitta, Gazdar, Minna, in collaboration with Eisenbarth, Scearce).

Twelve hybridoma cell lines which produce antibodies reacting with the rat islet cell line RIN-m were generated by fusing spleen cells from BALB/c mice immunized against RIN-m cells with mouse myeloma cells. Analysis for specificity showed that 1) antibodies from 5 of the cell lines reacted with rat fibroblast cells as well as RIN-m cells; 2) one antibody reacted with tissue sections from the transplantable rat islet cell tumor and with sections from normal rat pancreas; 3) four antibodies reacted with tissue sections from the transplantable rat islet cell tumor but not with sections of normal rat pancreas; 4) seven antibodies were cytotoxic for RIN-m cells. These antibodies should be useful reagents in studies of the physiology and pathophysiology of the islet cell plasma membrane.

3.12 Establishment and characterization of a rat islet tumor cell line RIN-m and clones secreting insulin, somatostatin, and glucagon. (Drs. Oie, Gazdar, Sims, Biologist, in collaboration with Drs. Chick, Weir, Bhathena, Smith and Recant).

We have established and described a continuous line RIN-m of islet cells from a transplantable rat insulinoma. These cells secrete insulin (IRI), somatostatin (SRIF), and glucagon. By using media supplemented with conditioned medium from a rat pituitary tumor line GH3 (which produces prolactin and

growth hormone), thirty primary clones were isolated. With respect to IRI and SRIF, all four possible hormone secretion pattern phenotypes (IRI+/SRIF+;IRI+ /SRIF-; IRI-/SRIF+; and IRI-/SRIF) were isolated. Instability in their hormone expressions led us to reclone two high hormone secreting primary clones C1-5 (IRI+/SRIF-) and C1-14 (IRI-/SRIF+). The resultant subclones were stable in their hormone secretion phenotypes and included clones of the classes IRI+ /SRIF; IRI-/SRIF+; and IRI-/SRIF-. No clone which stably produced high levels of both IRI and SRIF were found. From analysis of both primary and secondary clones of RIN-m, the following conclusions can be reached: 1) on a clonal basis, IRI and SRIF are expressed independently in this islet cell tumor; 2) both "stable" and "unstable" hormone producing clones can be isolated; clones producing high levels of both hormones appear to be unstable; 4) in contrast to the variability of hormone expression, all clones and subclones consistently retained high levels of L-dopa decarboxylase, the key APUD cell enzyme, indicating that variation in hormone secretion cannot be explained by loss of APUD properties; 5) for optimal growth, rat islet cells require a factor(s) (possibly growth hormone and prolactin) produced by the rat pituitary tumor line GH3, suggesting that these factor(s) may have a physiological role, possibly during fetal pancreatic growth.

Hormone secretion by islet cells can be increased by treatment of the cells with secretagogues. During studies to stimulate hormone production by RIN-m and its clones with various secretagogues, glucagon was found to be secreted by some of the cell lines tested with C1-14 secreting the most. However, analysis of 19 subclones of C1-14 showed that little of no glucagon was being produced by unstimulated subclones. In RIN-m cells and its clones, glucagon appears to be under tight control and is secreted only after exposure to secretagogues.

RIN-m cells and its clones and subclones have specific receptors for insulin, somatostatin, and glucagon. Analyses of Scratchard and affinity plots of RIN-m, high IRI secretors, and high SRIF secretors showed that 1) insulin secreting clones bound less insulin than somatostatin and other non-insulin secreting clones, indicating that insulin receptors were "down" regulated in the presence of high levels of insulin while somatostatin receptors showed just the opposite effect; 2) somatostatin secreting clones had more receptors to all 3 hormones while insulin secretors resembled parent cells in binding properties; 3) the presence of specific receptors for IRI, SRIF, and glucagon on all cells and the low affinity of these receptors for the hormones suggest autoregulation of hormone production.

3.13 HLA type of patients with cutaneous T-cell lymphomas (Drs. Bunn, Rosen in collaboration with Dr. Teraski).

In contrast to previous reports we have shown that there is no association between any HLA type and mycosis fungoides or the Sezary Syndrome.

3.14 Storage of cell and serum from patients with cutaneous T-cell lymphomas (Drs. Bunn, Carney, Gazdar and Russell Chemist).

In order to facilitate labratory studies of many types we are storing

frozen cells collected from lymph nodes (obtained at surgery), perpheral blood (obtained by leukapheresis) and tumors (obtained by surgery). We are also obtaining and freezing serum before and after treatment.

3.15 Red blood cell deformability in experimented cancer chemotherapy. (Dr. Cohen).

Abnormal red blood cell deformability (RBCD) has been found to develop in animals bearing L1210 leukemia and Lewis lung carcinoma. It was further found that RBCD could be improved by red cell metabolic substrates, such as inosine and pyruvate and that RBCD also improved 4-5 days after effective chemotherapy. Consequently treatment experiments were initiated in mice 7 days after transplantation of 10^5 L1210 cells. Treatment of mice after optimal restoration of RBCD resulted in 44% cures. Best cure rate in any of the conrol groups was 2% cures. Therefore introduction of non-toxic methods to correct abnormal RBCD significantly improved chemotherpay efficacy in this animal model.

3.16 Experimental pulmonary toxicity of chemotherapy. (Dr. Cohen).

Bilateral interstitial pulmonary disease is being recognized as a complication of chemotherapy with increasing frequency. A mouse model for reproducably producing this toxicity with either BCNU, cyclophosphosphamide or mitomycin C, but not with adriamycin, has been developed. Pulmonary toxicity correlates with impairment of red blood cell deformability and can partially be corrected by restoration of deformability. Optimal treatment programs to prevent pulmonary toxicity are being developed.

3.1601 Angiotesin converting enzyme as a monitor of pulmonary toxicity (Dr. M. Cohen).

Preliminary experiments utilizing the above model indicate that angiotensin converting enzyme (present in pulmonary endothelial cells) decrease with development of lung toxicity. Decreases are noted in both blood and bronchial washings. Based on these results trials are being initiated in small cell lung cancer patients to follow serum levels of this enzyme as a possible indicator of lung toxicity.

3.17 Mechanisms of bone lysis in cutaneous T-cell lymphomas (Drs. Bunn, Matthews, Brigham, Bradley in collaboration with Drs. Schechter, Horton, Wahl and Dunnick).

We have demonstrated that CTCL patients may develop osteolytic lesions, generalized osteoporosis or osteosclerotic lesions. We have shown that the malignant T-cells from some patients secrete a substance (osteoclast activating factor) which can cause bone resorption in vitro. We are continuing to evaluate patients.

3.18 Laboratory studies on the kinetics of human malignancies. (Drs. Bunn, Carney, Gazdar, Krasnow and M. Schlam Microbiologist).

3.1801 Kinetic studies in lung cancer (Drs. Bunn, Krasnow and Schlam, Microbiologist).

We are studying the cell cycle distributions, doubling times, cell cycle times in patients, in tumor cells grown in vitro and as in nude mouse heterotransplants.

3.1802 Studies of automated cytology in lung cancer (Drs. Bunn, Carney, Gazdar, Matthews, Krasnow, and Schlam, Microbiologist).

We have compared results of standard cytologic evaluation of direct patient samples with DNA content analysis by flow cytometry (FCM). In over 200 samples obtained from metastatic sites there was an excellent correlation between the 2 procedures. In about 20% of instances, however, FCM was normal while cytology showed large numbers of malignant cells. Our cell culture studies have demonstrated that this is due to the fact that the tumors have diploid DNA content. We have shown that 80% of small cell tumors have aneuploid DNA content while 90% of non small cell tumors are aneuploid. We are attempting to improve detection by using dual parameter FCM employing DNA content analysis and monoclonal antibody staining.

Results from bronchial washings were less satisfactory particularly because of increased mucus and debris. To circumvent this problem we are attempting to culture specimens for 2 days in serum free chemically defined medium to get rid of debris and normal cells.

3.1803 Kinetic studies in cutaneous T-cell lymphomas (Drs. Bunn, Krasnow, Carney, Gazdar and Schlam, Microbiologist in collaboration with Dr. Shackney).

We have demonstrated that circulating Sezary cells are not derived from the skin but appear to migrate from lymphoid areas where the rate of cell production is greater than the rate of cutaneous production. Sezary cells do not divide in the blood. Extracutaneous Sezary cells proliferate at a relatively rapid rate but the rate of cell loss is also high and the clinical doubling times are long. Patients with aneuploid tumors appear to have a worse prognosis than those with diploid tumors and aneuploid tumors seem to proliferate at a more rapid rate. We have demonstrated that certain mitogens can preferentially stimulate malignant T-cells to proliferate. We are attempting to identify small numbers of CTCL cells in patients using dual FCM by combining DNA content analysis and monoclonal antibody binding analysis.

3.1804 Cell Kinetic studies in multiple myeloma. (Drs. Bunn, Krasnow, and Schlam, Microbiologist in collaboration with Dr. Schechter).

We have demonstrated that over half of all myeloma patients have cells with aneuploid DNA content at diagnosis and that 90% of patients have developed such cells by the time of death. Aneuploidy is significantly associated with advanced stage and renal failure and has a poor prognosis. Aneuploid tumors seem to have higher fractions of cells in S-phase although this needs to be confirmed in dual parameter studies. We are continuing to study patients with single parameter DNA and dual parameter DNA/myeloma protein studies.

3.1805 Cell kinetic studies in ovarian cancer. (Drs. Bunn, Carney and Schlam, Microbiologist in collaboration with Drs. Ozols, Young and Whang-Peng).

The DNA content light scatter properties of cells from patients with ovarian cancer are being determined and compared with clinical results, cytogenetic analysis and re-analysis of specimens in vitro.

3.1806 Use of FCM as a signature for cell lines (Drs. Bunn, Carney, Gazdar and Minna).

With the rapid expansion of the number of cell lines maintained in the lab, and the literature reports of frequent cross contamination of cell lines, it is important to have a rapid method for identification of cell lines. We have shown that DNA content analysis is a useful technique for this purpose.

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, Non-Hodgkin's lymphomas (e.g., Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, and Ewing's sarcoma are studied. In leukemia, we have devised therapeutic regimens wich may allow maximum tumor cell kill while minimizing sequellae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high dose-therapy and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, autologous bone marrow rescue, total parenteral nutrition) in permitting such therapy. Phase I-II trials are also conducted in refractory tumors; agents include ICRF-187, poly I:C/poly-l-lysine, iphosphamide, dihydroxyanthracenedione, and 2'-deoxycoformycin. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function. The psychosocial concomitants of cancer are explored.

Objectives:

1. To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill, while leaving intact effective elements of the immune response and minimizing therapeutic sequellae -- especially in the CNS.

2. To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, and Ewing's sarcoma.

3. To study the possible role of high-dose ("ablative") chemotherapy in advanced solid tumors, and the role of supportive care (platelet and white cell transfusions, laminar-flow protection, bone marrow rescue, hyperalimentation) in permitting such ablative therapy.

4. To study the biology of selected tumors, including kinetics, immunology, virology, prognostic markers, karyotypes, biochemistry, and drug sensitivity.

5. To assess the pharmacology of selected new agents, e.g., high-dose methotrexate, dihydroxyanthracenedione, ICRF-187, poly I:C/poly-l-lysine, 2'-deoxycoformycin, and Iphosphamide in humans.

6. To study the short- and long-term effects of malignant disease and cancer treatment on growth, development, and organ function.

7. To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Methods and Major Findings:

A. Therapeutic Regimens for Acute Lymphocytic Leukemia (ALL)

1. Protocol 72-1 - was instituted as a consequence of Mathe's report in 1969 suggesting a beneficial effect of immunotherapy in childhood All. The 72-1 study was designed to compare the effectiveness of chemotherapy plus immunotherapy versus chemotherapy alone as maintenance treatment for newly diagnosed patients with this disease. The study was designed to expose patients to immunotherapy early, following remission induction, rather than after long periods of chemotherapy. Remission induction therapy consisted of repeated five day courses of POMP (prednisone, vincristine, methotrexate, and 6-mercaptopurine). All patients were then treated with systemic consolidation chemotherapy and central nervous system prophylaxis. The latter consisted of 2400 rads to the whole brain and intrathecal medication given during the period of cranial radiation and monthly thereafter for 30 months. Following completion of consolidation chemotherapy and cranial irradiation, patients were randomly assigned to a 34-month course of maintenance treatment. Patients received either chemotherapy alone, or chemotherapy plus immunotherapy with non-irradiated, live allogeneic leukemia cells and BCG. A total of 70 patients were entered on this

study which was closed to entry in 1975. Sixty-three patients (90%) achieved complete remission. There has been no significant difference in the relapse rate or remission duration between those patients who received chemotherapy alone and those who received chemotherapy plus immunotherapy. At the present time 27 patients (43%) are in their initial complete remission, having completed therapy and off all treatment. Five patients have been off therapy for three years, seven for four years, four for five years, and eleven for six years. Six of 33 patients (18%) have relapsed after completing therapy. In terms of prognostic factors, it is notable that 55% of the patients who entered this protocol had an initial white blood count >25,000 per mm^3 and/or were less than two or greater than ten years of age. Eighty percent of the relapses have occurred in patients with poor initial prognostic signs. In contrast, 80% of those patients currently in unmaintained remission were in a good prognostic category initially.

2. Evaluation of the Long-Term Effects of CNS Prophylaxis - The institution of central nervous system prophylaxis (cranial radiation and intrathecal methotrexate) has radically altered the treatment of ALL. Not only has it reduced the incidence of meningeal leukemia from 50-70% to 5-10%, but it has also been responsible for a marked improvement in overall remission duration and increased long-term survival. Nevertheless, attention has been directed to the possible adverse effects of CNS preventive therapy. Several POB studies have highlighted the potential dangers of the cranial radiation plus intrathecal methotrexate utilized in the current standard approach to CNS prophylaxis.

   a. Abnormal Computed Tomography Brain Scans in Children with ALL Following CNS Prophylaxis - A serious form of delayed neurotoxicity which has been observed in ALL patients treated with cranial radiation and either intrathecal or systemic methotrexate is necrotizing leukoencephalopathy. We have studied 32 asymptomatic patients with ALL who had received (on the 72-1 protocol) prophylactic cranial radiation (2400 rads), and (by randomization) either intrathecal methotrexate or cytosine arabinoside. These patients were evaluated by computed tomography 19 to 67 months after the initiation of prophylaxis. Seventeen of 32 (53%) had one or more abnormal findings. Although minor neurologic abnormalities were detected in some patients in the study group (see below), they did not correlate with the CT scan abnormalities. The unexpectedly high prevalence of such abnormalities contrasted with the essentially normal tomographic findings in a control group of patients with acute lymphocytic leukemia who received no CNS prophylaxis. The results of this study heightened concern over the potential adverse effects of CNS preventive therapy and suggested that alternative approaches to such prophylaxis should be sought.

   b. Neurological and Psychological Evaluation of ALL Patients Who Have Received CNS Prophylaxis - We have performed a comprehensive neurological evaluation of the 32 apparently asymptomatic patients discussed above. Abnormalities of both the peripheral and central nervous system were detected. However, these findings did not

correlate with the presence of CT abnormalities, nor with the particular type of intrathecal chemotherapy. Comprehensive psychological testing has been performed in those patients on the 72-1 protocol who remain in complete remission. All subjects were administered the Wechsler Intelligence Test and the Bender-Gestalt Test of perceptual motor function. These intelligence tests were administered to the CNS prophylaxis patients and to 3 control groups. The first control group included 34 siblings of the above ALL patients, with a mean age similar to that of the patients. This group presumably was also similar to the patients in genetic and environmental background. A second control group consisted of 13 survivng ALL patients who had never received any CNS therapy. (Siblings of these patients were also tested.) A third control group consisted of 22 cystic fibrosis patients (and their siblings), chosen because they represent a group of frequently hospitalized children. The healthy sibling controls attained significantly higher IQ scores than did the patients who had received CNS prophylaxis. In contrast, the ALL patients who did not receive CNS treatment, and the cystic fibrosis patients (who also did not receive any CNS treatment) obtained IQ scores that did not statistically differ from the IQ's of their respective sibling groups. Analysis of the results also revealed that younger patients had significantly greater decrements in Full Scale and Verbal Scale IQ scores than older patients. The results of this study indicate that CNS preventive therapy with cranial radiation and intrathecal chemotherapy can significantly impair both intellectual and perceptual motor functions. The findings of this study again suggest that it is appropriate to search for alternative methods of CNS preventive therapy.

c. Neuro-Endocrine Function in ALL Patients Following CNS Prophylaxis - In an attempt to identify possible adverse effects of CNS prophylaxis upon the hypothalamic-pituitary axis, we examined the hypothalamic-pituitary function of 23 ALL patients. Eighteen patients (Group A) had received cranial radiation (2400 rads) and multiple doses of either IT methotrexate or cytosine arabinoside as prophylaxis. The remaining 5 patients (Group B) received no cranial radiation. Nine patients in Group A (50%) had abnormally low growth hormone responses (<7.0 ng/ml). Seven of these 9 patients also demonstrated ventricular dilatation on their CT scans. In contrast, only 1 of the 9 patients in Group A with normal growth hormone responses demonstrated this abnormal CT scan finding. Thus, there was a significant correlation (P=.015) between ventricular dilatation on CT scan and abnormally low growth hormone responses. Since ventricular dilatation is believed to result predominantly from cranial radiation, this correlation is consistent with the hypothesis that abnormal GH responses may be due to an adverse effect of radiotherapy upon the hypothalamic-pituitary axis, although a possible contributory role for either systemic or intrathecal chemotherapy cannot be ruled out. None of the patients in Group B had abnormal CT scans or low growth hormone responses. Since the real test of GH secretion is linear growth,

the clinical implications of the abnormal GH responses are unclear. Examination of the linear growth curves in both patient groups did not reveal a correlation between impaired GH responses and short stature. Although eight of the eighteen (44%) evaluable patients (<12 years old at diagnosis) are in the 10th percentile or less, only four of these eight patients had peak GH responses <7.0 ng/ml, and only one patient's growth curve exhibited a persistent "fall-off" pattern indicative of inhibited growth. However, it is of concern that none of these eight patients has demonstrated "catch-up" growth following the termination of their treatment. It is notable that study of a group of Ewing's sarcoma patients who had received less intensive CNS prophylaxis (2000R and only a single dose of IT methotrexate) revealed neither CT scan nor neuroendocrine abnormalities. These data suggest that presence of CT scan and neuro-endocrine abnormalities is related to the intensity of the CNS prophylactic regimen.

3. Protocol 75-1 - Retrospective analysis of protocol 72-1 had demonstrated that patients could be divided into good and poor prognostic groups on the basis of certain "front end" factors. These include age at diagnosis, initial white blood cell count, presence of meningeal leukemia (prior to the institution of CNS preventive therapy) and failure to achieve remission with four induction courses of POMP therapy. In the 75-1 study, patients were segregated on the basis of these previously identified prognostic factors. Patients in the good prognosis group were treated conservatively with moderate dose chemotherapy on a regimen similar to that utilized in the 72-1 protocol. Patients in the poor prognostic group were treated with a more intensive chemotherapeutic regimen which utilized an early intensification course of L-asparaginase and cyclophosphamide and a late intensification course (approximately 6 months after induction of remission) with cytosine arabinoside and daunomycin. Twenty of the 21 patients achieved complete remission. However, 10 of the 16 patients of the poor prognosis group have relapsed in contrast to all 5 patients in the good prognosis group who remain in complete remission. Because the projected remission duration and survival data in the poor prognosis group patients was no different from that observed in our earlier 72-1 poor prognosis patients, the study was terminated at an early stage.

4. Treatment of Newly Diagnosed Acute Lymphoblastic Leukemia - Current Protocol (POB 77-02) - The current ALL treatment protocol addresses the two major therapeutic problems which emerged from previous NCI studies: 1) The need for improved treatment for patients possessing certain poor prognostic factors, and 2) improvement in the method of delivering CNS preventive therapy such that effective treatment can be administered with less morbidity. The main objective of this protocol is to investigate the efficacy of high-dose, protracted intravenous methotrexate infusions as central nervous system preventive therapy. Patients are randomized to receive CNS prophylactic therapy either with cranial radiation (2400 rads) plus intrathecal methotrexate, or with high-dose 24 hour, systemic methotrexate infusions. As noted previously, a major stimulus behind the development of this protocol

was our increasing awareness of significant toxic sequellae associated with the prophylactic cranial irradiation. The hypothesis to be tested in the present protocol is that CNS preventive therapy using high-dose protracted systemic methotrexate infusions alone is equally effective and less toxic than the current standard form of CNS prophylaxis (cranial irradiation and intrathecal methotrexate). An additional aim of this study is to assess the utility of an intensified systemic maintenance schedule which alternates standard maintenance treatment with periodic "induction-type" chemotherapy regimens. The rationale for using the systemic methotrexate approach rather than local therapy to prophylax the CNS is based on several considerations. First, there are ample data which demonstrate that intravenously administered methotrexate can cross into the CSF and achieve therapeutic CSF levels. We have successfully used the proposed intravenous regimen to induce complete remission in overt meningeal leukemia. Second, it is believed that the systemic route is more "physiologic," providing entrance of drug at all levels of the cerebrospinal axis and insuring better drug distribution throughout the CNS including the meninges, the site of origin of leukemic spread to the CNS. Third, moderate dose intravenous methotrexate without cranial irradiation has recently been shown in a randomized study to decrease the incidence of CNS leukemia. Intravenous methotrexate also has been used effectively in the treatment of CNS non-Hodgkin's lymphoma. Fourth, intravenous methotrexate has certain technical advantages over intrathecal administration, and obviates the necessity for frequent lumbar punctures. In addition, considerable clinical experience with prolonged intravenous methotrexate infusion and the accompanying citrovorum rescue has shown it to have acceptable systemic toxicity. Pharmacologically, this 24-hour high-dose methotrexate regimen provides more prolonged therapeutic CSF antifolate concentrations than does a standard dose of intrathecal methotrexate both in ventricular and lumbar cerebrospinal fluid. It is hoped that through the use of this methotrexate infusion technique central nervous system preventive therapy can be given which is as effective as, but less toxic than, the combination of cranial irradiation and intrathecal methotrexate. The 77-02 protocol also attempts to provide patients with optimal exposure to those chemotherapeutic agents considered most active against ALL by employing an escalated, intensified maintenance schedule which alternates standard maintenance treatment with frequent, periodic, "induction-type" chemotherapy utilizing varying reinduction regimens. It is hoped that the application of this intensified maintenance schedule will improve the prognosis for "high-risk" ALL patients. Experience to date has shown this intensified approach can be accomplished without a significant increase in drug related morbidity. To date, 76 patients have been randomized on this study. Seventy-five have achieved complete remission. Twenty-four of these individuals were randomized to receive cranial irradiation plus intrathecal methotrexate; 52 have been treated with the high dose, protracted intravenous methotrexate infusion. (The randomization is weighted on 2 to 1 basis.) There have been 5 CNS relapses (2 and 3 in each treatment group, respectively) and 6 bone marrow relapses (5 in the cranial radiation + intrathecal methotrexate arm). Although it is too early to draw statistically significant conclusions regarding the

utility of the methotrexate infusion arm, the data thus far appear very encouraging. Freedom from systemic relapse in the infusion arm is an unanticipated dividend, as is the overall survival improvement amongst poor prognosis patients. It is notable that toxicity associated with the intensive maintenance schedule has been relatively minimal. In mid 1979, two other institutions (The Children's Orthopedic Hospital, Seattle, and the Children's National Hospital Medical Center, Washington, D.C.) joined this protocol as collaborators and in 1980 an additional two institutions (Columbus Children's Hospital, Columbus, Ohio, and the Children's Hospital Medical Center of Northern California, Oakland, CA) joined our study. This collaboration should provide sufficient patient accrual to complete this study within 2 years.

5. Treatment of Acute Lymphoblastic Leukemia in Relapse - Although considerable success has been achieved in the treatment of newly diagnosed, previously untreated patients with ALL, the general experience in the treatment of patients who relapse while undergoing chemotherapy has been far less positive. Our recent experience with an intensive four drug reinduction regimen has, however, been encouraging. Between 1971 and 1976, 125 hematologic relapses of ALL were treated with a 4 drug regimen consisting of L-asparaginase, vincristine, daunomycin and prednisone (Asp-VDP). These relapses occurred in 56 patients whose multiple consecutive relapses were treated with the Asparaginase-VDP protocol. Of the 125 courses evaluated, 114 (91%) resulted in complete remission. Ninety-one percent (51/56) of patients who received their first reinduction course of this regimen attained complete remission. Eighty-nine percent (31/35) of patients also achieved complete remission on their second exposure to this regimen. Although the numbers are small, all of the patients who received 4 or more courses of this regimen for treatment of 4 or more relapses achieved complete remission. Surprisingly, no difference in response rates was noted in patients who received this reinduction regimen as treatment for multiple subsequent relapses, i.e., reinduction efficacy did not decline following repetitive exposures to this regimen. The current relapse protocol (POB 77-09) builds upon our experience with the four drug Asparaginase-VDP regimen discussed above. While remission reinduction rates are excellent, it is evident that more effective maintenance therapy is needed. Protocol 77-09 compares an aggressive maintenance treatment regimen to conventional maintenance therapy. Following induction therapy with the Asparaginase-VDP regimen, all patients receive intensification with cyclophosphamide as well as CNS reinforcement consisting of six weekly courses of intrathecal methotrexate which begin shortly after marrow relapse. Patients are then randomized to receive one of two types of maintenance therapy, an aggressive schedule which involves periodic "reinduction type" treatment alternating with conventional cycles of 6-mercaptopurine and methotrexate, or the conventional cycles alone. This protocol is employed at the time of first and second relapse. Individuals who relapse on one maintenance arm are reinduced as above, and crossed over to the other maintenance schedule. Thus far, 29 patients have been enrolled on the 77-09 protocol. Ninety percent of the 29 patients achieved complete remission. Among these patients there have been 19 relapses. The median

duration of remission is 345+ days. The limited accrual to date does not permit us to draw conclusions regarding efficacy of the different maintenance treatments.

B. Therapeutic Regimens for Acute Nonlymphocytic Leukemia (ANLL): Prototol 78-07 (BCRC)

The present treatment protocol (78-07) is designed to test three forms of maintenance therapy for this disease. Patients who achieve complete remission are randomized to receive either chemotherapy alone, chemotherapy plus splenectomy, or chemotherapy plus immunotherapy. All patients receive identical induction chemotherapy consisting of the combination of cytosine arabinoside (100 mg/M^2/day x 7 iv) plus daunomycin (45 mg/M^2/day x 3 iv). Patients who achieve remission on this induction regimen are then randomized to one of the three maintenance treatment arms. One-third of the patients receive intensive maintenance therapy every three months with cytosine arabinoside (100 mg/M^2 iv q 12 h) plus 6-thioguanine (100 mg/M^2 po q 12 h) given to marrow hypoplasia. A second group of patients receives maintenance chemotherapy (as above) plus immunotherapy with neuraminadase-treated allogeneic blast cells. A third group of patients receives chemotherapy plus splenectomy. This is a BCRC protocol which we have joined. It is too early to make a definitive assessment of the value of the various maintenance treatment arms, but thus far, there are no significant differences between them.

C. Studies on the Treatment of Overt CNS Leukemia: Treatment with Systemic (IV) Methotrexate Infusions

Our recent studies in patients and in a sub-human primate model have revealed that therapeutic concentrations of MTX can be achieved in cerebrospinal fluid following intravenous infusion. We have evaluated the utility of 24 hour continuous systemic MTX infusions. A relatively constant plasma drug level is maintained for 24 hours; 12 hours later (36 hours after the start of the infusion) citrovorum factor rescue is initiated. The infusion dose over the first hour (the priming dose) is given at a dose of approximately 1.5×10^{-7} times the desired plasma MTX concentration. This is followed immediately by a 23 hour maintenance infusion at an hourly rate of one-fifth the primary dose. Our studies have demonstrated that this technique is feasible and that IV doses which maintain a CSF MTX level of 1×10^{-5}M can be given without apparent systemic or neurological toxicity. We have also determined that the ventricular and lumbar CSF: plasma MTX ratios are similar, suggesting that systemic MTX administration has the advantage of providing consistent drug concentrations throughout the CSF space. This contrasts with intralumbar administration, which does not provide optimal drug penetration into the ventricular CSF. We have now treated 8 patients with meningeal leukemia and have observed complete responses in six. This approach will be utilized in our next major meningeal leukemia treatment protocol.

D. Studies on the Treatment of Metastatic Neuroblastoma

We have developed a collaboration (under an international agreement) with the Polish National Institute of Mother and Child to assess Phase II agents in patients with refractory metastatic neuroblastoma. The Warsaw institution is relatively unique in that it treats approximately 45 newly diagnosed cases of this rare tumor per year. Our initial treatment protocol, begun late in 1978, is designed to test the efficacy of streptozoticin in this disease. Patients selected for study are those who have been demonstrated refractory to established chemotherapeutic agents. Our interest in streptozoticin is based on the known efficacy of this agent against other neural crest tumors. As yet, there have been no therapeutic responses of significance. Nevertheless, this agreement offers a unique resource for the evaluation of Phase II agents in neuroblastoma.

E. Treatment of Metastatic Osteogenic Sarcoma: Protocol 77-03

This study employs total surgical debulking of pulmonary metastatic disease (Phase I) followed by intensive sequential treatment with various agents known to be active in osteogenic sarcoma (Phase II). Following resection, patients are given 1 week of high-dose methotrexate followed by exposure to 3 alkylating agents (L-Pam, DTIC, and cyclophosphamide). During the severe bone marrow hypoplasia which follows such treatment, patients are offered laminar-air-flow isolation, platelet and granulocyte transfusions, and autologous bone marrow rescue where appropriate. Following recovery of peripheral counts, patients are then exposed to 1 week of treatment with adriamycin and cisplatinum. Eighteen months of maintenance with high-dose methotrexate alternating with adriamycin then follow. Patients who cannot be totally debulked at the initiation of this study are treated with high-dose methotrexate (pharmacologic Phase I) in the hope that their lesions will be altered in such a way as to then permit total surgical resection, followed by the intensive sequential chemotherapy described above. Of the 30 patients entered on Phase II (intensive chemotherapy in the absence of macroscopic tumor), 25 are evaluable, of whom 10 (40%) are free of disease at a median of 36 months (18-52 months) after debulking. Since an earlier study by Martini et al. suggested some benefit from metastatectomy alone in osteosarcoma, it is not possible to say that our current result represents an improvement over previous experience, since we may simply be seeing the effect of aggressive pulmonary resection per se. On the other hand, the few patients with a past history of multiple episodes of pulmonary metastases, each separated by no more than several months, who now remain continuously disease-free for two or more years following entry onto this protocol comprise an encouraging anecdote, and it is tempting to believe that intensive chemotherapy delivered after extensive debulking of metastases may be an effective approach to the treatment of metastatic osteogenic sarcoma.

F. Study and Treatment of Rhabdomyosarcoma

1. Protocol 76-4 (primary treatment) - Previous studies have shown that combination chemotherapy has significantly improved the survival of children with localized rhabdomyosarcoma. In contrast to the situation

with localized tumors, combination chemotherapy has failed to improve the survival of patients with unresectable or metastatic rhabdomyosarcoma. For such patients, this protocol addresses the utility of intensive (escalating) combination chemotherapy (in conjunction with hematologic and antimicrobial support) versus conventional dose combination chemotherapy. To date, 28 patients have been entered on this study. The median age is 17 yrs (range 1-32 yrs). The majority of patients (95%) are Stage II-III. Alveolar histology predominates (16/28) and the primary sites include head and neck (10), genitourinary (6), and extremity (9). All Stage I patients attained a CR and remain in remission for 24+ months. Eight of the 13 Stage II patients have completed induction therapy and all attained a CR. However, 6/8 have relapsed (median duration 9 mos, range 1-13 mos). Of the 12 Stage III patients, 10 have completed induction therapy. Six of these patients were randomized to the standard dosage chemotherapy schedule and five attained CR. However, four patients subsequently relapsed and three died. Six patients were randomized to the intensive drug schedule. One patient died during treatment from radiation-induced pneumonia. Of the five remaining evaluable patients, four attained CR and one patient failed to respond to therapy. Only one of the five responders is disease-free. The overall failure rate for patients in this study is 16/26 (62%), reflecting the poor prognostic features (age, site, histology) of this patient group. Of greater interest is the fact that of the 15 relapses to date, 12 have been local (at least initially). This result suggests a possible role for either more extensive surgery or for the use of intraoperative irradiation and/or radiation sensitizers (including the potential application of total body irradiation in patients with metastatic disease).

2. Protocol 75-5 (relapse treatment)

We have evaluated an intensive chemotherapy regimen in relapsed patients: DTIC 250 mg/m^2 IV, days 1-5, adriamycin 35 mg/m^2 IV, days 1 and 2, and cyclophosphamide (CY) 45 mg/kg IV. We attempted to escalate the CY over four consecutive cycles at 4-6 wk intervals from 1 day to 4 days of therapy. In some patients, surgery and/or radiation were also utilized. Ten patients were studied [2 male, 8 female; median age 15 yrs (range 2-28)]. All patients had failed VAC (9 while still receiving therapy); 6 had also received adriamycin. Six patients had relapsed with generalized involvement, 4 with local recurrence. Histologic subtypes included embryonal (3), alveolar (6), and undifferentiated (1). Eight patients were escalated to a total of 3 days of CY (135 mg/kg) and 2 patients to 4 days (180 mg/kg). Six of 10 patients responded (4 CR, 2 PR), 2 patients stabilized, and 2 had progressive disease despite escalation. Of the responses, 3 followed the first cycle and 3 followed CY escalation. The two stabilizations also followed CY escalation. However, the median duration of response was only 2 mos (range 1-13). There were two treatment-related deaths. All patients developed profound granulocytopenia (PMNs<500/mm^3, median 15 days) with fever or infection and thrombocytopenia. While the duration of response does not suggest that this intensive therapy was useful for patients who had failed prior therapy, the response rate in

these refractory patients implies that dose escalation may be beneficial in the initial treatment of high risk patients.

G. Non-Hodgkin's Lymphoma

Three clinical protocols are presently open for patient entry. These are the primary protocol (77-04) for untreated patients with undifferentiated lymphomas (including Burkitt's lymphoma) and lymphoblastic lymphomas; an intensive protocol utilizing high dose cyclophosphamide, ara-C and (where cryopreserved autologous bone marrow is available for re-infusion) total body irradiation; and a protocol examining the activity of Ifosphamide as a single agent after failure of the intensive protocol. Sixty patients have been admitted to the primary protocol (77-04) and an analysis of the first 46 patients has recently been completed. The protocol employs the CHOP regimen followed in 10 days (without delay for neutropenia) by a high-dose, protracted methotrexate infusion. Excellent results have been obtained in lymphoblastic lymphoma and patients with completely resected undifferentiated lymphomas. Although a markedly different relapse pattern compared to previous protocols has been observed, which suggests more effective control of systemic tumor, patients with widely disseminated disease still have a poor prognosis (30-40% predicted disease-free survival at 3 years) and a future protocol will be directed at these patients. Overall survival in the protocol is currently about 70%.

Very poor results have been obtained with salvage protocols, although Ifosphamide is active even after failure of high dose cyclophosphamide. This drug will probably be incorporated into combination regimens for poor risk patients at presentation and for patients who relapse.

H. Ewing's Sarcoma

As a background to our current trials, we have completed an analysis of historical data at the NCI. A total of 117 patients with histologically documented Ewing's Sarcoma were treated at the NCI from 1964 until 1976; these patients received radiation to the primary lesion and adjuvant chemotherapy consisting of a series of progressively more intensive regimens (S1 - S4). S1 consisted of cyclophosphamide given either as a single dose or in a series of short courses. S2 combined cyclophosphamide and vincristine. S3 combined cyclophosphamide, vincristine, and actinomycin D and also employed 2000 rads of whole brain irradiation and a single dose of intrathecal methotrexate. S4 was similar to S3 with the exception that adriamycin was substituted for actinomycin D; patients were also treated with central nervous system prophylaxis. The overall analysis of results from protocols S1 through S4 indicated that the treatment groups were relatively unbalanced with respect to prognostic factors at presentation, in particular, site of primary disease and serum lactate dehydrogenase levels at presentation. Elevated lactate dehydrogenase levels and centrally located primary sites are associated with a poorer prognosis. These imbalances prevented any firm conclusions being drawn from the previous studies as to the overall efficacy of later (more intensive) treatment protocols when compared with earlier protocols. The results of the analysis also indicated that patients who presented with metastatic

disease had a poor therapeutic outcome irrespective of the treatment regimen employed, with median survival on the order of 22 months and maximum disease-free survival of 70 weeks (regimen S4).

With this background in mind, protocols were initiated in 1976 for patients presenting with metastatic and pelvic disease, and patients presenting with disease of the extremities without metastases. Patients presenting with pelvic or metastatic disease were treated with combination chemotherapy plus radiation to the primary site (and all metastatic sites) followed by autologous marrow storage, low-dose total body irradiation and intensive high-dose chemotherapy, and subsequent reinfusion of the harvested marrow. Patients presenting with lesions of the extremities were treated with combination chemotherapy, radiation to the primary site, prophylactic pulmonary irradiation, and maintenance chemotherapy. Patients who relapsed on prior protocols or on the present protocol were treated where possible with a regimen that was similar to that now used for patients with pelvic or metastatic disease. Patients who could not receive such treatment were placed on Phase I or II trials. Results of these protocols to the present time suggest that for patients presenting with disease of the extremities, or non-metastatic pelvic disease, these regimens yield results that are the equivalent of the best previous results obtained, but are not significantly better. However, results for patients presenting with metastatic disease are significantly improved compared with previous regimens. These results also suggest that total body irradiation can be combined with systemic chemotherapy (with appropriate support measures) and are well tolerated. There has to date been no mortality associated with these regimens. Since the results indicate that therapy is relatively well tolerated, a next step would be to increase the dose of total body irradiation (which is presently below a cytotoxic level) to a cytotoxic dose (using fractionation), and to see whether this will improve results for these patients. In vitro studies being carried on presently in the Radiation Branch will define the fractionation to be used. The total dose will be derived from previous experience at other centers with bone marrow transplant regimens. We note, as an aside, that the number of patients presenting with primary lesions of the rib (2 among the previous 117 patients) is 10 on the present protocols. Among these 10 patients, all of whom receive combined radiation and surgical therapy to the primary lesion (3000 rads of radiation followed by surgical resection, followed by 3000 rads of radiation), followed by the intensive regimen with autologous marrow harvesting and reconstitution and total body irradiation, there have to date been no relapses.

This compares strikingly with previous results for central axis patients wherein approximately 80% of patients would be expected to relapse. Some thought is therefore being given to more aggressive combined modality approaches to the primary lesion in cases where that is possible. With respect to the favorable prognostic cases, such as tumors of the distal extremities, we hope to acquire enough patients to perform a randomized study through collaborative arrangements with other centers treating Ewing's Sarcoma. One possible protocol would randomize patients between the best available current regimen and a regimen similar to the previous intensive

regimen used at NCI which appears to be well tolerated and yielded improved therapeutic results in the extremely high-risk group of patients.

I. Miscellaneous Phase I and II Studies:

1. Dihydroxyanthracenedione - Trials in pediatric patients are presently being completed. The maximum tolerated dose in patients with solid tumors appears to be 18 mg/m^2 given on a once every 4 weeks schedule. This compares with a maximally tolerated dose of 12 mg/m^2 observed in adults. The maximally tolerated dose in patients with acute leukemia appears to be 26 mg/m^2. The dose-limiting toxicity in patients with solid tumors is myelosuppression. The dose-limiting toxicity in patients with leukemia appears to be nausea and vomiting.

2. ICRF 187 - Pediatric trials with ICRF 187 appear to be reaching a maximally tolerated dose of 3000 mg/m^2 given daily x 3 and repeated every 28 days. This compares with a maximally tolerated dose in adult patients of 1250 mg/m^2 on the same schedule. The dose-limiting toxicity in both cases appears to be myelosuppression. The implication of these studies is that pediatric patients tolerate higher doses of these agents than do adults, and that new anticancer drugs require independent evaluation in children.

3. Pediatric vs. Adult Phase I Trials - As a result of these studies, we have systematically reviewed anticancer drugs which have had comparable trials in both adult and pediatric patients. To date there appear to be approximately 20 such compounds. The minimal ratio between the observed maximum tolerated dose in pediatric patients and that observed in adult patients is 0.83. The maximal ratio is approximately 2.3 for ICRF 187. For 16 of the 20 compounds, the pediatric maximally tolerated dose is higher than that observed in adults. If one examines the dose-limiting toxicity in pediatric and adult patients, for the vast majority of agents (17 of 20) dose-limiting toxicity is myelosuppression. The spectrum of toxicity for the various agents in adult and pediatric patients is similar but not identical, there being some differences in many cases. If one now looks for an explanation of why pediatric patients appear to tolerate more drug than do adult patients (for agents which have such a wide variety of mechanisms of action), one possible explanation arises from the fact that the dose-limiting toxicity for the majority of these agents is myelosuppression. It is possible that the number of bone marrow stem cells per unit surface area is larger in pediatric patients than it is in adult patients. It is also possible that the metabolism of these drugs is faster in pediatric patients per unit surface area than it is in adult patients. If one re-examines the data of Pinkel (Cancer Research, 1958) in which he studied the relative doses of methotrexate in younger versus older patients, he could account for the majority of the difference by changing from mg/kg to mg/m^2; however, there was some difference which still remained, the difference favoring pediatric patients. This could also account for part of the observed difference in our current trials. In any event, since the difference in maximally tolerated dose varies so widely, it would appear that the dose required to achieve an

equivalent biological effect in children may be quite different from that required in adults, again justifying independent trials in children.

4. Relapse ALL: Phase I Study of 2'-Deoxycoformycin - As part of an evaluation of promising new agents in relapsed patients with ALL, we recently conducted a Phase I study of 2'-deoxycoformycin in a cooperative effort with two other institutions (St. Jude Children's Research Hospital and the Sidney Farber Cancer Research Institute). This agent (2'-dCF) is a known inhibitor of the enzyme adenosine deaminase (ADA). ADA is an enzyme of known importance in lymphocyte metabolism. It is present in highest amounts in lymphoid tissues and absence of the enzyme, as occurs with genetic absence of ADA, results in defective lymphocyte maturation and function (i.e., severe combined immunodeficiency disease). Our interest in 2'-dCF as an anti-leukemia agent was stimulated by our observation that the leukemic blast cells of ALL patients with "T Cell disease" have considerably higher ADA levels than "Non-T, Non-B" lymphoblasts, prompting speculation that, should it be effective against leukemia, 2'-dCF may have "specificity" for poor prognosis patients with T lymphoblasts. Thus far, 26 patients with refractory ALL have been treated with 2'-dCF. Ten of these patients had T-cell disease, the remainder had non-T, non-B cell leukemias. The dose initially utilized for the study was 0.25 mg/kg iv for 3 days. It was subsequently increased to 0.5 mg/kg and then to 1 mg/kg. Mild toxicities noted with the drug include nausea, vomiting, diarrhea and conjunctivitis. Dose-limiting toxicity includes effects on the central nervous system (confusion, seizures, encephalopathy), the kidney (acute renal failure), and the liver. The 0.5 mg/kg dose has been associated with only mild toxicity and has been demonstrated to inhibit the adenosine deaminase of lymphoblasts. Of the 26 patients treated to date, 2 have achieved complete remission and partial responses were noted in 4 individuals. This study confirms the activity of 2'-deoxycoformycin in acute lymphoblastic leukemia; a second Phase I study is underway in which dCF is combined with adenosine arabinoside (ara-A). Since dCF will prevent the deamination of ara-A, the known anti-leukemic effect of the latter drug should be potentiated.

5. Phase II Trials of Polyriboinosinic-Polyribocytidylic Acid Stabilized with Poly-l-lysine, in Carboxymethylcellulose [Poly(ICLC)], a Highly Effective Interferon Inducer - Poly(ICLC) resists hydrolysis by primate serum (unlike the parent compound), induces high levels of serum interferon, and is effective in acute viral infections of subhuman primates. In a Phase I clinical trial, Poly(ICLC) induced significant serum interferon levels in 76% of trials, and the correlation between dose and peak interferon titer was linear. The maximum tolerated dose for all patients at a given drug dose was 12 mg/sq m; at this dose, the mean peak interferon titer was 1940 reference units/ml. We have undertaken several Phase II trials of poly(ICLC) at doses up to the MTD of 12 mg/sq M. In 26 NCI patients with AML, Burkitt's lymphoma, osteosarcoma, or Hodgkin's disease, there have been no significant responses. Among 18 patients with refractory ALL studied by the CCSG, there has been one initial response, but the patient died before the response

could be fully evaluated. Five patients have been studied at the University of Arizona with multiple myeloma; all 5 have experienced a decrease in their plasma or urine M-component. Finally, 2 children at Johns Hopkins with juvenile laryngeal papillomatosis have required less frequent surgery while receiving poly(ICLC). These results suggest that certain tumors may show a biological effect of interferon inducers, as they appear to do with interferon per se; the ultimate clinical significance of this effect remains unproven. Moreover, our observation that poly(ICLC), at a tolerable dose, is the first consistent inducer of high serum interferon levels in humans, suggests that it should be studied in certain human viral infections.

J. Parenteral Hyperalimentation as an Adjunct to Intensive Treatment

Adjuvant parenteral nutrition (TPN) has been proposed as having a major role in the multimodal therapy of cancer. To assess its benefit, we have evaluated in a prospective randomized study the use of TPN in a group of young patients receiving aggressive chemotherapy for metastatic or high-risk sarcomas. Fourteen patients were randomized to receive TPN and 18 to receive conventional nutritional (CN) support. During the study period (from first dose of chemotherapy to recovery from myelosuppression), the TPN patients received between 750 and 1950 calories/m^2/day (median 1650) and between 5.3 and 12.4 gm N/m^2/day (median 8.9), while the CN patients received between 380 and 880 calories/m^2/day (median 685) and between 0.0 and 3.7 gm/N/m^2/day (median 0.5). The mean daily nitrogen balance during the study period for the TPN group (-3.0 to 1.3 gm/N/m^2/day, median -0.7) was significantly higher (p=0.005) than that of the CN group (-6.2 to -0.7 gm/N/m^2/day, median -2.6). A least-squares linear regression analysis suggests that a calorie intake of 1875 calories/m^2/day and a nitrogen intake of 10.1 gm N/m^2/day are required in this stressed patient group to achieve nitrogen balance. Serum protein levels (albumin, total protein, and transferrin) did not differ between the two treatment groups. The proportion of patients responding to therapy and the long-term survival were similar between the treatment groups.

K. Psychosocial Concomitants of Cancer

We have previously reported on the families of patients who have life-threatening illness and on physicians who care for such patients. In particular, we explored the factors in the doctor-patient relation which promote anxiety in the doctor to the end that he is less effective than he might be. During the past year, our annual seminar for Clinical Associates on the Doctor-Patient Relation has continued with Dr. Harold Greenberg as the seminar leader. The seminar continues to be highly successful and has proven to be a model for many other institutions throughout the country, including cancer centers and general hospitals. We have also continued a collaborative research effort with the Laboratory of Developmental Psychology, NIMH (M. Yarrow, Chief). The objective of this research is to analyze developmental stages in young patients who have chronic and/or life-threatening disease, to learn the factors which promote normal or abnormal development, and the manipulations which may be of value in dealing with developmental abnormalities. We are continuing studies on the nature of

communication between staff, family and patient; the effects of isolation within the laminar-flow room as a function of the age of the patient; and the effects of mentation in patients who have received prophylactic irradiation of the central nervous system (as noted elsewhere, we have detected a significant drop in I.Q. among patients so treated).

Significance to Biomedical Research and the Program of the Institute:

Significant progress has been made in delineating the cell of origin in acute leukemia and in identifying factors which may be useful in predicting response to therapy. By further defining the acute leukemias vis-a-vis these prognostic factors, we hope to refine therapy to the end that remission rate and duration will further improve. In this Branch 43% of patients with ALL entered in our most recently completed study are free of disease after having been off all treatment for 4 - 7 years. We have learned that the acute leukemia cell has extraordinary antigenic and biochemical complexity; the effects of both specific and nonspecific immunotherapy in this disease have been discouraging, but our understanding of immunological and biochemical mechanisms involved in leukemia has increased. In this regard, we have identified a new agent (2'-deoxycoformycin, an inhibitor of lymphocyte adenosine deaminase) which may be effective in relapsed acute lymphocytic leukemia.

The central nervous system is a major site for relapse in acute leukemia. Our studies on route and scheduling of CNS chemotherapy promise an effective and relatively nontoxic approach to this important problem. It is further evident that the most important contribution that can now be made to the treatment of "good risk" patients with acute lymphocytic leukemia is to provide effective CNS prophylaxis without the significant toxicity we have detected in association with cranial irradiation (CT scan abnormalities; neurologic, intellectual, and psychologic dysfunction; growth hormone deficits). By exploiting the elegant monkey model for CNS pharmacokinetics developed in this Branch, the sophisticated monitoring techniques available to us (e.g., C.T. scans), and our experience in clinical pharmacokinetics, we hope to develop an effective CNS prophylaxis to be administered systemically and without significant sequellae.

We are beginning to make progress in the treatment of advanced solid tumors that have been refractory to conventional doses of combination chemotherapy. The response in non-Hodgkin's lymphoma has been particularly gratifying in that it now appears that we will achieve durable survival in about 70% of patients. We further believe that the concept of high-dose combination chemotherapy together with maximal supportive care (including, for example, autologous marrow rescue) will be useful in metastatic rhabdomyosarcoma and Ewing's sarcoma, although our evidence is disappointing in the case of metastatic neuroblastoma and osteosarcoma. Early intensive treatment of newly diagnosed patients with high-risk (but low bulk) disease may be of particular value (as hypothesized in the mathematical model of Norton and Simon, wherein the low growth rates of small tumor volumes necessitate high dosages of chemotherapy for effective cyto-eradication). While such aggressive therapy is not without toxicity, our experience to date suggests that it can be successfully delivered, and that it may be of benefit to such poor prognosis patients. In each of the solid tumors we are exploring other interesting observations: the synergistic possibilities of treatment with cis-platinum and adriamycin in the case of

metastatic osteogenic sarcoma, pharmacological debulking in the case of localized rhabdomyosarcoma, and the suggestive efficacy of total body irradiation and prophylactic pulmonary irradiation in the case of Ewing's sarcoma. With regard to new drug development, our finding that children can tolerate 2.3 times the adult maximum tolerable dose of ICRF-187 indicates the necessity for independent Phase I trials in children. We have made considerable progress in the development of multiresource approaches to the psychosocial concomitants of cancer in patients, their families, and therapists. We believe that this approach is exceedingly important, for failure to deal with these concomitants may be reflected in inadequate treatment of cancer. We believe that the Pediatric Oncology Branch has provided a model for coordinated research and care in the psychosocial concomitants of chronic illness which has proven applicable to many other diseases and institutions.

Finally, it should be noted that certain of our studies relate to clinical concerns that are not restricted to cancer treatment: For example, our clinical trials of poly(ICLC) have for the first time demonstrated consistent and significant interferon induction with tolerable toxicity in man. These data should prove useful in a wide variety of infectious disease problems, e.g., control of the hepatitis B carrier state.

Proposed Course:

All of the premier studies enumerated previously will be continued, but emphasis is shifting to poor-prognosis patients (those presenting with unresectable and/or metastatic disease, or other known high-risk factors). Aggressive protocols have been developed for acute leukemia patients who relapse from primary induction protocols but remain sensitive to extant chemotherapy. Increased emphasis will be given to Phase I-II trials, in particular studies of molecularly cloned interferon, dihydroxyanthracenedione, deoxycoformycin + ara-A, Iphosphamide, "orange crush," and ICRF-187. We shall continue to explore the pharmacokinetics of methotrexate so as to improve the therapeutic index of this drug as it is used in the treatment and prophylaxis of meningeal leukemia. Experiments on the use of high-dose chemoradiotherapy in newly diagnosed metastatic solid tumors will be expanded; in these studies, chemotherapy is assessed in a situation whereby maximum supportive care is possible, including autologous bone marrow rescue. Our interest in laminar-flow-room prophylaxis, granulocyte transfusions, total parenteral nutrition, and empiric antibiosis will continue pari passu with studies of high-dose chemotherapy. We plan to expand studies in clinical and molecular pharmacology and to utilize the results of such studies in planning further clinical chemotherapy trials. Both our clinical and basic studies in the kinetics of hematopoietic reconstitution and differentiation will be used to guide further experiments on clinical marrow reconstitution, using cryopreserved marrow (or stem cells obtainable from the peripheral blood). In collaboration with the Laboratory of Developmental Psychology, NIMH, we plan to expand our study of stages in normal and abnormal psychological development as they relate to the setting of chronic illness. Finally, we shall expand our interest in the etiology of cancer in the young with further studies of the 8:14 translocation in Burkitt's lymphoma, repair of alkylation injury in Ewing's sarcoma, and aryl-hydrocarbon hydroxylase inducibility in leukemia and solid tumors.

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Hematologic Support of Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Deisseroth	Head, Experimental Hematol. Sec.	PO C
	G. Messerschmidt	Guest Worker	PO C
Other:	J. Minna	Chief	VA C
	E. Glatstein	Chief	RO C
	D. Idhe; D. Glaubiger/P. Pizzo,	Senior Investigators	VA C; PO C
	B. Chesbro	Clinical Associate	PO C
	R. Ozols	Senior Investigator	MB C
	R. Trapani	Director, Immunology	MBA, Inc.
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	N. Anagnou/T. Shan/S. Fargion	Visiting Fellows	PO C

COOPERATING UNITS (if any)
Radiation Oncology Branch, NCI; VA-Medical Oncology Branch, NCI; Medicine Branch, NCI; Hazleton Laboratories, Inc.; Microbiological Associates, Inc.; Georgetown University; Lab. of Immunology, NCI; National Naval Medical Center

LAB/BRANCH
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	3.0

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hematopoietic reconstitution using autologous bone marrow cells is being evaluated as an adjunct to trials of intensive therapy in resistant sarcomas, small cell lung carcinoma, undifferentiated lymphoma, and testicular cancer. Augmentation of peripheral blood stem cell level (to facilitate stem cell engraftment) by pharmacologic methods is under intensive investigation in man and the canine model. Studies of the interaction between autologous peripheral blood and autologous bone marrow, with attainment of engraftment at low total numbers of cells, is also under study in the canine model. Studies in vitro and clinical trials are being undertaken to define the optimal platelet support of cancer patients. Extracorporeal immunoadsorption for the treatment of adenocarcinoma is being evaluated in humans and canines. The direct infusion of protein-A is being evaluated in canines for the treatment of adenocarcinoma. Multiple assays have been developed to measure stem cell function and pluripotent stem cells which give rise to megakaryocytes, erythrocytes, lymphocytes, and neutrophils. These assays are being used in the autologous marrow program, in developmental studies of transplantation in canines, and in evaluation of human and canine marrow cells grown in liquid culture.

Objectives:

1. To reconstitute patients with autologous hematopoietic stem cells following intensive chemotherapy and/or irradiation of Ewing's sarcoma, small cell carcinoma of the lung, soft tissue sarcomas, undifferentiated lymphomas, and testicular carcinomas, using stem cells collected from marrow or peripheral blood of man; to amplify the numbers of peripheral blood hematopoietic stem cells so as to permit collection of an adequate number of such cells during a single continuous flow centrifuge collection; to study the biology of hematopoietic reconstitution using animal models as well as model systems in man (e.g., post-ablation recovery); to characterize the interaction of marrow and peripheral blood in accelerating immune and myeloid recovery following intensive therapy, and to extend our data on the dose of CFUc/kg needed for engraftment in the animal model to man using both autologous peripheral blood and marrow cells. We are also studying the efficacy of autologous cryopreserved marrow cells to increase tolerance to subsequent maintenance chemotherapy.

2. The preclinical canine model will be used to determine if the amplification of committed stem cells, which we have observed in patients during hematopoietic recovery from conventional dose combination chemotherapy, also occurs in dogs during hematopoietic recovery from chemotherapy. If we are able to reproduce this amplification phenomenon in dogs, we will then collect varying numbers of mononuclear cells from the peripheral blood of animals containing amplified stem cells in the peripheral blood, and test these preparations for their ability to completely reconstitute animals following total body irradiation. If we find that the minimal dose of peripheral blood mononuclear cells required to reconstitute animals following total body irradiation is lower in collections made from animals with amplified populations of peripheral blood mononuclear cells, this result will imply that such preparations contain amplified levels of pluripotent stem cells. We will use this information to develop means of collecting adequate numbers of peripheral blood mononuclear cells from patients who require autologous stem cell reconstitution but whose autologous marrow cells cannot be collected. We are also examining the interaction of amplified peripheral stem cells and bone marrow stem cells to facilitate engraftment at low doses.

3. To extend the encouraging results of the capillary platelet migration inhibition test and the results of our retrospective analysis of determinants of good post-transfusion platelet increments in alloimmunized recipients. We wish to identify in a clinical trial which cancer patients will profit from the transfusion of HLA-matched platelets as opposed to single-donor unmatched platelet transfusions; to assess the incidence of HLA antibodies in these patients compared to aplastic anemia patients.

4. To assess the continuous flow centrifuge as a means of supportive treatment for a variety of diseases. Neocyte collection (young red cells) is being used to decrease the transfusion requirements of patients with aplastic anemia or other disorders associated with transfusion hemosiderosis (e.g., thalassemia). Among many other uses to which this technology is being put, collection of lymphocytes is underway in conjunction with the Surgery

Branch, NCI, to investigate in vitro activation of patients' lymphocytes and assess tumor cell killing in vivo.

5. The canine model is being used to evaluate critically the recent observation that passage of the animal's blood over staph A antigen (on nitrocellulose filters connected to a continuous flow centrifuge plasma line) will produce lysis of various canine neoplasms. Responders are being compared to nonresponders with respect to changes in immune complexes as they may relate to predictive indices of response and to the mechanism of tumor regression. In addition, clinical trials (Phase I and Phase II) are being carried out in man using this immunoadsorbent technique. A battery of in vitro tests of both humoral and cellular immune response is being employed to attempt to elucidate the mechanism of the tumor regressions seen to date using this procedure. Finally, eluates from these immunoadsorptive filters are being studied with the hope of developing preparations of antitumor antibodies and tumor-specific antigens for use in biological studies as well as in diagnosis. The canine model is also used to assess immunologic and tumor modifiers, i.e., intravenous protein A in the treatment of mammary adenocarcinoma.

Methods Employed:

In vitro assays are used to identify the numbers of stem cells of erythroid or myeloid origin present in marrow and peripheral blood samples. These assays involve incubation of nucleated hematopoietic cells in semi-solid culture media (methyl cellulose or agar). The number of colonies formed in 10-14 days constitutes an indirect measure of the stem cell content. Automated temperature-controlled instruments are used for the cryopreservation of marrow. Aminco continuous-flow centrifuges and IBM continuous flow centrifuges are used for the collection of granulocytes and platelets, as well as stem cells, neocytes, and lymphocytes. This latter component is also collected by conventional nonautomated centrifugational methods ("split bag method"). Collection of mononuclear cells from the peripheral blood is accomplished with the Aminco continuous-flow centrifuge. An intramural laboratory equipped with advanced culture and analytical techniques (cytofluorograph, scintillation spectrometers, ultracentrifuges, laminar flow hoods, phase bright-field and fluorescent microscopes) is supported by contract facilities in which canine experiments, in vitro assays of platelet compatibility, and histocompatibility phenotyping are conducted. A donor file of 20,000 individuals (all of whom are typed with respect to the A and B loci of the HLA system) is backed up by an interlocking network of 13 centers for donor procurement. Finally, a serum respository with at least 14,000 entries is maintained for clinical and laboratory investigations.

Major Findings:

A. Clinical Trials

1. Phase I Trial of Immunoadsorption in the Clinical Treatment of Malignancy - Five patients, one pilot and four protocol patients, were treated with immunoadsorption. The tumors included synovial cell carcinoma, colon carcinoma, melanoma, and esophageal carcinoma. Access

to the circulation was obtained and the whole blood was then separated into plasma and cellular elements. The cells were returned to the patient immediately. The plasma was passed over a filter that contained Staph aureus Cowan I strain that had been previously killed and fixed. A graduated dose was given to the patient, i.e., 0.1, 0.2, 0.4, 0.8, and 1 gm/kg. The patients were treated initially and then again 1-2 weeks later. No tumor responses were seen in this Phase I trial. Physiologic alterations in cardiopulmonary function were the major toxicities. After cell separation was obtained and immunoadsorbed plasma was being returned to the patient, a shaking chill developed. This was usually accompanied by mild hypertension and an increasing cardiac output. Soon after this initial manifestation, the systemic vascular resistance began to progressively decrease, followed by a fall in mean arterial pressure. Cardiac index continued to increase. All immunoadsorption procedures required fluid administration, and 5 required pressor agents to maintain a mean arterial blood pressure over 60 mm Hg. Two deaths occurred during this trial. Both were pulmonary deaths in patients who had advanced cancer that resulted in a severe reduction in pulmonary function prior to the procedure. It appears that the major toxicity is cardiopulmonary, but tolerable if pulmonary compromise is not present. An empiric observation was that when less plasma was infused or at a slower rate, the physiologic changes were less marked. This suggests that a vasoactive factor is generated during the filtering process. The same physiologic changes have been observed by David Terman at Baylor College of Medicine. He has also observed that a slower rate and less plasma results in little or no toxicity and tumoricidal effect is still observed.

2. Phase II Trial of Immunoadsorption in the Treatment of Malignancy - The Phase I trial was technically difficult to perform. Also, because of the toxicity, experimentation with purified Protein A was begun in the dog model to find a less toxic but effective procedure. A product was developed by Baxter-Travenol that housed the purified Protein A attached to activated charcoal. This was prepared in the manner described by Terman and shown to have activity in dogs and humans. In using this new product, patients are treated with small volumes of immunoadsorbed plasma that is infused very slowly. Inclusion into this protocol will initially be limited to adenocarcinoma patients. This phase will begin when FDA approval is obtained and will require approximately 12 to 18 months to complete. The first five patients will be treated in the intensive care unit to obtain cardiopulmonary data. When toxicity is considered acceptable, patients will then be treated on the ward. All patients will be treated weekly for 24 weeks. Periodic assessment of disease status and response will be made. Twenty patients will be treated initially. Extensive immunologic data will also be generated by Drs. Ron Herberman and Terry Phillips.

3. Autologous Bone Marrow Transplantation in Support of Chemotherapy Trials - Autologous bone marrow transplantation is provided for the Pediatric Oncology Branch, VA-Medical Oncology Branch, and the Medicine Branch, NCI.

Three major protocols use autologous bone marrow transplantation in conjunction with intensive tumor therapy in the Pediatric Oncology Branch. In Burkitt's lymphoma, complete response is achievable in up to 90% of cases. However, some patients relapse and may then benefit from intensive chemotherapy and irradiation. This is supported by bone marrow transplantation. Nonresponders are also given intensive therapy with bone marrow transplantation. Ewing's sarcoma patients have been treated at the NCI since 1964. Past experience showed that patients with metastatic disease at diagnosis had a uniformly poor prognosis. Also, patients with central axis primary lesions had a high incidence of relapse. Therefore, intensive adjuvant therapy with bone marrow transplantation was implemented. The use of intensive therapy plus autologous marrow has improved the results significantly in patients presenting with metastatic disease. Patients with soft tissue sarcomas also have marrow stored. This is usually done during induction and given back after intensive therapy in an adjuvant setting.

Autologous bone marrow transplantation is also used in the treatment of small cell carcinoma of the lung by the VA-Medical Oncology Branch. Patients with extensive stage small cell sarcoma have a low rate of complete remission with standard chemotherapy. The patients are then given intensive radiation therapy and chemotherapy followed by autologous bone marrow transplantation in an attempt to decrease relapse and increase the number of complete remissions. Autologous bone marrow transplantation is also used by the Medicine Branch in the treatment of poor prognosis cases of bulky testicular carcinoma. Those patients that do not obtain a complete remission after moderate-dose chemotherapy are surgically debulked and given intensive chemotherapy, followed by autologous bone marrow transplantation.

4. Evaluation of the Variables that Determine Engraftment of Autologous Bone Marrow in Humans - Presently, an intensive review of all cases transplanted at the NCI with autologous bone marrow is being conducted. (The first case was in 1974.) A total of 157 patients have had their marrow stored, and 55 have had marrow reinfused. The present evaluation has determined what factors have an effect on marrow engraftment. Multi-variant analysis of approximately 25 factors has suggested that previous irradiation and chemotherapy before storage of marrow cells is a major determinant of engraftment.

5. Platelet Transfusion Studies

   a. Frequency of anti-HLA antibodies in patients with leukemia, solid tumors, and aplastic anemia - Thrombocytopenia is a frequent accompaniment of intensive chemotherapy for malignant disease; however, hemorrhagic complications have been much reduced by liberal transfusion of platelets. Studies in patients receiving repeated administration of platelets (usually for therapy of primary bone marrow failure) have indicated that alloimmunization often occurs, and this phenomenon is positively correlated with refractoriness to transfusion. HLA-matching of platelet donor and recipient has, therefore, been utilized in an attempt to improve platelet survival

and preserve the efficacy of transfusion. We retrospectively examined both the transfusion records and HLAantibody screens (by lymphocytotoxicity studies, Dr. P. Terasaki) of the following groups of patients: 1) All patients treated by the Pediatric Oncology Branch (POB) for acute lymphocytic leukemia since 1968; 2) patients with Ewing's sarcoma treated with the current POB protocol; and 3) patients with childhood nonHodgkin's lymphoma treated with the POB "BACT" protocol (BCNU, Ara-C, cyclophosphamide, and 6-thioguanine). These groups were chosen because of the intensive nature of the chemotherapy administered and the consequent high rate of thrombocytopenia and platelet transfusion. Preliminary data indicate that more than 60% of those patients do not become alloimmunized despite fairly large quantities of platelets given over an average of seven months of treatment. Of the 28% who did develop anti-HLA antibodies, half reverted to negativity within two months and remained so despite continued administration of platelets over an average period of ten months.

b. Clinical trial to evaluate utility of HLA-matched versus unmatched platelet transfusions - It is estimated that 106 patients are required for this prospective study to be interpretable. At this time, 59 patients have been entered onto the protocol, 21 of whom have required platelet support. Only 7 of the 21 have been crossed over between the two arms. The average number of transfusions required by the 7/21 patients who have become refractory to matched or unmatched platelets was 7.8, while those 14/21 patients not refractory to the matched or unmatched arms have required 8.7 platelet transfusions on the average. Thus, most patients entered onto the trial (38/59) have not yet required platelets. Of the patients requiring platelets, most remain responsive to either matched or unmatched (14/21). There is no difference between the number of patients crossing over from the matched or unmatched arms, but the numbers are very small at this time. Also, the anti-HLA, platelet migration inhibition, and lymphocytotoxicity tests are all negative for evidence of alloimmunization. Although more patients are needed for definitive analysis, it appears that no benefit is derived from the use of HLA-matched platelet transfusions in a cancer patient population which contains very few patients with AML.

B. Studies in the Preclinical Canine Model

1. Assessment of Peripheral Blood Cell Plus Marrow Cell Interaction: Engraftment with and without Lithium and with and without Peripheral Stem Cell Expansion - Amplification in circulating CFU-c has been previously found to be inducible in the canine model and humans. At the time of elevated CFU-c induced by drugs, collected cells are capable of engrafting lethally irradiated dogs. Lithium has been shown to increase granulocytes under many circumstances, and an interaction between peripheral blood cells and bone marrow cells has been shown in colony stem cell assays. Using these data, a dog trial was designed to evaluate, 1) if peripheral blood plus bone marrow cells could reconstitute hematopoietic function after lethal irradiation in a synergistic

fashion, 2) if expansion of peripheral stem cells by cytoxan is necessary for an interaction, and 3) the effect of lithium given prior to collection of peripheral blood and marrow on subsequent engraftment. Initial results suggest a synergism between bone marrow cells and peripheral cells in engraftment. Continued canine trials will be needed to answer these questions.

2. Immunoadsorption with Purified Protein-A on Various Matris - Originally, killed and fixed staph aureus, Cowan I strain bacteria were used as a source of Protein A in the treatment of canine adenocarcinoma. However, toxicity in the human trial was at times severe. Because of this, alternative forms of Protein A were evaluated for less toxicity and equal efficacy. Protein A bound to sepharose was screened in 4 dogs with spontaneous mammary carcinoma. No significant responses were seen. However, toxicity appeared to be less, and one dog had a partial response with mast cell sarcoma.

3. Direct Infusion of Protein A in the Treatment of Spontaneous Adenocarcinoma - Initially, Protein A was postulated to remove blocking factors from the serum and enhance cell-mediated cytotoxicity, resulting in tumor regression. However, the previous dog trials have shown a relationship between the volume of plasma adsorbed and the response. Also, very small amounts of Protein A still have tumoricidal activity in the face of very limited Fc binding capability. These observations suggest that another mechanism is active and not simply removal of immune complexes. We therefore have begun to infuse purified Protein A directly into tumor-bearing dogs. We postulate that the effect occurring on the column containing Protein A should also occur within the vascular space. Again, small doses are being employed and with these small doses no toxicity is seen. Our trial is too early to evaluate response in these dogs.

4. Transplantation of Stem Cells after In Vitro Culture - Canine stem cells are being maintained in long-term culture. These stem cells will be removed from the longterm system and reinfused into the same dog from which they were obtained. After 900 rads lethal irradiation, multiple assays for CFU-GM, BFU-C, and CFU-GEMM will be performed on the culture system, and on the dogs prior to and after transplantation. Assessment of time to engraftment will be evaluated.

C. Laboratory Studies: Development of Stem Cell Assays for CFU-GM, BFU-C, and CFU-GEMM - The understanding of hematopoietic differentiation was increased dramatically by the development of stem cell assays. These assays are also of critical importance to optimization of autologous bone marrow transplantation. Recently, these assays have been introduced to the Experimental Hematology Section. Assays for granulocyte-macrophage progenitors, erythroid progenitors, and stem cells giving rise to megakaryocytes, eosinophils, and lymphocytes have been developed. These assays are used in both human and canine studies. Presently, different growth factors are being evaluated to optimize colony formation. The serum-free media is also being modified to optimize these assays.

Significance To Biomedical Research and the Program of the Institute:

Our studies on the role of alloimmunization in yielding poor post-transfusion increments (in single-donor platelet transfusion) has indicated that for 85% of solid tumor or leukemia patients, single-donor HLA-mismatched infusions are adequate. This information should assist in the development of national policies for effective resource allocation in cancer centers.

Our analysis of over 50 episodes of hematopoietic reconstitution following intensive therapy has suggested that the portal of irradiation employed in a patient and previous chemotherapy before marrow storage both play a role in determination of hematopoietic reconstitution after intensive therapy. These studies will permit identification of patients at risk for failure to engraft following attempted autologous stem cell reconstitution.

Studies on the interaction of peripheral blood and marrow stem cell populations, and lithium carbonate and cytoxan-induced amplification of peripheral blood stem cell pools, have shown that it is possible to increase the reconstitutive capability of autologous stem cell preparations to restore hematopoietic function when compared to marrow alone (after intensive treatment). These findings may provide a means for averting the failure of hematopoietic reconstitution seen in those patients exposed to large pelvic fields of irradiation.

The studies of immunoadsorption in the preclinical canine model and in clinical trials are designed to prove whether this form of biological response modification is of any benefit in cancer tratment.

Proposed Course:

Autologous bone marrow reconstitution will continue to be used for the support of patients undergoing intensive therapy. This technique is being extended to patients with carcinomas of the testis or lung who are receiving intensive therapy. Trials in the preclinical canine model on the effect of lithium carbonate and cytoxan in inducing stem cell amplification, and on the reconstitutive capability of combined peripheral blood and marrow autologous stem cell populations, will be completed. If current trials of immunoadsorption in dog and man suggest the efficacy of staph A Protein A treatment, this modality will be extended to a larger number of patients for evaluation.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06830-11 PO

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	K. Kraemer, Senior Investigator	CB CB
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COOPERATING UNITS (if any)
University of Florida; Lab. of Clin. Investigation, NIAID; Biometrics Research Branch, NCI; Washington Hospital Center; Clinical Pathology, CC; Chem. Branch and Metabolism Branch, NCI

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Infectious Disease Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant disease. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients. The role of oral absorbable antibiotic prophylaxis is being evaluated in patients receiving standard doses of chemotherapy, and the role of protected environments (laminar air-flow room isolators) is being studied in patients receiving intensive chemotherapy. We are also evaluating new methods for detecting occult infections in neutropenic patients. Empiric and specific antibiotic treatment of granulocytopenic patients with fever of unknown origin or proven infections is being prospectively studied, and the roles of newer antimicrobial agents are also being investigated. The role of chemically-defined immunoregulatory agents is being evaluated in in vitro and in vivo models to develop methods of improving host defenses following immunosuppressive chemotherapy, and to help reduce the incidence and severity of infectious complications.

Objectives:

1. To assess the current etiologies of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, underlying disease, and granulocytopenia.

2. To determine the most appropriate means for the evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.

3. To improve methods by which occult infections may be detected and treated.

4. To assess the possibility of reducing the incidence of infectious complications in granulocytopenic cancer patients by the use of prophylactic antibiotics.

5. To assess the efficacy and toxicity of empiric and specific antibiotic regimens for patients who become febrile and/or infected during periods of granulocytopenia. The role of antifungal therapy in high-risk patients will also be determined.

6. To improve the utility of the "total protected environment" in reducing the incidence of severe infection in patients with various lymphomas and solid tumors who are undergoing very intensive chemotherapy (with or without autologous marrow rescue), and to learn whether the intensive treatment possible in this circumstance results in improved tumor response and prolongation of survival.

7. To evaluate methods for bolstering host defenses and stimulating granulocyte recovery following chemotherapy-induced immunosuppression as an adjunct to the treatment and prevention of infections.

Methods Employed:

Patients considered to be at high-risk for developing chemotherapy-induced granulocytopenia are randomly assigned to receive oral absorbable antibiotic prophylaxis or a placebo prior to the onset of granulocytopenia and fever. Patients are followed clinically to determine if subsequent fever (and/or infection) does occur, as well as to detect antibiotic toxicity and/or the development of microbial drug resistance. Patients who do become febrile while granulocytopenic are carefully assessed for the possibility of an infectious etiology and begun empirically on broad-spectrum antibiotics. Random assignment to subsequent antimicrobial therapy is stratified according to a number of variables in the pattern of fever and/or infection. All patients are closely monitored with pharmacokinetic and metabolic studies which relate to antibiotic levels and (potential toxicity, and surveillance for changes in antibiotic sensitivity is carried out. Selected patients are the subject of newer diagnostic studies including gallium scintiscanning with or without leukocyte transfusions, ELISA-inhibition assay for Candida antigens, and assays for Enterobacteriaceae anti-core glycolipid. Assessment of the total protected

environment [laminar-air-flow room (LAFR), oral nonabsorbable plus absorbable antibiotics, cutaneous and orificial antisepsis] includes serial patient and environmental surveillance as well as longitudinal assessment of physical compliance and psychological tolerance. Laboratory investigations of immunomodulating agents include lymphocyte blastogenesis and function studies, reverse plaque inhibition assays, in vitro bone marrow culture, and challenge with infectious agents.

Major Findings:

A. Evaluation of Fever in Cancer Patients

Between November, 1975, and November, 1980, 1001 episodes of fever in 324 cancer patients were prospectively studied. 20.8% of the episodes occurred when patients were nongranulocytopenic and the remaining 79.2% when patients were granulocytopenic. Infectious etiologies were analyzed, clinical presentations compared, and recommendations regarding initial therapy generated.

B. Management of Documented Infections

1. The incidence of colonization and infections due to the JK bacteria in cancer patients was prospectively studied and guidelines for surveillance developed.

2. The value of routine microbiological surveillance and daily blood cultures was assessed in 652 episodes of fever and granulocytopenia, demonstrating that routine body cultures are costly and of no utility in clinical management. Daily blood cultures, however, even in febrile-granulocytopenic patients receiving antibiotics, can provide information leading to major therapeutic modifications and should be performed in patients receiving maximum supportive care.

3. A retrospective study of the treatment of bacteremia in cancer patients showed a significant reduction in second infections when patients with protracted granulocytopenia (> 7 days) were treated with broad spectrum antibiotics rather than specific antimicrobial therapy. Prospective studies are in progress to confirm this.

4. A study in which cancer patients with dermatomal Herpes zoster are treated with PUVA (psoralin plus ultraviolet light in the A range) has been initiated. Two patients have been treated to date.

C. Management of Fever of Unknown Origin (FUO)

1. Approximately half of the 762 episodes of fever and granulocytopenia evaluated on study are FUO's. Of these, 61% are defined as "low risk" and have resolution of fever and granulocytopenia within one week after initiation of antibiotics, none with subsequent infectious complications when antibiotics are discontinued.

2. To assess the benefit of continued antibiotic therapy in patients remaining granulocytopenic for greater than a week, patients who become afebrile after starting empiric antibiotics are continued on therapy for 14 days. At that time, afebrile patients with persistent granulocytopenia are being randomized to continue or discontinue antibiotics. Fourteen patients have been randomized to date, and preliminary observations suggest a benefit to continuing antibiotics while patients are granulocytopenic.

3. FUO patients with persistent fever and granulocytopenia after a week of empiric antibiotics do poorly when antibiotics are discontinued, but appear to benefit from the addition of empiric systemic amphotericin-B to the broad spectrum antibiotics and their continuation until resolution of fever and granulocytopenia. A prospective study comparing amphotericin-B to the new antifungal agent ketoconazol is being initiated.

D. Prevention of Infection

1. A double-blind randomized trial comparing the combination of trimethoprim/sulfa (Bactrim) plus erythromycin to a placebo has been conducted during the last 2 years in 150 patients (541 episodes) to prevent infection in granulocytopenic patients. Analysis suggests a significant benefit only in patients who fully comply with the protocol (i.e., medication) requirements, and especially in patients with leukemia receiving maintenance therapy.

2. The value of total protective isolation for patients undergoing intensive chemotherapy has been assessed in 75 patients. Current studies are evaluating the need for isolation when patients are also receiving autologous bone marrow reconstitution.

3. The role of total parenteral nutrition (TPN) in shortening the degree and duration of myelosuppression in patients receiving intensive chemotherapy has been evaluated in a randomized trial; no benefit of TPN could be defined with respect to recovery from bone marrow suppression.

E. Experimental Studies

1. The cyanoaziridine, azimexon, has been compared to lithium chloride, bestatin, and BCG in inducing leukocytosis in rodents. Azimexon produces a significant sustained granulocyte elevation, raising the possibility that it may be useful in accelerating recovery from the myelosuppression of cytotoxic chemotherapy. Studies are in progress to define the mechanism of action in both in vivo and in vitro model systems.

2. Lithium chloride, azimexon, and bestatin have been shown to result in a significant increase in the production of Interleukin 2. Effects on other lymphokines and the impact of these agents on hematopoiesis are under study.

Significance to Biomedical Research and the Program of the Institute:

Infection remains the leading cause of morbidity and mortality in the cancer patient. The majority of infections occur as a consequence of disease and/or treatment-induced alteration of host defenses (especially granulocytopenia), and they are the major impediment to the delivery of cancer chemotherapy. Consequently, effective supportive management of the patient is essential if the potential benefits of chemotherapy are to be achieved. This includes an understanding of the natural history of infectious complications in the compromised host, especially their early recognition and diagnosis. Our studies to date have helped to define the appropriate evaluation of the febrile, neutropenic cancer patient, as well as the specific management of particular infections and fevers of undetermined origin. These changes in management have resulted in a significant reduction in the morbidity and mortality related to infection.

Moreover, our studies of infection prevention (both the protected environment and empirically administered antibiotics) suggest that the frequency and morbidity of infectious complications can be significantly reduced, thus permitting the optimal delivery of cancer chemotherapy.

Proposed Course:

We shall continue our studies of the natural history of infectious complications in cancer patients as outlined in the progress report, since this evaluation will help further to define high-risk patients and assist in their diagnosis and management. Rapid diagnostic assays which do not depend on culture of the organisms will be studies in order to provide the most rational basis for immediate antibiotic and anti-fungal management. Our studies on the optimal empiric use of antibiotics in febrile, neutropenic patients will be continued, as will our clinical trials related to the specific management of septicemia, local bacterial infections, and viral, protozoan, and fungal complications. Our studies on antibiotic prophylaxis will be continued, and the use of chemical or immunological adjuvants which might shorten the period of granulocytopenia will be assessed. Similarly, our studies of the protected environment will be continued, with emphasis on the early intensive treatment of high-risk tumors prior to the emergence of chemotherapy resistance.

The ultimate challenge is the development of effective cancer treatment methods which are tumor-specific and which do not produce the significant compromise of host defenses which result in infectious complications. However, until this goal is realized, we will continue to investigate more effective and less toxic methods for treating and preventing infection in immunosuppressed patients. Our major emphasis will be directed at prevention. We will seek more effective methods for suppressing and/or eliminating the host's endogenous microbial flora with absorbable and nonabsorbable antimicrobial agents. Our major research target will be to develop methods for immunostimulation of the host's defenses during chemotherapy-induced immunosuppression. Methods to activate cellular and humoral immunity, the macrophage-monocyte system, and mechanisms which expand and/or protect the neutrophil mass following chemotherapy will be sought through the use of chemically-defined immunoregulatory agents. While combining these host bolstering techniques with prophylactic antibiotics, we will also

explore chemotherapeutic schedules which may have a more selective effect on tumor cells.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06840-06 PO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biology and Immunology of Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project the biology and immunology of acute leukemia are studied, with particular emphasis on investigation of acute lymphocytic leukemia. Leukemic lymphoblasts are being characterized on the basis of cell surface markers and specific antigens detectable by various monoclonal antibodies and heteroantisera. Such characterization permits comparison of leukemic and normal lymphoid cells in terms of cellular differentiation and malignant transformation. Biochemical studies, such as determination of the role of the purine pathway enzymes in lymphoid leukemia and lymphoma, permit insight into the relationship between malignant and normal lymphoid cells. Certain immunologic functions of leukemic lymphoblasts are studied such as their immunoregulatory capacity. Study of the role of the monocyte-macrophage system in the lymphoid malignancies is also in progress.

Objectives:

1. To develop methods of distinguishing between immunological subtypes of leukemic lymphoblasts and further to characterize such subtypes.

2. To define possible correlations between immunological cell surface markers and various biochemical characteristics of leukemic lymphoblasts.

3. To study the biochemical status of the purine enzyme pathways in malignant lymphoid conditions in an attempt to assess their role in lymphoid differentiation.

4. To evaluate heterologous anti-leukemia antisera and hybridoma-produced antibodies as potential diagnostic and therapeutic tools in the treatment of acute lymphoblastic leukemia.

5. To evaluate the status of immunoglobulin genes in acute leukemic lymphoblasts.

6. To assess whether acute leukemic lymphoblasts are capable of manifesting immunologic "helper" and/or "suppressor" function, using in vitro immunologic assays.

7. To explore the role of the monocyte-macrophage in disease states (including leukemia) and to assess the functional heterogeneity of this cell system during normal maturation.

Methods and Major Findings:

A. Immunological and Biochemical Correlations Involving Leukemic Lymphoblasts

Our recent research efforts have been aimed at elucidating biochemical markers of potential diagnostic and therapeutic value. Five enzymes have been studied in this regard, including adenosine deaminase, 5'nucleotidase, purine nucleoside phosphorylase, terminal deoxynucleotidyl transferase and acid phosphatase. Our results suggest that a "biochemical profile" of acute lymphoblastic leukemia cells provides the mechanism for further defining subsets of ALL and offers an avenue for new diagnostic and therapeutic approaches. Our studies have been extended to other lymphoid malignancies, including the lymphomas and the Sezary syndrome.

B. Study of Hybridoma Antibodies and Heterologous Antisera in Acute Lymphoblastic Leukemia

We have studied the reactivity of leukemic lymphoblasts with a variety of recently developed hybridoma antibodies both as an aid to classification and as a means of understanding the biology of leukemic cell differentiation. Our results demonstrate the presence of a wide variation in the expression of antigenic determinants on acute lymphoblastic and acute myelogenous leukemia cells. In collaboration with Drs. Ronald Billing and Paul Terasaki, we have been developing a variety of hybridoma antibodies

to antigens on the leukemic lymphoblasts of our patients. The antibodies developed are being evaluated: 1) to determine their usefulness as an aid in the sub-classification of ALL, 2) to assess their potential role in increasing diagnostic sensitivity in the assessment of bone marrow relapse, and 3) as a possible means of in vitro immunotherapy for this disease. A murine model has been developed to study this latter approach.

C. Studies of Immunoglobulin Genes in Acute Leukemic Lymphoblasts

Under the direction of Dr. Stanley Korsmeyer, and in collaboration with Dr. Thomas Waldmann, studies were initiated to examine the status of immunoglobulin genes in ALL lymphoblasts.

D. Role of Acute Lymphocytic Leukemia Cells in Suppression of the Immune Response

We have studied the ability of leukemic lymphoblasts to effect suppression of antigen-induced lymphocyte responses. Our studies have shown that the interaction of HLA-DR sera with antigens on the leukemic cell surface induces a strong signal for suppression of antigen-induced lymphocyte responses.

E. Role of the Macrophage in Leukemogenesis

We have studied the role of the mononuclear phagocyte in the development of Friend-virus induced leukemia. We have determined that the susceptibility of the newborn animal to viral infection and subsequent leukemia is based on an immunologic mechanism specifically involving a defect in macrophage function.

Significance to Biomedical Research and the Program of the Institute:

Our studies on the characteriztion of leukemic cells using immunological and biochemical markers have revealed the presence of distinct biochemical differences among leukemic lymphoblasts of different immunologic subclasses. In addition, extension of these studies into other lymphoid malignancies has revealed that development of a biochemical profile may provide a useful mechanism for classifying lymphoid malignancies in terms of their state of lymphoid differentiation. These findings may be of both prognostic and therapeutic significance. In addition, evaluation of the immunological functions of these cells, e.g., immune suppression and Fc receptor status, is providing valuable insight into the degree of differentiation of the various leukemic lymphoblast populations. Our studies of the utility of specific anti-leukemic antisera and hybridoma antibodies may yield information helpful in the diagnosis and treatment of acute lymphoblastic leukemia.

Studies in recent years have shown that the macrophage-monocyte system plays a central role in the host defense against tumor and infection. The development of assays of human monocyte function which are capable of detecting specific clinical abnormalities is of singular importance in furthering our understanding of the monocyte-macrophage system in human disease.

Our studies on the role of the mononuclear phagocyte in leukemogenesis in the Friend leukemia virus system, although preliminary, may confirm a major role for this cell type in the leukemogenic process, underscoring the need for further definition of the role of the mononuclear phagocytic system in patients with leukemia.

Proposed Course:

We are planning to expand our efforts to characterize acute leukemic lymphoblasts both biochemically and immunologically. In particular, we are extending our evaluation of the role of the purine pathway enzymes not only in acute lymphoblastic leukemia but also in other lymphoid malignancies. Preliminary data suggest that determination of a biochemical profile by assessment of these enzymes may yield important information as to the state of maturation and differentiation of both normal and malignant lymphoid conditions. We are also planning to pursue the prognostic and therapeutic relevance of our findings. Our interest in heteroantisera to acute leukemic lymphoblasts has been expanded. Specific antileukemic hybridoma antibodies are being developed which may be of use in the diagnosis and the treatment of acute lymphoblastic leukemia. Investigation into the role of the macrophage - monocyte in both normal and disease states will include further studies examining the functional heterogeneity of this system as well as the biochemical nature of these cells during the activation process. We are pursuing our studies on the role of the mononuclear phagocyte in leukemogenesis in the Friend virus leukemia system. These studies should yield important information regarding the role of the mononuclear phagocyte in the initiation of the malignant process. Immune surveillance of patients with malignancies will continue with special emphasis on the assessment of the mononuclear phagocytic system.

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October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis

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8.0	5.0	3.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the organization and expression of SV40 and adenovirus 2 genomes contained in Ad2-SV40 hybrid virus DNA molecules. The hybrid viruses are also used as models for understanding the behavior of integrated genomes. We are also analyzing the behavior of SV40 and Ad2-transformed cells (and somatic cell hybrids of these parents) as a function of the molecular conformation of viral nucleic acids within these cells, as a function of events which may control (or be controlled by) viral replication, transcription, and translation, and as a function (in vivo) of host immune recognition and killing. Secondly, we are analyzing the state and expression of the endogenous AKR murine leukemia virus genome in mice with varying expression of leukemia and virus production, as well as exploring the evolution, transmission, and relation to leukemogenesis of various murine RNA viruses. The techniques of restriction endonuclease mapping and molecular cloning are being applied to this genetic analysis of ecotropic, xenotropic, amphotropic, and recombinant murine leukemia viruses.

Objectives:

1. To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.

2. To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, translation and maturation. The early antigens of SV40 and Ad2 and their role in cell transformation and tumor rejection are of particular interest, using as a model somatic cell hybrids of SV40 + Ad-transformed parents.

3. To enhance our understanding of the etiology and natural history of murine leukemia by biochemically and genetically analyzing various murine leukemia viruses, both those occurring naturally as well as laboratory-derived strains. We believe that such analyses will not only improve our understanding regarding leukemia-causing viruses, but also our understanding regarding the nature of high-, low-, and non-leukemic strains of mice. A further objective is to explore the evolution and transmission of these viruses, using cells from a variety of wild rodents and related species, and using comparative viral genetic maps derived through the use of restriction endonucleases and molecular cloning of viral nucleotide sequences.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed or adapted. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use. This laboratory routinely employes equilibrium density centrifugation, analytical ultracentrifugation, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromatography, and the in vitro synthesis of viral DNA probes. An extensive virus purification and tissue culture facility is maintained; plaque assays and CF or IF tests for viral-specific antigens are performed. Radiolabelled proteins are analyzed via utoradiography or fluorograpohy; fingerprint analysis of the tryptic peptides of purified proteins is performed by a two-dimensional procedure involving high-voltage electrophoresis and thin-layer chromatography. Mouse breeding experiments are performed in association with studies on the genetics of RNA tumor viruses. Viral DNA sequences, either in pure form or integrated into the cellular DNA, have routinely been molecularly cloned using bacterial or plasmid-vector systems. Restriction endonuclease mapping of unintegrated, integrated, or molecularly cloned viral DNAs is performed. New viruses are characterized using single cell cloning, fluorescent antibody assay techniques, virus titration, reverse transcriptase assays, sucrose and caesium density

gradient purification, and Southern blotting techniques, in addition to the methods described above. The somatic cell hybrid technique is employed for the study of transformation and oncogenicity.

Major Findings:

A. Studies on the Murine Leukemia Viruses

1. Structure of endogenous murine leukemia virus DNA in mouse genomes. By using molecularly cloned ecotropic AKR murine leukemia virus (MuLV) DNA, a 400-base-pair ecotropic type-specific segment in the env region has been identified. This DNA segment and other defined viral subgenomic fragments have been used as ^{32}P-labeled probes to identify and analyze the structure of integrated ecotropic viral DNA sequences in uninfected mouse genomes. Those mice from which endogenous ecotropic MuLV of the AKR type have been isolated contained at least one virtually complete linear copy of the viral genome. Strains from which ecotropic MuLV has not been isolated lacked ecotropic-specific sequences. All inbred mouse strains tested also contained MuLV DNAs of genomic length whose restriction endonuclease digestion pattern was characteristic of xenotropic viruses.

2. Restriction endonuclease mapping of murine leukemia viruses isolated from wild mice. The genomes of murine leukemia viruses (MuLV) isolated from wild mice have been studied. Detailed restriction endonuclease maps of the 8.8 kb unintegrated linear viral DNAs were derived for five ecotropic and five amphotropic MuLVs from California field mice, for Friend-MuLV, and for one ecotropic and one xenotropic MuLV from Mus musculus castaneus. In general, the California MuLVs were similar in their leftward 6 kb (corresponding to the leftward long terminal repeat [LTR], gag, and pol) and rightward 1 kb (7.8-8.8 kb, corresponding to p15E and the rightward LTR). For the region spanning 6.0 to 7.7 kb (which includes the sequences that encode gp70) the amphotropic MuLVs shared few enzyme sites with the ecotropic MuLVs, although the California ecotropic MuLVs were highly related to each other in this region, as were the amphotropic MuLVs. Cross hybridization studies between amphotropic and California ecotropic MuLV DNAs indicated that they were not homologous in the region 6.3-7.6 kb; the California ecotropic viral DNAs crosshybridized in this region to AKR ecotropic MuLV. When the California viral DNAs were compared with AKR ecotropic viral DNA, many differences in enzyme sites were noted throughout the genome. The U3 regions of the wild mouse LTRs showed partial homology to this region in AKR MuLV. The LTR of Moloney MuLV was highly related to that of the California MuLVs, while the LTR of Friend MuLV appeared to be a recombinant between the two types of LTRs. The M. m. castaneus isolates were most closely related to ecotropic and xenotropic MuLVs isolated from inbred mice. One amphotropic MuLV DNA has been cloned from supercoiled viral DNA at its unique EcoRI site in pBR322. Viral DNAs with one and two LTRs were isolated. Following digestion with EcoRI, DNAs of both types were infectious. It is concluded that ecotropic and amphotropic MuLVs differ primarily in the region which encodes gp70.

3. Origin of mink cytopathic focus-forming (MCF) viruses: Comparison with ecotropic and xenotropic murine leukemia virus genomes. Restriction endonuclease maps have been developed for the viral DNAs from nine xenotropic and eleven MCF murine leukemia viruses (MuLV) isolated from AKR and other mouse strains. In contrast to the highly related nature of ecotropic viral DNAs isolated from inbred mice and from M. m. molossinus, each xenotropic and MCF viral DNA was unique. Xenotropic MuLV DNAs could be divided into two classes, which correlated with previously reported serological and biochemical data; one xenotropic MuLV isolated from an AKR mouse showed features of both classes. Ecotropic viral DNA hybridized poorly or not at all to a 1.2 kbp segment of xenotropic viral DNA located in env 6.3-7.5 kbp from the left end of the viral DNAs. All MCF viral DNAs contained non-ecotropic sequences in a portion of the env gene region, but some MCF viruses were composed principally of non-ecotropic sequences. The non-ecotropic regions of the MCF viral DNAs were related to xenotropic MuLV DNA, but many MCF viral DNAs contained sequences not found either in xenotropic or ecotropic MuLV DNA. It was concluded that these MCF viruses probably arose via recombination between ecotropic MuLV and endogenous MuLV DNA sequences; sequences of recombination include a portion of env, but need not be limited to this region. The polytropic host range of MCF viruses may represent an endogenous viral function.

4. Mink cell focus-forming (MCF) viruses: Their cellular origin and their role in murine thymic lymphomas. We have recently developed restriction endonuclease maps of the DNAs of ecotropic, xenotropic, and MCF viruses isolated from AKR and other mouse strains. As already predicted from the earlier studies, each MCF virus was composed of ecotropic and non-ecotropic sequences, and each contained non-ecotropic env sequences which shared restriction endonuclease sites with xenotropic env sequences. However, some sites were unique to MCF viruses, suggesting that the non-ecotropic sequences were not actually derived from proviruses of known xenotropic viruses. Some MCF viruses differed from ecotropic viruses only in their rightward 3 kb, which includes env and the LTR, suggesting that this region plays a critical role in MCF-induced disease. For Friend MuLV induced splenic erythroblastosis, which is also associated with MCF virus production, the oncogenic region has been localized directly to the env-LTR segment.

   In one virus, however, the span of the inferred segment of non-ecotropic sequences extended over virtually all of pol and env. This latter observation suggested that the non-ecotropic sequences of the MCF viruses might be derived from endogenous sequences which were organized in cell DNA as endogenous proviruses and that the MCF in vitro host range might be encoded completely by these sequences. We also found a consistent difference between the DNA genomes of those MCF viruses which do and those which do not accelerate disease. The former contained an XbaI site in env at 7.7 kb (map locations are given according to their distance from the left [5'] end of the viral DNA) which is also present in ecotropic MuLV DNA; MCF viruses which do not accelerate disease did not have this site. These results suggested, but did not

prove, that the XbaI site might be derived from the ecotropic viral parent.

In the present study, we have used viral DNA probes specific for ecotropic and for MCF-xenotropic sequences to analyze the viral sequences in high molecular weight DNA from mouse embryos and from spontaneous and MCF virus induced tumors. Our findings indicate that endogenous MCF-like env sequences are part of proviral DNAs which do not contain the XbaI site noted above. This MCF-like env appears to have been acquired relatively intact in at least some MCF viruses which do not accelerate disease. By contrast, all tumor tissue contains MCF-like env sequences linked to the ecotropic XbaI site.

5. Metabolism of viral RNA in murine leukemia virus-infected cells: Evidence for differential stability of viral message and virion precursor RNA. Molecular hybridization techniques were used to examine the stability of viral message and virion precursor RNA in murine leukemia virus (MuLV)-infected cells treated with actinomycin D (Act D). Under the conditions used, viral protein synthesis continues, but viral RNA synthesis is inhibited and the cells produce noninfectious particles (Act D virions) lacking genomic RNA. Kinetic analysis of total RNA in virions revealed that the amount of hybridizable viral RNA decreased steadily following the addition of Act D and by 8 h was 10% of the control value. Sucrose gradient fractionation of viral RNA showed that this low level of hybridization was due to residual 70S RNA in the Act D virion population. These results indicated that viral RNA which is destined to be encapsidated into virions has a half-life of approximately 3 h. In contrast, other intracellular virus-specific RNA molecules appeared to be quite stable and persisted for a long period of time, with a half-life of at least 12 h. These observations support the idea that two independent functional pools of viral RNA exist within the infected cell: one serving as message and the other precursor to virion RNA. The existence of two viral RNA pools was further documented by the finding that 12 h after the addition of Act D, when virion precursor RNA is depleted, 35S and 21S viral mRNA species could be identified in polyribosomal RNA as well as in total poly(A)-containing cell RNA.

Surprisingly, 35S mRNA appeared to decline more rapidly than 21S mRNA, which was stable or possibly increased in amount.

B. DNA Tumor viruses: Ad-SV40 Interactions

1. Somatic Cell Hybrids of Transformed Cells - Three Ad2 transformed hamster embryo cell lines (Ad2HE) and two SV40 HE cell lines were obtained from Dr. A. Lewis (NIAID). Mutant Ad2HE and SV40 HE cell lines were established which lacked either thymidine kinase (TK^-) or hypoxanthine-guanine phosphoribosyl transferase ($HPRT^+$). Various combinations of $HPRT^-$ Ad2 HE cells and TK^- SV40 HE cells have been fused and the resulting hybrid cell lines have been characterized for chromosome number, morphology, and viral T antigen expression. All hybrid lines were cloned from media in which only cells that are $HPRT^+$

and TK^+ can propagate; all hybrid lines examined contained 10-15% fewer chromosomes than expected for a tetraploid cell line. The hybrid cell lines were of two classes which differed in cell morphology and viral T antigen expression. One class expressed only SV40 T antigens and the morphology of these cells was indistinguishable from that of nonhybrid SV40 HE cells; these cells also lacked Ad2 DNA. The second class of hybrids expressed both Ad2 and SV40 T antigens; the morphology of these cells either resembled that of nonhybrid Ad2 HE cells or was intermediate between that of A2 HE and SV40 HE cells. The chromosome analysis of the hybrid and nonhybrid cells has been limited to determining the chromosome number in selected hybrid lines. We are presently attempting to determine if chromosomal markers can be identified by examining banding patterns in hybrid cell lines and in their nonhybrid progenitors.

2. Oncogenic analysis of Ad2-SV40 hybrid cell lines - Ten hybrid cell lines (five of each class) and their nonhybrid mutant parents were tested for their ability to induce tumors in newborn or adult syngeneic and adult allogeneic hamsters. All hybrid cell lines readily induced tumors in newborn syngeneic hamsters. The hybrid cell lines that expressed only SV40 T antigen, like nonhybrid SV40 HE cells, induce tumors in both adult syngeneic and allogeneic hamsters, and the histomorphology of these tumors is indistinguishable from that of nonhybrid SV40 HE tumors. In contrast, the hybrid cells that express both Ad2 and SV40 T antigens induced tumors only in syngeneic animals and the histomorphology of these tumors was indistinguishable from that of nonhybrid Ad2 HE tumors. Unlike nonhybrid Ad2 HE cells which rarely induce tumors in adult syngeneic hamsters, and only then at high doses of transformed cells, one line of hybrid cells consistently induced tumors in adult syngeneic hamsters at low doses of transformed cells. The histomorphology of these tumors was indistinguishable from that of tumors induced by Ad2 HE cells. The sera from the animals bearing hybrid-induced tumors contained antibodies which reacted with SV40 T antigens as well as with Ad2 T antigens. Thus, even though the SV40 antigens were expressed and recognized by the host immune system, the Ad2 antigens apparently determined that the transformed hybrid cells would not propagate in allogeneic hosts.

3. Regulation of viral transcription in cells infected with iododeoxyuridine-substituted Simian Virus 40 as a model for the activation by iododeoxyuridine of latent viral genomes - 5-iododeoxyuridine (IdUrd) induces the expression of viruses in a variety of cell lines that harbor latent viral genomes. Moreover, IdUrd stimulates cellular as well as viral RNA synthesis in certain cells. In order to understand better the action of IdUrd on RNA metabolism, we have examined viral RNA synthesis in monkey cells infected with IdUrd-substituted simian virus 40 (SV40). Extensively substituted SV40, in which 18 to 35% of the thymidine residues were substituted by IdUrd, was 100-fold less viable (by plaque analysis) than was unsubstituted SV40, although the substituted virus induced 30 to 50% as much viral-specific RNA as did the unsubstituted virus. In contrast, SV40 containing only 10 to 15%

IdUrd substitution was almost as viable as unsubstituted virus, and the substituted SV40 induced 5-fold more viral-specific RNA, as well as longer viral messenger RNA transcripts, than did the unsubstituted virus. These results suggest that the lightly substituted, mutagenized SV40 genome may produce defective proteins which fail to regulate their own transcription. Cellular DNA into which halogenated pyrimidines have been incorporated may also induce the synthesis of defective regulatory proteins, including cellular repressors of transcription which normally maintain the latent state of integrated viral genomes.

Significance to Biomedical Research and the Program of the Institute:

In studies on the mouse-tropic, xenotropic, and amphotropic murine leukemia viruses, our objective is to correlate specific leukemia virus sequences with specific biological events, especially leukemogenesis. Comparative study of the genetic information specified by different murine leukemia virus sequences in different strains of mice is now feasible, and we believe that it will be possible to determine the evolutionary origin and transmission of murine leukemia virus infection. Our studies have explained why only some mice produce virus and develop leukemia even though all mice may contain MuLV antigens. Moreover, it has been established that a major mouse chromosomal locus for the production of MuLV is in fact composed of MuLV nucleotide sequences, and is not merely a host locus which permits the expression of remote viral functions. Finally, our recent studies suggest that specific recombination between the genomes of various naturally occurring murine viruses may be the necessary and sufficient event which eventuates in leukemogenicity. This and other hypotheses can now be tested quite directly via the manipulations made possible by molecular cloning. In this research, we have recently succeeded in molecular cloning of the first murine leukemia virus DNA known to be integrated and infectious. It should be emphasized that these studies concern known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not been characterized and where the genetics of the disease are not known and cannot be controlled.

Whether a specific viral infection will lead to virus production or to cell transformation depends entirely upon the interaction between the incoming virus and the host cell involved. At the molecular level, such interactions may occur between virus-specific macromolecules and macromolecules specified by the host, and similar interactions may occur between the products of two viral genomes (e.g., "enhancement"). Our studies with Ad2 and SV40 are directed toward building a model of these interactions; our hope is to provide a better understanding of the mechanisms of viral infection and transformation by examining the effects these two genomes have on each other during co-infection. Similarly, our studies of somatic cell hybrids derived from SV40- and Ad2-transformed parent cell lines may indicate how interactions between these two viral genomes influence oncogenicity, and specifically, how subtle differences in vival-encoded proteins (possibly at the cell surface) may promote or inhibit host "immune surveillance" (recognition and killing of transformed cells). This Ad + SV40 hybrid cell system is thus a powerful tool for separating transformation from oncogenicity.

Proposed Course:

The emphasis in this laboratory during the next year will be on our SV40 + Ad somatic cell hybrid systems. It is possible that Ad T antigen expression (or the expression of some other Ad protein) renders cells more vulnerable to the host's cellular immune system than does the expression of SV40 proteins. Dr. J. Cook (Denver) has shown that untransformed HE cells and SV40 HE cells resist lysis by activated macrophages in an in vitro assay. In contrast, HE cells transformed by Ad2 and Ad12 are readily lysed in this assay. We shall therefore test our Ad2-SV40 hybrid cell lines for sensitivity to lysis by activated macrophages to learn if in vitro, as well as in vivo, the Ad2 phenotype behaves dominantly. Dr. J. Hibbs (Salt Lake City) has proposed that SV40 transformed HE cells resist macrophage lysis as a consequence of a reduced dependence on aerobic respiration and oxidative phosphorylation. Accordingly, we shall also compare the respiratory patterns of Ad2-SV40 hybrid HE cells with those of Ad2 HE and SV40 HE cells.

Other laboratories (R. Sager, Boston) have shown that hybrid cells formed from nontransformed and SV40-transformed mouse 3T3 cells are 1000-fold less oncogenic in nude mice than nonhybrid SV40-transformed 3T3 cells. They suggest that the suppression of oncogenicity is a gene dosage effect since the ratio of T antigen to cell DNA would be less in hybrid cells than in nonhybrid cells. This explanation is unlikely in our case since our hybrid cells which have a tetraploid chromosome number but which lack Ad DNA are just as oncogenic as nonhybrid SV40 HE cells. A similar reduction in oncogenicity which accompanies hybridization of transformed cells to nontransformed cells has been reported by H. Harris (London). These workers suggest that transformation is a mutation-selection process and that the untransformed genotype is dominant. We are currently in the process of forming hybrids between transformed and untransformed HE cells to determine whether the oncogenicity of transformed hamster cells is also attenuated by hybridization to normal cells.

Furthermore, we have available Ad2-transformed HE cells which express only one or several Ad proteins; Ad12-transformed cells which exhibit an oncogenic pattern intermediate between those Ad2 and SV40; and SV40 cloned hamster lines which themselves vary exponentially in oncogenic potential. Hybrid matings in all of these situations should help further to define the immunologic (and molecular) mechanism(s) by which transformed cells do or do not induce tumors.

Finally, it is possible that Ad T antigens determine the oncogenicity of Ad2-SV40 transformed hybrid cells by interacting with or otherwise modifying the physical structure, the biochemical activity, or the intracellular distribution of the SV40 T antigen. Attempts are currently in progress to compare SV40 T antigen expression in nonhybrid SV40 HE cells with that in Ad2-SV40 hybrid cells by electrophoretic mobility, electrofocusing pH, DNA binding affinity, and distribution in fractionated cells.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06860-05 PO

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Clinical and Molecular Pharmacology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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5.0	3.0	2.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns clinical and pre-clinical studies of anti-cancer drugs: We are interested in the development of clinically useful assay systems for anti-cancer drugs, and clinical drug interactions are investigated both in man and in animals. On a pre-clinical level, we have focused on investigations of resistance and sensitivity of murine leukemia cell lines to anthracyclines. Using human cells, we have started to evaluate relative resistance and sensitivity using in vitro cloning methods which allow for measurement of sensitivity to anti-cancer drugs as well as isolation of resistant clones. As a consequence of studies related to cloning of human tumor cells, we have begun a limited study on the utility of monoclonal antibodies against specific tumor cells for possible use as part of treatment schemes involving autologous marrow reconstitution.

Objectives:

1. To develop and apply clinically useful assay systems for chemotherapeutic agents.

2. To characterize the pharmacokinetics and distribution of chemotherapeutic agents and related compounds in order to enhance their clinical utility.

3. To investigate mechanisms of resistance and sensitivity of tumor cells to chemotherapeutic agents both in vivo and in vitro.

4. To utilize in vitro biochemical and biophysical measurements to characterize interactions of chemotherapeutic agents with whole cells and subcellular components, and to examine biochemically the effects of such interactions with regard to damage and repair of subcellular components; to elucidate the molecular bases for chemotherapeutic resistance and sensitivity.

Major Findings and Methods:

A. Anthracycline resistance - We are studying mechanisms of chemotherapeutic resistance using as a model murine leukemia cells which have been stably resistant to anthracyclines for 8 years. Our studies indicate that these cells are not resistant to anthracyclines on the basis of increased ability to detoxify superoxide radicals. The cells are approximately the same in volume as sensitive cells, but are of markedly different morphologic appearance and have different intracellular composition and membrane composition with regard to lipid constituents. These quantitative differences are reflected in different transport properties and intracellular distributions for a number of agents including anthracyclines. We are presently attempting to correlate in more detail membrane composition, physical properties of membrane structures, and relative sensitivity and resistance to antineoplastic agents. We believe that such characterization may give insight into general mechanisms of resistance in tumor cells.

B. Human tumor stem cell assay - The human tumor stem cell assay is a double layer soft agar technique which permits the preferential growth of tumor cells compared with normal stroma from primary tumor explants. While the method shows promise and allows direct isolation of resistant clones, it is also beset by significant problems. Among these problems, we have been particularly interested in dealing with the low plating efficiency of most tumors and eliminating the difficulties associated with visual counting at 2-3 weeks subsequent to plating, when distinguishing tumor cell colonies from aggregates is difficult. Utilizing a modification of the medium employed in the assay and tritiated thymidine incorporation as a measure of cell growth, we have been able to improve the plating efficiency for most human tumor cells without increasing the contamination with normal stromal elements, and we have been able to make measurements of chemosensitivity at 5 days subsequent to plating with an accuracy equal to that obtained at 3 weeks using the previous system. Using this technique, we hope to characterize the spectra of resistance to specific chemotherapeutic agents in specific tumors.

C. In vitro drug screening - In addition to screening new agents with the method outlined in the previous paragraph, we have utilized human neuroblastoma cell lines and tritiated thymidine incorporation to screen potential new agents. We have shown that there is a concordance between results obtained using incorporation of labelled material into cell lines, and the more standard soft agar technique, for a number of new chemotherapeutic agents as well as for established agents. We hope to extend this effort to be able to utilize cell lines of a number of different tumor types, an approach which would have the advantage of utilizing reproducible sources for the screening of new agents with anti-cancer activity.

D. Monoclonal antibody - As part of the work with human tumor cell lines, we have been collaborating in a limited study of the utility of a specific monoclonal antibody against a human tumor (neuroblastoma) in the in vitro treatment of marrow contaminated with tumor cells. We hope to use the antibody itself in a diagnostic test for detection of tumor cells and to use a cytotoxic variant of the antibody as a therapeutic method to remove these contaminating cells. Experiments involving concomitant use of anti-cancer drugs and specific monoclonal antibodies in this setting are also underway.

E. Methotrexate plus probenecid - We have previously shown that probenecid inhibits the clearance of methotrexate from plasma and CSF, in animal models and in patients. We have shown, using an in vitro soft agar cloning technique, that probenecid inhibits methotrexate cytotoxicity in mouse L1210 leukemia cells, that this effect is dose-related, and that it may be related to probenecid perturbation of the cell cycle. Two independent measurements of intracellular methotrexate levels as a function of probenecid concentration indicate that there is no significant effect of probenecid on intracellular methotrexate levels.

We have also shown that concentration x time cytotoxicity of methotrexate in L1210 appears primarily related to the time of exposure above the threshold concentration. The same effects have been verified in a number of Burkitt cell lines at this point. The reason for the effect is unclear. We are presently investigating the use of cell cytofluorimetry to monitor the cell cycle.

F. Psoralens - We have been studying the psoralen class of compounds (which are light-sensitive bifunctional alkylating agents), utilizing both L1210 mouse leukemia cells and human lymphoblastoid cells. Results to date are compatible with results previously obtained in in vitro experiments wherein psoralens were reacted directly with DNA, and also indicate that in human cells cytotoxicity is directly proportional to the number of crosslinks formed.

Significance to Biomedical Research and the Program of the Institute:

The potential utility of in vitro chemotherapeutic sensitivity tests which could, in principle, be equivalent to antibiotic screening tests is clear. Studies of the human tumor stem cell assay as such a test are therefore also

clearly of value. The test is presently primitive as outlined in the text; however the refinements which we have introduced appear to be improving the clinical utility of the test by shortening the time frame and eliminating some of the observer bias currently associated with the test. A critical evaluation in a variety of clinical settings is required before expanding utilization of the test, as well as the solution to certain of the other problems which remain, particularly the method of drug exposure. In conjunction with this approach, the exploration of mechanisms of cellular resistance to chemotherapeutic agents is also of interest. Initial impressions from the experimental data gained so far on the subline of mouse leukemia cells which are resistant to anthracyclines indicate that cancer cells may have only a finite number of mechanisms of resistance, when presented with toxic agents such as anticancer drugs. One of these general mechanisms of resistance may lie in membrane modification of the cell such as is implied by the data obtained on the subline of P388 murine leukemia cells resistant to anthracyclines.

Proposed Course:

It is our intention to pursue molecular mechanisms of sensitivity and resistance in tumor cells using the methods that are outlined above, and to continue to try to elucidate the problems we have observed with regard to individualized in vitro screening for tumor sensitivity. Hopefully, this elucidation will allow an improved testing system. On a clinical level, we plan to continue our efforts in developing new assays where appropriate, to study the pharmacokinetics of anticancer agents in man and lower animals, and to continue studies relating these measurements to both cytotoxicity and antitumor effect.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06870-05 PO

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Hematopoietic Differentiation in Human Leukemia Cell Lines and their Cell Hybrids

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COOPERATING UNITS (if any)

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6.0	4.0	2.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the defective differentiation known to characterize human leukemia populations by: Characterizing the genetic mechanisms underlying regulation of expression of fetal and embryonic programs of globin gene expression in leukemia cells, as well as the decreased levels of alpha globin gene expression seen in patients with acute myelogenous leukemia, by use of somatic cell hybrids and the techniques of molecular biology. We are also studying the changes in mRNA and proteins in the cytoplasm during commitment to myeloid or monocyte differentiation induced in the HL60 cell line by the addition of dimethyl formamide or phorbol esters. Finally, we are studying the regional localization of human alpha globin genes on human chromosome 16 to determine if this gene is closely linked to an unstable region known to exist on this chromosome.

Objectives:

1. To characterize the genetic mechanisms leading to embryonic and fetal globin gene expression in the K562 chronic myelogenous leukemia cell line through the use of cell hybrids.

2. To investigate the origin of decreased human alpha globin gene expression in acute myelogenous leukemia (in patients from non-Mediterranean European origin in whom congenital abnormalities in the expression of these genes do not exist) by use of somatic cell hybrids; to determine if this mechanism mediating the abnormal alpha globin gene expression is related to the evolution of leukemia.

3. To extend our studies on decreased alpha globin gene expression in acute leukemia to geographic regions in which non-deletion and alpha thalassemia has evolved in several genetically isolated populations, so as to permit the identification of acquired defects of human alpha globin gene expression which arise concomitantly with leukemia and may not by themselves lead to expression of clinically significant thalassemic syndromes.

4. To establish the regional mapping positions of the human alpha globin gene locus and the adenine phosphoribosyl transferase locus on human chromosome 16.

5. To characterize the protein and mRNA populations present in the HL60 human promyelocytic leukemia cell line before and after exposure of this cell line to agents associated with the induction of myeloid (dimethyl formamide) or monocyte (phorbol myristate acetate esters) differentiation; this characterization will be achieved by 1- and 2-dimensional gel electrophoresis of proteins synthesized by HL60 or produced in vitro by cell-free translation of the cytoplasmic RNA from this cell line.

6. To extend our morphological studies at the light microscopic level on differentiating populations of HL60 to the electron microscopic level, before and after exposure of cells to agents associated with development of myeloid or monocyte phenotypes.

7. To establish in vitro determinants of full human alpha globin gene expression in hybrid mouse erythroleukemia cell lines.

Methods Employed:

The techniques of cell biology (cell culture and cloning, chromosomal analysis by light and fluorescent microscopy, velocity sedimentation fractionation of hematopoietic cells at different steps of differentiation, functional assays of myeloid differentiation, isozymal analysis, radioisotopic analysis of enzyme activity and protein synthesis, cell fusion, and development of in vitro systems for the study of gene expression) are combined with those of molecular biology (molecular hybridization assays for the presence and expression of unique gene sequences, oligo dT chromatography of mRNA, hydroxylapatite chromatography of DNA, synthesis of cDNA corresponding to the globin mRNAs of man and mouse, density gradient ultracentrifugation, gel electrophorectic separation of

nucleic acids, restriction endonuclease mapping, Burke-Sharp analysis, southern and northern transfers, cell-free systems for the translation of putative mRNA populations from hybrid cells, 2-dimensional gel electrophoresis) in order to study mechanisms governing regulation of gene expression in mammalian cells. A tissue culture laboratory equipped with environmental control devices is employed, as well as a complete laboratory for the study of gene expression at the molecular level.

Major Findings:

A. Study of Human Alpha Globin Gene Expression in Hybrid Mouse Erythroleukemia Cells which Contain Human Chromosome 16 from the K562 Leukemic Cell Line: We have used the availability of a hybrid mouse erythroleukemia cell which contains human chromosome 16 from the K562 cell line in the absence of all other human chromosomes. K652 is a cell line derived by Andersson from the pleural fluid of an adult patient, in blast crisis of chronic myelogenous leukemia, which exhibits expression of globin genes as well as a number of other erythroid markers which are specific for embryonic and fetal life, and which exhibits no detectable expression of human beta globin (adult non-alpha globin mRNA and globin chains). Exposure of this cell line to 20-50 micromolar hemin results in elevations of intracellular levels of these embryonic and fetal globin mRNA's and globin chains. We have made in our laboratory hybrid mouse erythroleukemia cells which contain human chromosome 16 from the K562 line and exhibit low levels of human alpha globin mRNA as shown by northern transfer analysis, when incubated in the presence of dimethyl formamide. We propose to determine the levels of expression of the human zeta (embryonic alpha globin gene) and human alpha globin gene expression in these hybrids at the level of mRNA, globin chain, and hemoglobin synthesis before and after exposure of the cells to dimethyl formamide and/or hemin. (The former agent induces the expression of globin genes in mouse erythroleukemia cells, and the latter agent induces the expression of globin genes in the K562 cell.)

B. A Study of Decreased Alpha Globin Gene Expression in Human Myelogenous Leukemia: Disruption or alteration of the orderly maturation of committed precursor cells into functional and mature differentiated hematopoietic cells is characteristic of leukemic states. In order to identify the types of abnormalities which underlie disordered states of differentiated gene expression in leukemia cells, we have chosen to study acute myelogenous leukemia patients in whom abnormalities of globin genes have occurred during the evolution of acute myelogenous leukemia. We have generated hybrid mouse erythroleukemia plus human leukemia cell lines derived from patients in whom a non-deletion form of hemoglobin H disease arose during the evolution of either preleukemic or frank myeloblastic leukemic states; these patients were previously known to be hematologically normal. In these patients, the very low levels of expression of human alpha globin mRNA and human alpha globin chains (alpha/beta ratios less than or equal to 0.08) are consistent with derepression of all human alpha globin genes on the two separate chromosomes (human chromosome 16) present in a diploid cell. Restriction endonuclease studies of the DNA from two of these patients has shown that there are no detectable deletion mutations. The presence of an acquired mutation affecting at least four separate alpha globin genes on

two separate chromosomes in the patients' cells is suggestive, in this acquired disorder, of a single mutation on a chromosome other than human chromosome 16, or a mutation on human chromosome 16 which acts in a cis and trans effective manner. In the hybrid cells derived from one of these patients, we have shown that at least one of the pair of human chromosomes 16 present in the patient's cells exhibits low levels of human alpha globin gene expression in the absence of other human chromosomes. In addition, we have, in collaboration with the laboratory of Dr. Weatherall, identified restriction endonuclease markers in proximity to the alpha globin genes of two of the three patients which we are using to identify each of the two different members of the diploid complement of human chromosome 16 present in the patients' cells.

We have also isolated mouse erythroleukemia cells which have retained both members of the diploid set from each of two patients. We have multiple independent fusion events in each case and are currently trying to characterize the level of human alpha globin gene expression in each one of these hybrids at the level of mRNA and globin chain synthesis. These results are being compared to a set of similar hybrid cells (multiple hybrids from various independent fusion events) in which the human chromosome 16 is derived from donors in whom alpha globin gene expression is normal.

C. Regional Localization of the Human Alpha Globin Gene on Human Chromosome 16: We have obtained from Dr. Jay Tischfield a set of human X mouse hybrid cells which contain translocations of different regions of human chromosome 16 on autosomes. We are in the midst of characterizing the chromosomal composition in detail of each of these cloned hybrid cells (using Giemsa-trypsin banding and Hoechst 33258 staining) and are also determining if the human alpha globin gene is present or absent in each cell line. We have already characterized each cell line with respect to the presence or absence of the enzyme adenine phosphoribosyl transferase, a gene also known to be on human chromosome 16.

The completion of these studies will result in the regional localization of both the adenine phosphoribosyl transferase and the alpha globin gene on human chromosome 16. In particular, regional localization will also tell us whether the human alpha globin gene is near the so called "fragile site" on human chromosome 16.

D. Study of mRNA and Protein Populations in the HL60 Cell Line During Development of Myeloid and Monocyte Phenotypes: We demonstrated that the human promyelocytic leukemia cell line HL60 represents a bipotent cell population which will withdraw from the cell cycle and exhibit the biochemical and functional characteristics of polymorphonuclear leukocytes or macrophages when treated in culture with dimethyl formamide (DMF) or 12-O-tetradecanoyl phorbol 13 acetate (TPA), respectively. We further demonstrated that the commitment to macrophage differentiation by TPA is irreversible after 6 hours of treatment, and is not affected by subsequent addition of DMF to the culture. In contrast, the commitment to myeloid differentiation requires continuous exposure of the cells to DMF, and can be overridden by exposure of the cells to TPA to yield a cell population with a mixed myeloid-macrophage phenotype.

The proteins of untreated control, TPA-, and DMF-treated HL60 cells were labeled in culture with ^{35}S-methionine, isolated, and analyzed by 1- or 2-dimensional electrophoresis and autoradiography. Preliminary 2-dimensional electrophoretic studies carried out in our laboratory indicated numerous quantitative and qualitiative differences in the spectrum of newly synthesized cytoplasmic proteins in control, TPA-, and DMF-treated HL60 cells. These studies show that non-coordinate changes occur in several populations of mRNA in HL60 after DMF or TPA, and will be extended to molecular cloning of cDNA's for the mRNA's (and ultimately genes) which are specifically expressed during the time course of DMF- and TPA-mediated differentiation.

E. Changes in the HL60 Promyelocytic Leukemia Cell Line at the Electron Microscopic Level During Acquisition of the Phenotypes of Myeloid and Monocyte Differentiation: We have previously characterized the morphological, biochemical and physiological changes which occur in a cloned population of HL60 cells during the development of myeloid and monocyte phenotypes induced by exposure of these cells to the low molecular weight compounds Dimethyl formamide (DMF) and phorbol myristate acetate (TPA). We have now extended these studies to the electron microscopic level. Preliminary analysis suggests that there are significant numbers of cells in uninduced populations of HL60 which have features displayed by HL60 after development of the myeloid and monocyte differentiated programs induced by DMF and TPA. This presence of the morphological features of both myeloid and monocyte differentiation before exposure to TPA and DMF in this cloned HL60 population suggests a significant tendency to spontaneous differentiation of the HL60 cells to monocyte as well as myeloid differentiation in the absence of exogenous inducing agents. This finding extends previous results from our lab as well as others suggesting that the cell which gave rise to HL60 was the common granulocyte-macrophage precursor cell.

Significance to Biomedical Research and the Program of the Institute:

Our objective has been to clarify the genetic and molecular mechanisms which control the orderly division and maturation of hematopoietic cells. Virtually all leukemic states involve a breakdown in the orderly maturation of one of the types of differentiated cell, or the complete arrest of the ability of these cells to mature normally, resulting in the accumulation of immature committed precursor cells and the cessation of production of the normal myeloid, erythroid, or lymphoid cells necessary for the survival of the patient. We have also chosen to study alterations in the expression of globin genes associated with leukemia because we have molecular probes for analysis of the state of the genes and their gene products, at the level of globin DNA, mRNA, and globin chain synthesis. We are able to assess levels of expression of the alpha globin gene in the mouse erythroleukemia cell hybrid, and we are able to assess these processes for developmentally specific patterns of globin gene expression. We have designed experiments to clarify the type of genetic change which results in defective expression of the erythroid program in leukemic cells, as well as resulting in reversion to patterns observed before birth. Characterization of the way in which differentiation is altered in hematopoietic cells at the genetic level during the evolution of leukemia may add to our knowledge of how globin gene expression is regulated during development,

as well as the manner in which leukemia arises and perhaps how we may better control its course.

The failure of myeloid precursor cells to commit to and carry out their normal program of cellular differentiation leads to the development of the leukemic state in man. A detailed analysis of the molecular biology of the HL60 cells should significantly add to our understanding of the processes operative in normal and abnormal cellular growth and differentiation. By extending these studies to other human leukemic cell lines, it is hoped that a detailed understanding of the biology of the leukemic disease state will emerge.

The regional localization of the human alpha globin gene and the adenine phosphoribosyl transferase gene will be of value to biologists and geneticists as well as to molecular biologists who are attempting to develop methods of gene transfer. Finally, we are interested in identifying if the chromosomal regional location of the alpha globin genes will have any significance to the manner in which the evolution of leukemia results in altered states of expression of these genes.

Proposed Course:

We shall complete the studies discussed above which involve the analysis of human alpha globin gene expression, using Northern transfer and solution hybridization to assess human alpha globin mRNA levels, as well as gel and column techniques for the assessment of human alpha globin gene expression at the level of globin chains. If we find persistence of a low level of expression in the hybrids which contain human chromosome 16 (in the absence of all other human chromosomes), a defect carried with human chromosome 16 into the mouse erythroleukemia cell is suggested. If we find high levels of expression from both of the members of the human chromosome 16 pair present in the donor cell after transfer to the hybrid cell, then a defect is indicated, involving a chromosome other than human chromosome 16, which affects the expression of human alpha globin genes on human chromosome 16. If a mixed pattern is seen, a defect which acts in a cis and trans effective manner is suggested.

We are also in the midst of developing a K562 cell which is deficient in adenine phosphoribosyl transferase (currently, the cell line is resistant to 1 microgram per cc of 6-diaminopurine and will be taken to resistance levels 100 times this level before use). We will then introduce into these deficient cells the human chromosome 16 from a human donor cell on which there is a human alpha globin gene varient (hemoglobin G Philadelphia), the expression of which can be followed independently from the human alpha globin genes of the K562 cell. We will then test whether the level of human alpha globin gene expression from a normal cell is altered when introduced into the environment of the K562 cell. We wish to determine from these experiments whether the differential expression of embryonic globin genes observed from human chromosome 16 in the K562 cell is also seen following its transfer to a cell (mouse erythroleukemia) in which adult globin genes, not embryonic, are expressed. Since hybrid mouse erythroleukemia cells which contain human chromosome 16 in the absence of other human chromosomes can be obtained, one can determine in these experiments whether the expression of the embryonic program in the K562 cell is stable after transfer to the mouse erythroleukemia cell, and if its expression in K562

is dependent on the presence of other human chromosomes than human chromosome 16. If embryonic globin gene expression is lost after transfer to the mouse erythroleukemia cell, then a role for the participaton of gene products from chromosomes other than human chromosome 16 is implicated. If, on the other hand, we observe persistence of expression of the embryonic program in mouse erythroleukemia cells when the K562 is the donor (embryonic globin gene expression is not seen in other mouse erythroleukemia cell hybrids which contain human chromosome 16), a modification of some locus on human chromosome 16 in the K562 is suggested.

We will use thin layer hemoglobin isoelectric focusing, triton X-100 gels, and carboxymethyl cellulose chromatography to analyze the types of hemoglobin as well as globin chains in the hybrid cells. Northern transfer analysis is being used to characterize the globin gene expression at the level of mRNA. We have genomic probes specific for the embryonic and adult alpha globin gene mRNA, and the methods listed above are ongoing in our laboratory.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06880-04 PO

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Experimental Approaches to the Treatment of CNS Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental approaches to the treatment of meningeal leukemia and other meningeal and non-meningeal CNS neoplasms are explored. A unique subhuman primate model which allows sterile, repetitive access to the cerebrospinal fluid is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities attendant upon various chemotherapeutic and radiotherapeutic treatments; and to evaluate and screen, in a preclinical setting, newer CNS treatment modalities and drug schedules. Information gained from studies with this model is then applied to the design of clinical protocols used to treat patients with meningeal and non-meningeal malignancies.

Objectives:

1. To explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.

2. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.

3. To assess the neurotoxicity of chemotherapeutic agents used in the treatment of CNS malignancy.

4. To better understand the physiology of the blood-brain barrier, using the subhuman primate model.

5. To utilize neurophysiologic and neuropharmacologic information gained in the experimental primate system as a basis for designing new clinical approaches to the treatment of CNS malignancy in man.

Methods Employed and Major Findings:

A. Pharmacokinetic Studies Using the Subhuman Primate Model

We developed a subhuman primate system which allows for repetitive sterile sampling of CSF over an extended period of time in unanesthetized animals. The model involves the subcutaneous implantation of an Ommaya reservoir in rhesus monkeys. Studies to date have demonstrated that this model provides CNS pharmacokinetic data which are similar to that obtained in man.

B. Experimental Methods of Improving CNS Antifol Therapy

We have investigated potential methods of improving methotrexate therapy to the central nervous system, and have shown that administration of methotrexate by the hyperberic intrathecal technique results in improved cerebrospinal fluid drug distribution. We have also studied the influence of body position on ventricular cerebrospinal fluid methotrexate concentrations following intralumbar administration, and have shown that maintenance of either the flat or Trendelenberg position for at least one hour following intralumbar administration of methotrexate results in substantially greater drug levels within ventricular CSF. We have evaluated the feasibility of utilizing high-dose intravenous methotrexate infusions to treat CNS leukemia. Clinical studies of this approach, which was piloted in the monkey, are currently underway. We have also studied the CSF pharmacokinetics of Aminopterin in the subhuman primate model.

C. Studies of CSF Pharmacokinetics of Other Antineoplastic Agents

We have studied a variety of antineoplastic agents with respect to their penetration, following IV administration, into the CNS, as well as their CSF pharmacokinetics following intrathecal injection. Agents evaluated

include AZQ, dihydroxyanthracenedione, aclacinomycin, cytosine arabinoside, L-asparaginase, and m-AMSA.

D. Effect of Alteration of Drug Metabolism on Cerebrospinal Fluid Pharmacokinetics of Intrathecally Administered Agents

In these studies we explored the effect of tetrahydrouridine on the pharmacokinetics of intrathecally administered Ara-C following either intrathecal or intravenous tetrahydrouridine administration. Inhibition of Ara-C deamination by THU resulted in a profound effect on Ara-C pharmacokinetics. This approach may have potential therapeutic value in man.

E. Studies on the Neurotoxicity of Methotrexate and/or Cranial Radiation

We have developed a subhuman primate model of methotrexate leukoencephalopathy. Studies in our model confirm the synergistic role of methotrexate plus cranial irradiation in the pathogenesis of this entity.

F. Studies of the Effect of CNS Treatment on the Neuroendocrine System

Abnormalities in hypothalamic-pituitary function have previously been reported in children given cranial irradiation for CNS prophylaxis in ALL. We have been studying the effects of graded doses of cranial irradiation on the hypothalamic-pituitary axis in subhuman primates, and have demonstrated evidence of a marked dose-response curve. These observations will be of considerable aid in planning the treatment of patients with CNS malignancy.

Significance to Biomedical Research and the Program of the Institute:

Rational treatment of central nervous system neoplasms requires knowledge of the physiology of the blood-brain barrier and a clear understanding of the CNS pharmacokinetics of antineoplastic agents. Detailed pharmacologic investigations in humans is limited by the lack of a ready route of access to cerebrospinal fluid. The development of the subhuman primate model facilitates such studies in a setting that approximates the human situation. In addition, the model provides for the study of chemotherapy and radiotherapy-related neurotoxicity, allowing for delineation of factors predisposing to toxicity as well as for identification of methods useful in monitoring the development of toxicity. Data obtained in this model have led to a variety of new treatment approaches now being assessed in man.

Proposed Course:

Use of the primate model to screen agents of potential value in treating CNS malignancy will continue. Studies of a variety of agents, including the antifols, platinum compounds, nitrosoureas, radiosensitizers, and Phase I agents (e.g., 2'-Deoxycoformycin) are in progress. Particular emphasis will be placed on the intravenous approach to the treatment of CNS malignancy, and studies will be designed to assess the penetration of intravenously administered compounds, e.g., methotrexate, into brain tissue. Exploration of various combination chemotherapeutic approaches to treating the CNS will also

be continued. Finally, a comprehensive study of post-therapy leukoencephalopathy is underway in an attempt to learn more about those factors which predispose to the development of this syndrome.

Publications

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06890-02 PO

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Lymphoma Biology and Epstein-Barr Virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies utilizing fresh lymphoma cells or derived cell lines are carried out with the primary objectives of understanding lymphomas in the context of normal lymphocyte differentiation, and identifying the normal counterpart cells of specific malignant lymphomas. Detailed phenotypic characterization of tumors and cell lines is carried out and studies of immunoglobulin secretion and the characteristics of the secreted molecular species are in process. The development of heteroantisera and hybridoma antibodies with specificity for non-endemic Burkitt's lymphoma is proceeding and has led to the recognition that American tumors differ phenotypically from African tumors. Characterization of these antigenic differences is in process. Studies of the biological effects of various EBV strains on normal lymphocytes continue.

Objectives:

1. To obtain tumor-derived cell lines from lymphoma patients and to use these in the study of lymphoma biology.

2. To induce differentiation in vitro and to determine the correlation between cell surface differences and in vitro behavior, including infectibility with Epstein-Barr virus (EBV) and response to plant lectins.

3. To study the cytogenetics of lymphomas and derived cell lines.

4. To compare isolates of the EBV which have been obtained from different clinical settings (e.g., African Burkitt's lymphoma, American Burkitt's lymphoma, infectious mononucleosis), and to determine whether differences can be discerned which might clarify the diverse biologic and pathologic expressions associated with this virus.

5. To investigate the role of other cofactors (e.g., C-type viruses) in the oncogenic expression of EBV.

6. Since the ability of a virus to remain latent may play a role in oncogenesis, factors which modify virus expression, transcription, and production will be investigated.

7. To investigate the immunostimulatory effects of EBV on human lymphocytes: The classes of immunoglobulins secreted in response to EBV stimulation; the role of T-cells in EBV stimulation (both as helpers and independent responders) and whether the response differs with T-cells obtained from seropositive vs. seronegative individuals. In addition, the manner in which different strains of EBV (i.e., transforming vs. infecting) stimulate immunoglobulin production will also be investigated.

Methods Employed:

Cell culture. Primary (i.e., tumor-derived) and established lymphoid cell lines are grown and maintained in RPMI 1640 plus 10-20% fetal calf serum at 37°C. Physical characteristics and karyotype are routinely examined. Immunological techniques are utilized in the study of cell lines including examination for surface immunoglobulin and various surface receptors. Various sub-types of complement receptors are examined (C3b, C4b, C3d) as well as EBV receptors, utilizing purified reagents and rosetting techniques. Cell membrane 'microviscosity' is studied by examining the rotation of a hydrophobic, fluorescent probe, diphenyl hexatriene (DPH), incorporated into cells or cell membranes. Rotation is measured in terms of fluorescence polarization anisotropy after excitation of probe molecules in a single plane of polarization. Virus-producing cell lines are grown in 6- to 12-liter quantities at different temperatures (32-37°C) for 5-14 days during which time the virus is labeled with ^{3}H-thymidine. Supernatants are harvested, clarified, concentrated by ultrafiltration and ultracentrifugation, and virus purified by dextran velocity and isopycnic sucrose sedimentation. Radiolabeled virus-containing bands are ultracentrifuged and dialysed. Purified viruses or cell suspensions are detergent lysed and subjected to enzymatic digestion of protein and RNA followed by

phenol extraction and ethanol precipitation. Certain viral DNA preparations are purified by isolation of 55s DNA on sucrose gradients followed by organic extraction. Viral DNA preparations are banded to equilibrium in cesium chloride and have an A260/280 nm ratio of 1.80-1.95. Viral antigens, particles, and biologic activity are assayed by immunofluorescence (EA, VCA, EBNA). Virus particles are quantitated in cells by electron microscopy. EBV infectivity is assayed by induction of EA in RAJI cells while transformation is determined by the genesis of EBNA-containing cord blood lymphoid cell lines following EBV infection. Sheared radiolabeled purified viral DNA is mixed with an excess of sheared unlabeled cellular (or viral) DNA, denatured, incubated at 65°C, and timed-samples assayed by hydroxyapatite chromatography to determine the degree of hybridization. Thermal elution profiles are determined on certain incubation mixtures utilizing graded temperature increments and hydroxyapatite chromatography. Radiolabeled-EBV is digested with purified restriction enzymes, analyzed by electrophoresis on agarose slab gels, and detected by fluorography or autoradiography. Immunoglobulin secretion is studied using the reverse hemolytic plaque assay in which Staphylococcal A protein is coupled to sheep red blood cells (SRBC) and then mixed with washed target cells, incubated on agar, and assayed with rabbit IgG antibody to human immunoglobulin(s) in the presence of complement. Immunoglobulin-secreting cells are detected by the presence of lysis of the SRBC--visible as a clear plaque. EBV is preincubated with purified populations of B cells, T cells, and cell mixtures. Proliferative capacity is measured by ^{3}H-thymidine incorporation.

Major Findings:

A. Studies of Immunoglobulin Synthesis by Tumor Cells

A method of analyzing secreted immunoglobulins on polyacrylamide gels has been developed, and using this, we have screened our library of lymphoid cell lines of various origins. Undifferentiated lymphoma lines of American origin consistently secrete more IgM than African lines, in which immunoglobulin secretion is frequently undetectable. We have observed a significant correlation between the absence of methionine in secreted light chains, and the presence of EBV DNA in the cell line. Further exploration of the significance of this phenomenon is under way. We have initiated studies of patients' sera in view of the finding of IgM secretion by cell lines, and in a small number of cases, fresh tumor cells. Initial results have demonstrated the presence of monoclonal immunoglobulins by thin layer agarose electrophoresis. More detailed studies are in progress.

B. Attempts at Inducing Differentiation: Analysis by 2-Dimensional Gel Electrophoresis

Use of a variety of inducing agents has led to the demonstration of enhanced complement receptor expression in several cell lines. Analysis of the influence of these agents by cell surface phenotyping, cytochemistry, 2-dimensional gel electrophoresis of radiolabeled proteins, and study of secreted immunoglobulins is under way.

C. Development of Specific Antisera

We have been able to demonstrate the presence of antigens on American Burkitt cell lines which are not present on African cell lines by raising heteroantisera in goats and absorbing these with cord blood lymphoblastoid cell lines or African Burkitt's lymphoma cell lines. Data so far has been collected by employing indirect immunofluorescence and flow cytofluorimetry. Currently, attempts to characterize these antigens are under way.

D. Characterization of Complement Receptors

We have confirmed the existence of two separate receptors for C3b utilizing blocking with highly purified C3b or C4b. One receptor will bind C4b and one will not. The latter also appears to have a lower affinity for C3b than the former. The non-C4b binding receptor is expressed preferentially by the majority of our tumor derived cell lines, the C4b binding receptor by cord blood lymphocyte-derived cell lines and normal lymphocytes.

E. Identification of Transforming Virus in the P3HR1 Cell Line

We have demonstrated that the P3HR1 cell line which is widely accepted as releasing only so-called "lytic" virus, does in fact release virions capable of transforming human cord blood lymphocytes. The use of monoclonal antibodies specific for transforming strains of EBV has confirmed this. Further studies of the relationship between the ability of EBV to induce immunoglobulin secretion and its ability to transform B lymphocytes are in progress.

F. EBV as a Polyclonal Activator of B Cells

Studies on differences between EBV strains with regard to their ability to induce immunoglobulin secretion and to transform human cord blood lymphocytes continue.

Significance to Biomedical Research and the Program of the Institute:

Attempts to comprehend lymphomas are doomed to failure if a purely morphological approach is used. As data accumulates, the resemblance of neoplastic lymphoid cells to their normal counterparts becomes more and more striking. Moreover, it is becoming clear that the behavior of lymphomas which have been identified precisely, e.g., by detailed immunochemical phenotypic characterization, can be predicted on the basis of what is known of the normal cell counterpart. Further, response to therapy and the approach to therapy also differs according to cell type when accurately determined, as opposed to utilizing a purely histological approach. Thus, our objective--to comprehend at a cellular level the biology of lymphomas--should result ultimately in an improved ability to separate different pathological entities, therefore to improve our ability to analyze the results of therapeutic trials and, hopefully, to indicate possible novel approaches to treatment.

EBV is closely associated with at least three human diseases--infectious mononucleosis, African Burkitt's lymphoma, and nasopharyngeal carcinoma. As such,

it represents a potential human tumor virus, and clarification of its role in the diseases with which it is associated is of paramount importance. Studies on the induction of EBV with corticosteroids may clarify the relationship of EBV to the host cell since they suggest that the viral genome may be influenced by mechanisms which regulate the transcription of unique genes in differentiated cells. Such information is pertinent to an understanding of the association between latent virus infection and oncogenesis--both of which are important characteristics of the herpes virus group as a whole. Studies of different strains of EBV will help to determine whether or not differences in virus properties are relevant to the association of EBV with sub-clinical, benign, and malignant diseases. Studies of the modification of normal lymphocyte behavior induced by EBV, and T-cell regulation of these phenomena, will provide insights into the spectrum of EBV-associated diseases, and may also lead to the definition of specific genes related to different biological properties, e.g., transformation versus immunoglobulin production.

Proposed Course:

The studies in lymphoma biology and EBV will be continued. In particular, we would like to attempt to characterize B-cell malignancies in terms of lymphocyte differentiation antigens. This would involve the generation of monoclonal antisera with mouse myeloma hybridomas. Such reagents would potentially provide objective identification of individual lymphoid malignancies, and lead to definitive answers to such questions as the relationship between undifferentiated B-cell lymphomas of various types. Such antisera may also be useful in studying EBV and its influence on B cells, e.g., are there specific B-cell populations which EBV infects? What are the differences in transcription (in terms of immunologically identified proteins) between lymphocytes which produce Ig in response to EBV but are not transformed, and transformed cells? Further studies are planned in conjunction with the Laboratory of Clinical Investigation on the relationship between EBV and complement receptors, and the significance of this to an understanding of the nature and evolution of virus receptors--a concept of considerable importance in the case of latent viruses which can function as horizontal transmitters of genetic information.

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ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

OCTOBER 1, 1980 to SEPTEMBER 30, 1981

The Radiation Oncology Branch continues in a transition. In the last two years, the turn over has been virtually complete of all staff, physician, technician, biology, and administrative.

The three main goals of the Radiation Oncology Branch continue unchanged:

1. Major emphasis on clinical trials of a combined modality nature, predominantly collaborative with other clinical branches.

2. Strong radiation biology program with heavy emphasis on basic science and clinical questions of relevance.

3. A training program in radiation therapy, equivalent in statue to the present programs in Medical, Surgical, Pediatric Oncology Branches within the National Cancer Institute.

At the moment, clinical trials are progressing in a wide variety of fronts, as will be discussed below. The biology program is up and running, although it has been somewhat difficult to optimize, because of problems with holding facilities for animals. The present B2 facility within Building 10 leaves much to be desired for long term animal experimentation, which is the major thrust of the in vivo work that goes on in this branch. The frequent outburst of infections that takes place on the B2 facilities has meant frequent interruptions and sacrifice of ongoing experiments that are designed to last 12 to 24 months. It is in fact limitations of the B2 facility that have caused the ROB to plan as a major portion of the future renovation to be carried out on the B3 level a major commitment of space to an entirely new animal facility that permits long term rodent experimentation.

Concerning the training program, a provisional approval has been obtained from the AMA Residency Review Committee. This program, in conjunction with the Uniformed Services University of the Health Sciences, working through Walter Reed Army Medical Center and National Naval Medical Center in Bethesda, consists of a three year training program under the direction of Dr. Eli Glatstein. Essentially 18 months will be within the National Cancer Institute, one year within Walter Reed Army Medical Center and six months within Bethesda Navy Medical Center. The necessity of sharing the training experience with other Medical Centers is required by the fact that the Cancer Institute patient base is limited to specific diseases. The clinical material that the military centers complements the Cancer Institute material nicely, with major emphasis on gynecologic neoplasms, head and neck cancers, and genitourinary cancers. These are areas in which the Cancer Institute patient base is presently lacking.

Although the radiation biology program is still in its early phases, due to limitations of space, there is an in vivo program and in vitro program. The latter has been hindered by inadequate radiation facilities, recently corrected with the purchase of a cobalt unit for radiation biology experimentation of cells. This will allow Dr. James Mitchell to work predominantly in the area of low dose rate radiation, an area in which he is considered an expert. In addition, experimentation of chemotherapeutic agents and hyperthermia is ongoing, and plans are underway for experimentation of photo-sensitivity as well. The in vivo work, under the direction of Dr. Elizabeth Travis, will concentrate on normal tissue tolerance and the impact of radiosensitizing and radioprotecting compounds. Ongoing work has centered around human CFUc, in collaboration with other branches.

The clinical program within the Radiation Oncology Branch is centered around combined modality studies. Most of these are collaborative with other branches. The most important of these revolves around the study of small cell carcinoma of the lung, in collaboration with the VA Medical Oncology Branch. This study consists of a controlled prospective study of the value of radiation therapy to the chest in patients with limited small cell disease. Preliminary results suggest significant benefit can be achieved with combined modality treatment over what can be obtained by chemotherapy alone. Nonetheless, additional patients are required before a final conclusion can be made. In addition, a collaborative venture is ongoing for advanced oat cell carcinoma of the lung that includes consolidation of a chemotherapeutic response with short term radiation therapy followed by marrow ablative cytotoxic treatment and bone marrow reconstitution. Another study in collaboration with the VA Medical Oncology Branch revolves around electron beam treatment for mycosis fungoides. This study is well underway, although it was interrupted with mechanical problems on the linear accelerator which have now been corrected. Such treatment of whole skin electron beam treatment is carried out in only a few medical centers in the United States.

There are also collaborative ventures with active participation with the Surgery Branch in soft tissue sarcomas and with the Pediatric Oncology Branch in Ewing's sarcoma, rhabdomyosarcoma, and lymphomas. In addition, limited experience has been obtained with whole body radiation in multiple myeloma, in conjunction with the VA Medical Oncology Branch.

Primary ROB studies presently center around intraoperative radiation therapy. Presently, patients are operated on the 10th floor, with massive surgery carried out for carcinoma of the pancreas, stomach, or retroperitoneal sarcomas. Maximum surgery is performed and the patient is then transported through the hallways and elevators to the ROB while under general anesthesia. They are then taken to the treatment room, transposed to the treatment couch, and re-opened under anesthesia so that a large single dose of electron beam treatment can be applied intraoperatively to the tumor bed, with critical normal viscera moved out of the way. This has been done in conjunction with misonidazole and the enthusiasm runs high for this investigational approach for these extremely difficult management problems. Randomized studies are being carried out in these diseases to delineate the benefit of this approach. At the present time, the import at

aspects revolve around clear delineation of the safety of this approach. Ultimately these are seen as first steps to later studies that will incorporate chemotherapy as well.

Another series of studies within the ROB have revolved around radiosensitizing compounds. Intravenous misonidazole has been studied in some detail, and the pharmacology has been delineated. The first target for radiosensitizing compound was carcinoma of the esophagus. Initially patients were treated with pre-operative irradiation, half the patients being randomized to receive the intravenous misonidazole with each fraction of radiation. After the first 8 cases, it became apparent that the combination of pre-operative radiation followed by surgery was potentially highly lethal, in terms of acute respiratory failure. The exact explanation for this remains unclear, but may be related to concentration of oxygen that is utilized during the surgery. In any event, respiratory failure has not been seen in patients who have not received surgery. As a consequence of this situation, the study was altered to consist exclusively of radiotherapy alone, with half the patients receiving the radiosensitizing compound. No surgery has been scheduled. Preliminary results suggest that no major benefit will be achieved by the misonidazole over what can be obtained by radiotherapy alone. In any event, additional patients are required before a final conclusion can be made.

Another major ROB study revolves around Stage I and II breast cancer, comparing radical surgery to radical irradiation with preservation of the breast. This study, in conjunction with the Surgery Branch, has accrued almost 50 patients in the first 18 months. This modest number is considered a major accomplishment, in view of the facts that no prior patient base has been recruited for such a study, and the extreme difference of the two arms makes for a difficult randomization. Nonetheless, the study appears to be accruing reasonably well and we anticipate that the accrual will improve further with time.

A final ROB study is going on in the treatment of locally unresectable osteogenic sarcoma and chrondosarcoma. This study deals with intravenous misonidazole, the hypoxic cell sensitizer. Despite the fact that this tumor is reported to "radioresistant", 7 of the 8 patients who have been treated thus far have responded, with overt tumor shrinkage, and at least one patient has been followed for over two years without obvious growth of the tumor mass. Additional patients and the longer followup is obviously important before any conclusion can be made.

The addition of Dr. Jan Van de Geijn, along with Dr. Joel Tepper, has allowed for full incorporation of CT scanning into our radiotherapy treatment planning. The major of patients who are treated are now scanned in the treatment positions and computerized treatment plans are generated, superimposed on CT sections. Dr. Van de Geijn has developed the only program which allows for accurate dose calculations with blocks placed in the fields. The treatment plans that are now generated from the ROB are extraordinarily sophisticated compared to what can be done in other medical centers. Such treatment plans are employed routinely on our esophagus patients, breast patients, and most of the soft tissue sarcomas. The only limitation has been the down time of the CT scanner itself, and the relatively small aperture available on the CT scanner.

Ultimately, we anticipate that in less than a year's time, we will be able to incorporate the scans and computerized treatment planning through any plane, not just simply cross sectional. It is also our intention to interface ultrasound into the program and compare it to CT for treatment planning ability.

A major portion of time has been devoted to plans for the new Radiation Therapy Treatment facility. The extraordinarily long delays in construction, along with what was felt to be an exorbitant cost estimate for completing the facility, led to the discharge of the contractor, who is already more than 24 months behind on an 18 month contract. As of this writing, a new contractor is not yet installed, but is expected shortly. When the facility has been completed, and new equipment is fully installed, the branch will move into its new home, after which full renovation of the present B3 facility will be made to convert it into a full-fledged radiation biology laboratory. Such laboratory space will consist of two major floors, one for radiologic physics and tissue culture radiobiology, and the other committed to in vivo radiobiology with a large rodent facility. In addition, the 4th bay within the new department will be renovated into an intraoperative facility. At the present time, the intraoperative program can only be carried out once a week, because of the disruption that it causes within the department. With the bay dedicated to intraoperative radiation in terms of operating space and radiation device in that room, such procedures will be performed on a more frequent basis. The major advantages of the intraoperative program appear to be the speed of the precise location of the tumor, and the ability to eliminate critical normal tissues (or at least protect them) from the typical radiation field. In addition, it offers a unique opportunity in which to combine radiosensitizing drugs, and even hyperthermia in the treatment of intra-abdominal neoplasms. It will also probably prove to be an ideal approach for retroperitoneal nodes in pelvic neoplasms.

In the forthcoming year, one new area of investigation is anticipated. A pilot study, in conjunction with the Medicine Branch, is anticipated in advanced cervix cancer. Patients with positive paraaortic nodes will be treated with adjuvant chemotherapy after primary treatment has been completed. In addition, those patients who have truly massive cervical lesions will be candidates for hyperthermia, as soon as a new cervix applicator with a built in microwave source has been obtained. This will allow investigation of hyperthermia in a high risk group of patients, and serve as the investigational approach for this interesting area of research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00650-26 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Service Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Allen S. Lichter, M.D.	Head, Radiation Ther. Section	ROB, NCI
Others:	Joel Tepper, M.D.	Sr. Investigator, Rad. Ther. Sec.	ROB, NCI
	James Schwade, M.D.	Head, Rad. Biological Section	ROB, NCI
	Tim Kinsella, M.D.	Sr. Investigator, Rad. Ther. Sec.	ROB, NCI
	Marilyn Glover, R.N.	Clinical Nurse	ROB, NCI
	Andrea Zola, R.T.	Medical Radiation Technician	ROB, NCI
	Sandra Franklin, R.T.	" " "	ROB, NCI
	Joy Greig, R.T.	" " "	ROB, NCI
	Barbara Kelly, R.T.	" " "	ROB, NCI
	Vicky Iler, R.T.	" " "	ROB, NCI
	Pat Webster, R.T.	" " "	ROB, NCI
	Kathy Yeakel, R.T.	" " "	ROB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to provide expert radiotherapy, consultation and radiation therapy treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the NCI. Support is given to the Medicine Branch, Surgery Branch, Pediatric Oncology Branch, VA Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and other Federal Hospitals in the area where technical expertise and technical equipment dictate a need for such consultation.

Project Description:

Objectives: To provide consultation and radiation treatment for Clinical Center patients.

Methods: Formal and informal consultation with referring physicians and application of radiation therapy where appropriate with x-rays and electrons in accordance with standard radiation therapy practice as well as modified programs where necessitated by adjuvant concomitant therapies.

Major Findings: There were 550 patients seen in formal consultation and an additional approximately 300 telephone consultations provided "ad hoc" advice on treatment or general information. Approximately 450 patients will be treated in this fiscal year with the majority of these being service patients and the remainder being protocol patients in the Radiation Oncology Branch.

Proposed Course: To continue.

Publications:

Tepper, J. E., Glaubiger, D., Lichter, A. S., Wackenhut, J., Glatstein, E.: Local control of Ewing°s sarcoma of bone with radiotherapy and combination chemotherapy. Ca 46:1969-1973, 1980.

Jentzsch, K., Binder, H., Cramer, H., Glaubiger, D. L., Kessler, R. M., Bull, C., et al.: Leg function after radiotherapy for Ewing°s sarcoma. Cancer 47:1267-1278, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00684-26 RO

PERIOD COVERED
October 1, 1980 to Sept 30, 1981

TITLE OF PROJECT (80 characters or less)

Nonclinical Irradiation Services

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.N. Padikal	Senior Staff Fellow	ROB	NCI
Other:	F. Harrington	Engin. Tec.	ROB	NCI
	B. A. Fraass	Staff Fellow	ROB	NCI
	J. van de Geijn	Head, Rad. Phys. and Comp. Auto Section	ROB	NCI
			ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS: .4 | PROFESSIONAL: .1 | OTHER: .3

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) [illegible] X (c) LITTER

(a1) MINORS (a2) INTERVIEWS

ABSTRACT OF WORK (200 words or less - underlining keywords)

Summary: The physics section provides radiation physics services, equipment, and advice on experiments involving radiobiology. Cells, tissue cultures, mice, rats and dogs were irradiated for radiobiology experiments.

PHS-2040

Project Description:

Objectives: To provide radiation physics expertise and equipment to researchers involved with radiobiological projects.

Methods Employed: Dosimetric investigations have been made to assist radiobiologists in irradiating cells, tissue cultures, mice, rats, and dogs using both linear accelerators and the 250 kVp X-ray unit. Many devices have been fabricated to hold animals in specific positions relative to the radiation beams while shielding certain critical organs.

Major Findings: Cells, tissue cultures, mice and rats, were irradiated. The A.F.F.R.I. 50MV accelerator was again used for a series of dog studies.

Significance to Biomedical Research and the Program of the Institute: Radiation physics support is essential to the Radiobiology Section, Radiation Oncology Branch.

Proposed Course: To be continued. Dosimetry in these difficult cases will be improved. Continuing technical support will be provided.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00998-03 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Radiation Sensitizers in Carcinoma of the Esophagus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schwade, M.D.	Acting Head, Radiobiology Section	ROB	NCI
Other:	E. Glatstein, M.D.	Chief	ROB	NCI
	Other Staff of the Radiation Oncology Branch			

COOPERATING UNITS (if any)

Surgery Branch, COP, DCT, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study radiation sensitizing compounds and their ability to increase the effect of radiation in sterilizing neoplastic cells. The plan is to take patients who have carcinoma of the esophagus that appears to be clinically confined to the mediastinum and give all patients irradiation. Half the patients will receive misonidazole, a hypoxic cell sensitizer, administered by an intravenous method. Following completion of the radiotherapy, survival, freedom from lapse, and tumor will all be evaluated. In addition, these patients, who are frequently cachectic, will serve as the basis for controlled studies of total parenteral nutrition. In addition, this study utilizes an innovative fractionation schedule for the irradiation (400 rad twice weekly, rather than 200 rads daily, 2 days per week).

Project Description

Objectives: By assessing the survival, freedom from lapse, and tumor response, we hope to be able to determine whether or not misonidazole is effective in augmenting radiation effects in patients with carcinoma of the esophagus. If this compound does appear to increase radiation effectiveness, then further work in radiosensitizing compounds is clearly warranted.

Methods Employed: Patients with previously untreated carcinoma of the esophagus confined clinically to the mediastinum will be accessioned and treated with irradiation. Half the patients will receive on a randomized basis intravenously administered misonidazole with each fraction of irradiation.

Major Findings: Ten patients have completed therapy. Of the patients who have completed therapy, there appears to be little difference between patients treated with or without the radiosensitizer.

Significance of the Research Program to the Institute: Radiation sensitizing drugs may represent an avenue to increase effectiveness of therapy by augmenting the effects of an existing modality, radiation. This study represents an attempt to evaluate the efficacy of the drug currently available and thought to be most effective, misonidazole.

Proposed Course: Patient accrual continues, since no definitive conclusions can be made at this time because of small patient numbers.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06310-02 RO

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Surgery versus Radiation Therapy in Treatment of Primary Breast Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Allen S. Lichter, M.D., Head, Radiation Therapy Section, ROB, NCI

Others: Other Staff within the Radiation Oncology Branch, NCI.

COOPERATING UNITS (if any)

Surgery Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study whether techniques of treatment that preserve the breast (lumpectomy followed by radiation therapy) provide equal survival opportunity when compared to women treated with standard surgical techniques (mastectomy) for primary breast cancer. After a work-up confirms localized disease the patients are randomized to treatment with either mastectomy or lumpectomy plus radiation therapy. Both groups have axillary node dissections and are treated with chemotherapy should the nodes be positive.

Project Description:

Objectives: Survival and recurrence figures are comparable for the two treatments, it should be far more acceptable for women to be treated with less than radical surgical procedures for localized breast cancer. The cosmetic result of localized treatment will be carefully evaluated. The psychological, sexual, and sociological impact of mastectomy versus lumpectomy will be noted. The ability to combine radiation therapy with aggressive chemotherapy in node positive patients will also be assessed.

Methods Employed: Patients with previously untreated carcinoma of the breast clinically and radiographically confined to the breast and axillary lymph nodes will be accessioned into the study. They will be randomized to have treatment with lumpectomy and radiation therapy verus mastectomy. Patients with positive axillary lymph nodes will receive chemotherapy.

Major Findings: This study has been active for 19 months. Currently 49 patients are enrolled and it is far to early to assess results.

Proposed Course: Patient accrual will continue.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06313-02 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Dose to Lung and Opposite Breast vs. Technique for Primary Breast Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.L. Roberson	Staff Fellow	ROB	NCI
Other:	A. S. Lichter	Head, Clin. Rad. Ther. Sec.	ROB	NCI
	H. A. Fredrickson	Comp. Systems Analyst	DCRT	NCI
	J. van de Geijn	Head, Rad. Phys. and Comp. Auto. Sect.	ROB	NCI
	A. Bodner	Summer Student	ROB	NCI

COOPERATING UNITS (if any)

Computer Systems Laboratory, DCRT, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS: 1.0 | PROFESSIONAL: .7 | OTHER: .3

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline Keywords)

Treatment planning techniques for primary breast irradiation are investigated to optimize dose to areas at risk while minimizing dose to critical structures. When the high-dose volume is increased to include the internal mammary chain (IMC), dose to lung and opposite breast increase. This effect has been investigated extensively with both treatment planning and dose measurements.

Project Description:

Objectives: To quantify the dose to critical structures such as lung and opposite breast, as a function of treatment techniques which include the internal mammary chain (IMC) within the high-dose volume.

Methods Employed: Dose distributions for many treatment techniques were simulated using CT scans from 15 patients. Dose to lung was calculated, three dimensionally for some patients. Dose to opposite breast was also calculated. Extensive film, thermoluminescent dosimetry (TLD), and ion chamber measurements have been made in water and polystyrene phantoms. These measurements have been used to verify the computer results, and have been related to the surface TLD measurements made on patients under treatment.

Major Findings: Typical radiographic verification and simulation films are misleading with respect to the volume of lung irradiated. No single technique is optimal for all patients. Dose to the opposite breast has been quantified.

Significance to Biomedical Research and the Program of the Institute:
An improvement in therapeutic ratio (dose to area at risk/ dose to normal tissue) is possible if the treatment technique is determined individually for each patient.

Proposed Course: To investigate techniques for modifying the dose to the opposite breast, and to investigate the feasibility of the combined photon and electron treatment technique.

Publications: P.L. Roberson, A. Bodner, A.S. Lichter, H. A. Fredrickson, T.N. Padikal, B.A. Kelly, and J. van de Geijn, Int. J. Rad. Oncol. Biol. Phys 6: 1416 (1980). Treatment Planning in Primary Breast Irradiation: The Influence of Technique on Lung Dose and Dose to Opposite Breast.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06317-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Toxicologic and Pharmacokinetic Studies of Misonidazole

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schwade, M.D.	Acting Head, Radiobiology Sec	ROB	NCI
Other:	E. Glatstein, M.D.	Chief	ROB	NCI
	Other staff of the Radiation Oncology Branch			

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to develop more effective radiation sensitizers, as well as more intelligently utilize those currently available, information must be gained regarding the toxicity of current compounds as well as their pharmacology. With regards to the latter point, studies regarding the modification of the pharmacology may lead to the ability to use the currently available compounds, most notably misonidaozle, with less toxicity.

PHS-6040 (Rev. 2-81)

Project Description:

Objectives: Pharmacokinetic parameters, in plasma, normal tissues and tumors is to be studied.

Methods Employed: Patients have received twice weekly doses of misonidazole intravenously noted to determine base line pharmacokinetic parameters and toxicity.

Major Findings: Elucidation of the pharmacokinetic parameters of misonidazole in the intravenous form have been determined, as well as maximum tolerated dose in a twice weekly fractionation (1.5 gm/m^2 2X/wk X 5 wks).

Significance to the Research Program of the Institute: Radiosensitizing drugs may allow more effective use of an existing modality of treatment, radiation therapy, by augmenting the effects of radiation killing in a select population of tumor cells, while having little effect on normal tissue. The Radiation Oncology Branch is the only group in the country currently investigating misonidazole in an intravenous formulation.

Proposed Course: Phase III studies in esophogeal carcinoma and osteosarcoma using irradiation and misonidazole IV. Continue current pharmacokinetic studies involving other radiosentizers.

Publications:

Strong, J.M., Schwade, J.G., Shoemaker, D. and Gangji, D.: Misonidazole dose and tumor level relationship: effects of individual variation on rate of misonidazole metabolism and absorption from the gastrointestinal tract. (Presented at CROS Radiosensitizer/Radioprotector Conference, Key Biscayne, October, 1979, manuscript in press) Clinical Trials. Jan, 1981.

Schwade, J.G., Strong, J.M. and Gangji, D.: IV misonidaozle (NSC 261037)--report of clinical experience. Clinical Trials, January, 1981

Schwade, J.G., Strong, J.M., Rowland, J., Kelley, P., Makuch, R., Tepper, J.E., Lichter, A.S., Glatstein, G.: Phase I Study of Intravenous Misonidazole (NSC 261037) submitted to Cancer Treatment Review, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06318-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Locally Unresectable Osteogenic Sarcomas and Chondrosarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James G. Schwade, M.D.	Acting Head, Radiobiology	ROB	NCI
Other:	Eli Glatstein, M.D.	Chief,	ROB	NCI
	Joel E. Tepper, M.D.	Senior Investigator	ROB	NCI
	Allen S. Lichter, M.D.	Head, Clin Rad Ther Sec	ROB	NCI
	Tim Kinsella, M.D.	Senior Investigator	ROB	NCI

COOPERATING UNITS (if any)

Surgery Branch, DCT, NCI

LABORATORY

Radiation Oncology Branch

SECTION

Clinical Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

ABSTRACT OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the hypoxic cell radiosensitizer misonidazole in conjunction with radiation therapy in the treatment of locally unresectable osteogenic sarcoma and chondrosarcoma. Patients who have a local neoplasm for which no radical surgery can be successfully undertaken will be treated with fractionation radiation therapy in conjunction with intravenously administered misonidazole. Because of the small number of patients who are appropriate for such a study, this will be a one armed study. Following completion of radiation therapy, combination chemotherapy will be administered to patients with osteogenic sarcoma with high grade neoplasms. Following completion of all treatment, survival, freedom from relapse, and complications of treatment will be carefully assessed.

PHS-6040
(Rev. 3-81)

Project Description:

Objectives: By assessing the survival, freedom from relapse, and complications of radiation therapy with intravenously administered misonidazole, we hope to be able to determine whether or not such treatment is appropriate for these neoplasms, which have here before been considered "radioresistant". Should the study prove that such treatment is effective, then such treatment could be contemplated for other osteogenic sarcoma patients.

Methods Employed: Patients with locally unresectable osteogenic sarcoma or chondrosarcoma, without overt metastatic disease, will be treated with radiation therapy in conjunction with intravenously administered misonidazole.

Major Findings: Seven patients have been entered on the study. In three local regrowth of tumor has not yet been seen. Tumor mass has not overtly regressed, but the bony matrix in all 3 patients appear to increase density. One patient remains apparently free of all disease. Another patient has had dissemination; this patient underwent an attempted "debulking" re-section prior to radiation therapy. The third patient remains clinically free of disease, but has suffered radiation injury to the bowel and ureter. Four other patients have been treated, 2 with brief response, 1 with no response, and 1 dying of intercurrent causes prior to correlation of therapy.

Significance to the Research Program of the Institute: Radiation sensitizing compounds represent a new avenue of investigation. These compounds are electron affinic and appear to mimic the effects of oxygen, and therefore appear to have the specific ability to make hypoxic cells within a tumor more responsive to radiation killing. Thus, these compounds take a known effect (e.e., radiation killing) and appear to be able to magnify it. If these compounds prove to be effective, then the need to invest large sums of money in research directions such as high L.E.T. radiation may be circumvented.

Proposed Course: Patient accrual is underway.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06319-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The use of prematurely condensed chromosomes (PCC) in the biological dosimetry of ionizing radiation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI
Other:	Nirmolini Soares, M.S.	Biologist	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine if the use of premature chromosome condensation (PCC) technique will improve the resolution of the lymphocyte biological dosimeter system for low total doses of radiation (<10 rad). With the PCC technique, chromosomal damage (gross breaks in chromosomes) of interphase cells can be studied immediately following radiation exposure. Assays will be made before the cells have had time to repair many of the initial breaks, thereby increasing the number of breaks counted as opposed to counting aberrations conventionally 24-48 hours after exposure in metaphase I and II.

Project Description:

Objectives: By scoring radiation damage in chromosomes (gross breaks) immediately following the exposure, we will construct radiation dose response curves which may provide greater resolution in the low dose region (1-10 rad). If this technique does provide greater resolution to the lymphocyte biological dosimeter system, then the determination of small radiation doses to accidentally exposed persons could be done with a considerable amount of confidence.

Methods Employed: Blood lymphocytes will be irradiated with graded doses of gamma photons and fused immediately with mitotic inducer cells. Slight modifications of the Rao and Johnson PCC technique will be used. Gross breaks in whole G1 PCC's chromosomes will be scored.

Major Findings: The study is in preliminary stages and the results are presently not available for assessment.

Significance to the Research Program of the Institute: Determination of low doses of radiation received by persons accidently exposed to radiation has been an issue of concern over the past 30 years. More precise methods of accessing low doses of radiation would be of value not only for accidental diagnostic exposure but also for environmental exposures to the general population.

In addition, these studies should provide better understanding as to the nature of chromosome breakage and repair.

Proposed Course: Project is currently underway.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06320-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Response of mammalian cells exposed to chemotherapeutic drugs and continuous radiation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI
Other:	Nirmolini Soares, M.S.	Biologist	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

ROB

SECTION

Radiobiology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

XX

SUMMARY OF WORK (200 words or less - underline keywords)

Cell killing and cell cycle kinetics will be studied for combinations of various chemotherapeutic drugs and continuous low dose rate radiation (5-300 rad/h). In addition, the morphological changes of cells exposed to these combinations of treatment will be documented by time lapse photography.

Project Description:

Objectives: The objectives of this project are to determine if there are combinations of continuous low dose rate radiation and chemotherapeutic drugs that will provide more cell killing to tumor cells (in vitro tumor cell lines) than to normal tissue cell lines.

Methods Employed: In vitro cell cultures will be exposed to the various agents mentioned above and assayed for cellular reproductive integrity using conventional tissue culture techniques.

Major Findings: The project is in the early stages of development. The cobalt 60 unit for continuous irradiation was installed in April, 1981. As soon as dosimetry has been completed, this project will start.

Significance to the Research Program of the Institute: These studies should provide a better understanding of interactions between radiation and drugs, which might be of value to clinical radiotherapy.

Proposed Course: Initiate preliminary experiments, calibrate cobalt-60 unit.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Dose-rate effects on aerated and hypoxic cells grown in culture.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James B. Mitchell, PH.D.	Radiation Biologist	ROB	NCI
Other:	Nirmolini Soares, M.S.	Biologist	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (300 words or less - underline keywords)

The purpose of this project is to study the effects of ionizing radiation with respect to cell killing and cell cycle perturbations to cells grown in culture. Particular emphasis will be placed on studying the response of cells to varying dose rates of radiation under both aerated and hypoxic culture conditions. Both continuous and fractionated irradiation schedules will be studied.

Project Description:

Objectives: The objective of the proposed project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the interrelationships of factors which influence radiosensitivity, with an emphasis on their implications for clinical radiotherapy.

Methods Employed: In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to various dose rates of radiation either under aerated or hypoxic conditions. Oxygen enhancement ratios (OER) will be determined.

Major Findings: The project is in the early stages of development. Cobalt-60 unit just acquired and we are in process of calibrating the unit.

Significance to the Research Program of the Institute: These studies should provide a better understanding of the effects of dose rate/fractionation on the OER. Since there is a good deal of information that indicates that hypoxic cells in tumors represent a problem for radiotherapy, these studies could lead to more efficient methods of sterilizing hypoxic cells.

Proposed Course: Basic hypoxic cell systems have been developed. As soon as Cobalt 60 unit is calibrated, project will begin.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06324-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protection of Radiation Injury in Normal Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI
Other:	Thomas N. Padikal, Ph.D.	Sr. Investigator	ROB	NCI
	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI
	David Brightwell	Stride Intern	ROB	NCI
	Daniel White	Stay in School	ROB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XX ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of sulfhydryl compounds have been developed by Walter Reed Army Institute, which protect normal tissues against radiation injury. WR 2721, one drug in this series, gives a dose modifying factor (i.e., ratio of radiation doses that give the same level of biological damage with the drug as without the drug) ranging from 1.2 to 2.5 depending on the normal tissue tested. Although this drug protects some murine tumors against radiation, the DMF in tumors is smaller than in tissues. This drug is now being used in clinical trials in radiation therapy. The purpose of this work is to: 1. study the ability of this drug to protect a rapidly proliferating and slowly proliferating tissue, i.e., skin and lung respectively, in mice; 2. to determine optimum timing of drug administration; 3. to determine if WR 2721 protects at small doses/fx in the clinical range; 4. to determine if there is a higher DMF for late than for early damage in mouse lung.

PHS-6040

Project Description:

Objectives: To assess the ability of sulfhydryl compounds to protect against radiation injury in skin and lung of mice; to determine the time of protection after various routes of administration; to determine if any of the drugs protect tumors.

Methods Employed:

Skin: One foot of a mouse is irradiated in a specially constructed irradiation jig. No anaesthetics are used for the procedure. Damage is assayed by quantifying the erythema, alopecia, dry and wet desquamation.

Lung: Both lungs of a mouse are irradiated in a specially constructed jig in which the rest of the body is shielded. No anaesthetic is used for the irradiation. Lung damage is assayed using a plethysmographic technique. (Travis et al., Brit. J. Radiol. 1979)

Major Findings: 1. Timing and route of administration experiments for skin protection have been completed. We have found that the DMF rises faster and to slightly higher values after IV than after IP administration of WR 2721 and 30 to 75 minutes is the optimum time between drug administration and irradiation to get the highest DMF. Paper is in progress.
2. The experiments on DMF after small doses/fx in lung are in progress, but data is not yet available.
3. There is a higher DMF for late lung damagee (1.5) then for early lung damage (1.3) measured by 2 assays, breathing rate and death. This experiment is being repeated.

Significance to the Research Program of the Institute: Radioprotectors may offer a means of improving the therapeutic ratio in radiotherapy.

Proposed Course: To be continued.

Publications: In progress

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06325-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

In vivo radiobiology.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI
Others:	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI
	Margaret Weston	Biologist	ROB	NCI
	David Brightwell	Stride Intern	ROB	NCI
	Danny White	Stay In School	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to establish an in vivo radiobiology research program. The major efforts of the research projects are directed towards radiobiology as it applies to clinical radiotherapy and towards basic radiobiology. The major emphasis of this program is on normal tissue injury after radiation and/or drugs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06326-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Early and late radiation damage in mouse lung.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI
Other:	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI
			ROB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Radiation causes two distinct histological changes in the mouse lung which occur at clearly separated times; radiation pneumonitis at 4-5 months after treatment (early damage) and fibrosis at 9 months post treatment (late damage). It is possible to dissociate these 2 types of damage, i.e., cause little early reaction while inducing a severe late reaction. This suggests that 2 different cell types or processes might be responsible for the 2 types of damage. It is the purpose of this study to define the conditions under which early and late damage can be dissociated, to define the radiobiological characteristics of the 2 types of damage, and define those cells which are involved in each type of damage. Answers to these questions may enable us to reduce late damage in the lung.

Project Description:

Objections: To define the conditions under which early and late damage in the lung can be dissociated; to define the radiobiological characteristics of early and late damage, to define the cells involved in each type of damage.

Methods Employed: The whole thorax of mice is irradiated with X-rays using a specially constructed jig which shields the rest of the body. Thus, only the lung injury is expressed. The procedure requires no anaesthetic. Fractionated doses of X-rays and low dose rate radiation will be used. Early and late lung damage is assayed by changes in breathing rate. Histological changes are scored and autoradiography will be used to look for proliferation of cells in the lung.

Major Findings: Experiments involving small doses given daily have been initiated and are in progress. Because the lung takes 6-12 months to exhibit both types of injury, no data is available at this time. Split dose experiments to measure the effect of time between doses (1 day to 56 days) have been started, but no data is available.

Significance to the Program of the Institute: Normal tissues are the dose limiting factor in radiotherapy, late fibrosis in the lung being a critical reaction. It is important that those conditions which can result in severe late fibrosis in the lung be defined and that conditions in which early reactions do not predict for late fibrosis be identified. Identification of the cell types involved in late damage may enable us to reduce this type of damage.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06327-02 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Normal tissue protection from drug induced injury.

NAMES, LABORATORY AND INSTITUTE AFFILIATION, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI
Other:	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiobiology

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sulfhydryl compounds, a class of radioprotectors, also protect against nitrogen mustard gas. Therefore, these compounds may be useful in protecting against chemotherapeutic drugs whose action is mainly alkylation. The purpose of this work is to study the ability of WR 2721 to protect critical normal tissues against damage from chemotherapeutic agents.

Project Description:

Objectives: To determine the ability of WR 2721 to reduce injury in the lungs and teeth of mice after administration of cyclophosphamide.

Methods Employed: Groups of mice will be given the protective drug at various times before administration of a range of cyclophosphamide doses. Other groups of mice will be given cyclophosphamide alone. Lung damage will be assayed in both groups of mice by measuring breathing rate. A system for scoring tooth deformities is being developed.

Major Findings: 1. WR 2721 protects against CTX bone marrow toxicity when equally given before or after CTX. However, better protection of CTX induced lung injury is achieved if WR 2721 is given after CTX, either as a single dose or as fractionated doses, thus keeping serum protection levels elevated. The corollary experiments in a tumor currently are in progress. 2. WR 2721 does not significantly protect against tooth injury in mice caused by CTX.

Significance to the Research Program of the Institute: Normal tissue toxicity is a limiting factor in the treatment of malignant diseases with chemotherapeutic agents. If radioprotectors reduce the damage to critical normal tissues such as lung, bone marrow, and gut, then the therapeutic ratio will be increased, i.e., there will be less morbidity.

Proposed Course: To be continued. A range of radioprotectors will be tested against a range of chemotherapeutic drugs. Various routes of administration will be tested for both drugs to determine the route which gives the best protection.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06328-01 RO

PERIOD COVERED
October 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (60 characters or less)

Field Configuration in Definitive Radiotherapy of the Intact Breast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas N. Padikal	Senior Staff Fellow	ROB	NCI
Others: A. S. Lichter	Head, Clin. Rad. Ther. Section	ROB	NCI
F. Harrington	Engin. Tec.	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda Maryland

TOTAL MANYEARS: 0.2 | PROFESSIONAL: 0.15 | OTHER: 0.05

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work has resulted in the development and implementation of a new irradiation technique to produce in a more reliable fashion a uniform dose distribution in the breast tissue and the supraclavicular area. The necessary numerical data for routine application are obtained by using a specially-developed computer progam.

Project Description:

Objective: To develop and implement a field arrangement for treatment of cancer of the breast. It is necessary to achieve a uniform dose across the entire treatment volume, while minimizing the dose to adjacent critical structures.

Methods: Extensive experimental work has demonstrated that a new method can be applied to generate a uniform matching of the supraclavicular field with the tangential breast fields.

Major Findings: This method is clinically applicable.

Significance to Biomedical Research and the Program of the Institute:
This work makes adequate treatment of all breast tissue routinely possible.

Proposed Course: Completed.

Publications: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Radiation Physics Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. van de Geijn	Head, Rad. Phys. and Comp. Auto. Section	ROB	NCI
Other:	P. L Roberson	Staff Fellow	ROB	NCI
	F. Harrington	Engin. Tech.	ROB	NCI
	V. Iler	Ther. Rad. Tec.(Dos.)	ROB	NCI
	B. A. Fraass	Staff Fellow	ROB	NCI
	T. N. Padikal	Senior Staff Fellow	ROB	NCI
	R. W. Miller	Commissioned Officer	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS: 3.7 | PROFESSIONAL: 2.0 | OTHER: 1.7

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The section provides expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning and the design and development of special equipment tailored to special clinical needs.

PHS-6040 (Rev. 9-81)

Objectives: To ensure high quality physics support for radiotherapy.

Methods employed: A new, efficiently graded system has been developed and implemented for monitoring the performance of the two linear accelerators, the simulator and the CT scanner. Special mechanical supports and measuring devices were developed to quantify the position of patients and to improve the reproducibility of daily patient setting-up. The data acquisition for treatment planning has been simplified and improved. A new method for computer-assisted treatment planning has been introduced. Considerable efforts have been invested in the dosimetry of intra-operative, totalbody and total-skin radiotherapy.

Major Findings: The introduction of beam monitoring jigs enables daily monitoring of output, beam flatness and symmetry and alignment of light field and X-ray fields for both linear accelerators. The method allows simple documentation of performance. The dosimetry of photon beam total body irradiation as well as that of total skin electron beam irradiation for Mycosis Fungoides requires further attention. Much attention had to be spent on TBI and MF dosimetry.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important improvement is the capability to image the effects of irregular shielding blocks which is of essential interest in the treatment of soft-tissue sarcomas, and cancers of the esophagus.

Significance to Biomedical Research and the Program of the Institute: The improvements in quality assurance, patient positioning and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies.

Proposed Course Continuation of further development of means and methods to improve the physical and technological basis of radiotherapy.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06330-01 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Extension of a 3-D Dose Field Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Johannes van de Geijn	Head, Rad. Phys. and Comp. Auto. Sect.	ROB	NCI
Cheng Po Cheng	Visiting Student	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	.3	1.0

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES XX (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams of the most general characteristics is of vital importance in radiotherapy. Conceptually, this model takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field" and mathematical expressions to describe the effect of variation of field size, depth and focal distance. This concept is applied to the effects of beam-modifying devices as well. The approach is attractive from a theoretical as well as a practical point of view. The current investigations concern the generalization for irregular fields modified by irregular blocks, both for photon beams and electron beams.

Objective: To develop a unified calculative model for the description of absorbed dose produced by beams of ionizing radiation, as a basis for computer-assisted treatment planning.

Methods employed

1) The variation of relative absorbed dose along the central ray with depth, field size and source surface distance (SSD) has been studied using published and locally-measured data. Mathematical representations have been established.

2) The variation of the relative absorbed dose across the beam has been studied as a function of field size, depth and SSD, for many radiation qualities, for both photons, electrons and neutrons. Mathematical representations for these variations have been established.

3) Over the present reporting period, special attention has been paid to verification of the model for the local radiation machines and to extension of the model to irregular fields modified by irregular blocks.

Major Findings: It has been found that the concept applies very well to the local facilities for regular rectangular beams including the use of wedges.

It has been established that the concept is applicable to irregular fields as well. Preliminary results for electron beams are most promising. The validity for neutron beams has been confirmed by investigators at Fermilab.

Many of these results have been incorporated in a clinical treatment planning system.

Significance to Biomedical Reserch and the Program of the Institue: The range of validity of the Dose Field Model determines the potential range of applicability of the clinical treatment planning program. In turn, the latter determines the degree of refinement in radiation treatment that can be scientifically documented.

Proposed Course: Continuation, with emphasis on electron beams.

Publications: none in this reporting period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06331-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Computer-assisted 3-D Radiation Treatment Planning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Johannes van de Geijn	Head, Rad. Phys. and Comp. Auto. Section	ROB	NCI
other:	Hal A. Fredrickson	Comp. Systems Analyst	DCRT	NCI
	Marie Chang	Comp. Specialist	ROB	NCI
	Daniel Syed	Head, CSL	ROB	NCI
	Cheng PoCheng	Visiting Student	ROB	NCI

COOPERATING UNITS (if any)

Computer Systems Lab

LABORATORY

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTION AND LOCATION

National Cancer Institute, Bethesda Maryland

TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.8 | OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES X (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is the development and clinical implementation of a generalized system for external beam treatment planning. It will enable the optimum utilization of existing treatment facilities. The system is based on a generalized 3-D dose field model which covers photon and electron as well as neutron beams. The computer program and most of its clinical implementation has been completed for the photon and electron fields available from the local 6 MV and 12 MV linear accelerators. The current capabilities include interactive simulation of most irradiation techniques, including the effect of most beam modifying devices. The system enables the display of dose distributions computed in several transverse contours and overlaid on corresponding CT scans.

PHS-0040

Project Description

Objectives: To develop and implement a generalized system for computer-assisted radiation treatment simulation.

Methods employed: The dose field model, developed elsewhere by the present PI, was further developed and experimentally tested for the local radiation facilities. The theoretical model was extended to cover irregularly-shaped beams as well as irregularly-shaped shielding blocks.

The associated computer program was also adapted for the local PDP-11/70 system, with expert assistance from the Computer Systems Laboratory. Great emphasis has been placed on optimization of interactive operational facilities and accommodation of input and hardcopy techniques to clinical demands.

A graphical input system, an option for introducing CT images in addition to or instead of mechanically-obtained patient contours, an interactive system for the variation of input parameters and a DEANZA color display system have been implemented.

Major findings: The system is now in routine use for clinical treatment planning. In comparison to other existing systems, it offers high speed computation and display of complete dose distributions in multiple slices, superimposed on CT images, including effects of wedge filters, irregular shielding blocks and diaphragm rotation. Several modes of display are available. The wide applicability of the Dose Field Model has been demonstrated at Fermilab, where it is applied for neutron beam therapy.

Significance to Biomedical Research and the Program of the Institute:
The convenient interactive manipulation of key beam parameters in combination with fast response is highly valuable in the complicated dosimetry problems encountered in special protocol studies.

Proposed Course

- Implementation of the Dose Field Model for regular and irregular electron fields

- Establishment of a "Slave Monitor System" to enable the display and limited modification of treatment plans during the daily Patient Conferences.

- Extension of the capabilities to compute and display dose distributions in sagittal and coronal sections of the patient.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06332-01 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (60 characters or less)

Clinical Use of a Match-line Wedge For Radiation Field Matching

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Fraass	Staff Fellow	ROB	NCI
Other:	J. E. Tepper	Civil Servant	ROB	NCI
	J. van de Geijn	Head, Rad. Phys. and Comp. Auto. Section	ROB	NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key words)

The purpose of this project is to develop a clinically useful method of matching adjoining megavoltage radiation fields so that the dose distribution through the match region is uniform. A "match-line wedge" has been developed which satisfies the above requirement. Simplicity of use will assure that the wedge will be effective clinically.

Project Description:

Objectives: To find a means of modifying the edges of adjoining radiation fields so that the dose distribution throughout the match region is uniform.

Method Employed: A match-line wedge has been desien designed so that a wide pseudo-penumbra is created when the wedge is placed in the edge of the radiation beam. This wide pseudo-penumbra makes the dose distribution in the match region less sensitive to setup errors and more uniform than is possible with normal matching methods when adjacent fields are matched. The design, mounting, simulation, setup, and treatment techniques have been developed so that use of the wedge is safe, useful, and simple. The dose distributions which result from variation of different parameters in the system have been studied in detail.

Major Findings: Use of the match-line wedge results in uniform dose distributions in the match region between adjacent radiation fields.

Significance to Biomedical Research and the Program of the Insititute:
Use of this device improves the uniformity of dose received by patients who are treated with matching fields, thereby improving the accuracy of treatment.

Proposed Course: Implementation of the match-line wedge will continue, until it is in routine clinical use. Use of the wedge with a wider range of treatment techniques will be explored.

Publications: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06333-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Dosimetry of Total Skin Electron Irradiation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.A. Fraass	Staff Fellow	ROB	NCI
Other:	P.L. Roberson	Staff Fellow	ROB	NCI
	V. Iler	Rad. Ther. Tec. (Dos.)	ROB	NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Compuer Automation Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.4	.1

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ( ) (b) HUMAN TISSUES ( ) (c) NEITHER

( ) (a1) MINORS ( ) (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A detailed study has been made of the dosimetry of total skin electron irradiation. This study has quantified and improved the whole skin treatments received by patients with mycosis fungoides.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To quantify the variation of dose to all parts of the body for patients receiving whole skin irradiation.

Methods Employed: The dosimetry system of the MeV XII accelerator has been substantially improved, allowing more precise definition of the total skin dose given. The absolute dose has been calibrated using various ionization chambers and thermoluminescent dosimeters (TLD). Extensive TLD measurements have been made on five patients. These measurements have made possible the mapping of the dose distribution over the whole body, and also have shown the daily variations and patient-to-patient variations which are possible with this treatment technique.

Major Findings: The dose to the skin is fairly uniform over the trunk, but the distribution of dose to legs, arms, and head is significantly different. Daily and patient-to-patient variations in dose are not overly significant.

Significance to Biomedical Research and the Program of the Institute: This work makes adequate treatment for mycosis fungoides possible with the whole skin irradiation technique.

Proposed Course: More work toward improving the absolute dosimetry is contemplated. Also necessary is the improvement of methods to assure uniform dose to the whole skin.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06334-01 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Dose to Gonads from Radiation Treatments for Lymphomas and Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	B. A. Fraass	Staff Fellow	ROB	NCI
Other:	T. Kinsella	Medical Officer	ROB	NCI
	V. Iler	Ther. Rad. Tec. (Dos.)	ROB	NCI
	R. Shering	Medical Officer	Endocrine Branch	NCI

COOPERATING UNITS (if any)

Endocrine Branch

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.1	.05

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS ( ) (b) HUMAN TISSUES ( ) (c) NEITHER

( ) (a1) MINORS ( ) (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Doses to gonads have been measured on patients who are irradiated as part of their treatment for lymphomas or sarcomas. Ion chamber and thermoluminescent dosimetry (TLD) measurements have been made to verify the measurements on patients.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To accurately determine the gonadal doses received by lymphoma and sarcoma patients who are treated with radiation.

Methods Employed: Thermoluminescent dosimetry (TLD) measurements have been made to determine the dose to testes and ovaries of patients treated with mantle, para-aortic, pelvic and leg radiation fields. Extensive ion chamber and TLD measurements have been made to verify the validity of the TLD measurements on patients.

Major findings: Gonadal doses can now be calculated retrospectively, as long as there is adequate information about radiation field and patient geometry. It may be possible to develop gonadal shielding useful for the above categories of patients.

Significance to Biomedical Research and the Program of the Institute: Gonadal doses will be correlated with fertility and hormonal function tests obtained by the Endocrine branch. The results will quantify the effects of radiation on fertility. Shielding which reduces the complications of these radiation treatments is clearly of major importance to the patients.

Proposed Course: The patient TLD and verification measurements will continue. Doses received by previously-treated patients will be calculated. Fertility and hormonal function test results will be correlated with gonadal doses. A useful gonadal shield will be developed.

Publication: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U. . DEPARTMENT OF HEALTH AND HUMAN S RVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06335-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comprehensive Quality Assurance Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. L. Roberson	Staff Fellow	ROB	NCI
Other:	J. van de Geijn	Head, Rad. Phys. and Comp. Auto. Section	ROB	NCI
	A. S. Lichter	Head, Clin. Rad. Ther. Section	ROB	NCI
	B. A. Fraass	Staff Fellow	ROB	NCI
	B. A. Kelley	Chief Ther. Tec.	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.15	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Summary: A comprehensive program that assures the correct operation and quality of the whole treatment system has been developed. The linear accelerators, simulator, and computer treatment planning system are all subjected to regular quality assurance checks.

Project Description:

Objectives: To develop quality assurance (QA) procedures that apply to each area of activities involved with patient treatment.

Methods Employed: Linear accelerator quality assurance checks are made each morning before treatment begins. Biweekly, quarterly, and annual checks are performed by the physics staff. Several devices have been fabricated which assure the physical stability of the QA test equipment. The simulator is subjected to a daily check. A basic set of data has been identified which is used to certify the correct operation of the computer treatment planning system. All dosimetric calculations are checked by computer before any treatment, preventing arithmetical mistakes.

Major Findings: A simple and effective Q.A. program has been instituted. Current machine operation is checked daily. Deviations from normal operation are found and corrected before patient care is affected.

Significance to Biomedical Research and the Program of the Institute: The Q.A. system is essential to the correct treatment of all radiation therapy patients.

Proposed Course: The CT scanner will be included in the Q.A. system. Attempts will be made to streamline some Q.A. checks, and to expand others. Continued effort will be devoted to expanding the scope of the Q.A. program.

Publications: None

INTRAMURAL SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM 06337-01 RO

PERIOD COVERED

October 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (80 characters or less)

Real-Time Radiotherapy Treatment Monitor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Fraass	Staff Fellow	ROB	NCI
Other:	T. N. Padikal	Senior Staff Fellow	ROB	NCI
	P. L. Roberson	Staff Fellow	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES X (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

ABSTRACT OF WORK (200 words or less - description in layman's terms)

The purpose of the project is to develop a real-time monitor for radiation treatments. Although routine quality assurance is the immediate aim, continued development will make feasible many projects which rely on real-time patient dose monitoring.

HHS-6040
(Rev. 2-81)

Project Description:

Objectives: To design and develop a real-time monitoring device for use with radiotherapy.

Methods Employed: A two-dimensional array of radiation-sensing diodes is used to monitor the radiation which is transmitted through the patient during a treatment. From knowledge of the transmitted intensity, patient dose information is obtained. A microcomputer - based system is used to accumulate and analyze the data from the diode array.

Major Findings: Initial hardware interfacing, computer programming, and diode selection have been accomplished. The system has been tested and found to be promising. Optimization of the many factors affecting system performance is now underway.

Significance to Biomedical Research and the Program of the Institute: This project is expected to improve the quality of radiotherapy in the Radiation Oncology Branch. Further development will lead to innovative and more precise types of treatments.

Proposed Course: To be continued. The present data acquisition system will be developed and refined. Design of the system to be used for automatic monitoring and recording of patient treatments will proceed. Investigation into the use of the diode array system for beam symmetry, quality, and calibration checks, compensating filter design, dynamic radiotherapy treatments, quality control, and real-time treatment analysis will continue.

Publication: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06338-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (60 characters or less)

Intraoperative Irradiation of Canine Retroperitoneum

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joel E. Tepper, M.D.	Senior Investigator, ROB, NCI
	William Sindelar, M.D.	Senior Investigator, SB, NCI
OTHER:	Elizabeth Travis, Ph.D.	Senior Investigator, ROB, NCI
	Thomas Padikal, Ph.D.	Senior Investigaror, ROB, NCI

COOPERATING UNITS (if any)

Surgery Branch, NCI
Armed Forces Radiobiologic Research Institute

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are presently exploring the use of intra-operative radiation therapy as a method of treating patients with abdominal and retroperitoneal tumors which have a low cure rate by conventional means. Although we have some information from previous clinical data on the tolerance of normal retroperitoneal structures to doses of intra-operative radiation therapy of approximately 2000 rads, full information is not available as to effects at high dose levels and the long term toxicity which may be expected. We have therefore proceeded on a series of experiments to evaluate the effect of intra-operative irradiation using canines as the experimental model. Dogs receive an exploratory laparotomy and then receive high dose radiation therapy to normal retroperitoneal structures. Toxicity has been evaluated at times up to 2 years after irradiation. Present results indicate that doses up to 3000 rads are acceptable with minimal toxicity but doses of 4500 rads may produce unacceptable toxicity. Toxicity has been specifically noted in intestines, ureter and kidney. We have not noted significant problems in large abdominal blood vessels or in the retroperitoneal soft tissue.

Project Description:

Objectives: By assessing the toxicity of intra-operative radiation therapy on canines, we hope to be able to evaluate the types of toxicities which we will see in clinical studies and also to try and determine what is likely to be a maximal acceptable dose level in humans. We also hope to be able to identify structures which would likely be more sensitive to intra-operative radiation therapy and which thus should be avoided in clinical studies. Present data indicate that modest levels of intra-operative radiation therapy are tolerable in humans, but detailed toxicity information is not available from present human studies.

Methods Employed: Full grown canines have exploratory laparotomy performed. While the abdomen is exposed the normal retroperitoneum including large abdominal blood vessels are irradiated with various doses of radiation from 2000-5000 rads. Animals are then followed long term for evidence of toxicity.

Major Findings: We presently have data at times up to 2 years following radiation therapy. We have seen minimal toxicity at doses of 2000 and 3000 rads to normal retroperitoneum. Doses of 4500 rads may produce unacceptable toxicity in the retroperitoneal structure. We have also noted significant toxicity in hollow visci such as small bowel, large bowel and ureter. We have also noted marked destruction of renal parenchyma when that structure is irradiated to high dose.

Significance to the Research Program of the Institute: There is presently a significant amount of interest in the use of intra-operative radiation therapy for patients with various abdominal and retroperitoneal malignancies. This interest exists because intra-operative radiation allows us the potential ability to irradiate to high dose a significant volumes of the retroperitoneum, while minimizing dose to sensitive normal abdominal structures such as small and large intestine, kidneys and ureters. If a higher effective biological dose can be delivered to the treatment volume, we may then be able to increase the cure rate of abdominal malignancies which presently have an extremly low cure rate.

Proposed Course: The present irradiation has been suspended pending the results on the animals who have already been irradiated. We will continue to follow the irradiated animals long term to determine any late toxicity.

Publications: Tepper, J., Sindelar, W., Travis, E., et al. Tolerance of Canine Anastomoses in Retroperitoneal Structures to Intra-operative Radiation Therapy. International Journal of Radiation Oncology, Biology and Physics, Vol. 6: 1362, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06339-01RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Total Body Radiation Therapy for Ewing's Sarcoma and Rhabdomyosarcoma.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joel Tepper, M.D.	Senior Investigator, ROB, NCI
OTHER:	Daniel Glaubiger, M.D.	Senior Investigator, POB, NCI
	Phillip Pizzo, M.D.	Senior Investigator, POB, NCI
	Timothy Kinsella, M.D.	Senior Investigator, ROB, NCI
	Albert Deisseroth, M.D.	Senior Investigator, POB, NCI
	Eli Glatstein, M.D.	Chief, ROB, NCI
	Arthur Levine, M.D.	Chief, POB, NCI

COOPERATING UNITS (if any)

Pediatric Oncology Branch

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/2	1/4	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although advances have been made in the treatment of pediatric Ewing's sarcoma and rhabdomyosarcoma, the cure rates in metastatic disease is still poor. Both of these tumors are very responsive to radiation therapy for the local disease. Because of the ability to limit the bone marrow toxicity with autologous bone marrow infusion, we are testing the efficacy of total body irradiation therapy as a systemic treatment modality for patients who have failed primary treatment in these diseases. We intend to assess the response rate and the duration of response in patients who have disease which has failed primary therapy. We will also evaluate the toxicity of high dose total body irradiation.

Project Description:

Objectives: By assessing the response rate and duration of response of patients with metastatic Ewing°s sarcoma and rhabdomyosarcoma we hope to be able to determine the relative efficacy of high dose total body irradiation therapy as a systemic treatment agent. If a high response rate is noted, we would then plan on proceeding to use high dose total body radiation therapy as part of the primary treatment modality combined with chemotherapy for patients presenting with poor prognostic features.

Methods Employed: All patients will have metastatic disease which have failed the conventional treatment modalities. Patients will be accepted with metastatic Ewing°s sarcoma or pediatric rhabdomyosarcoma. Patients will receive 400 rads TBI given on each of 2 days with 1 day separation. After the radiation therapy patients will receive autologous bone marrow re-infusion from bone marrow which was previously harvested and then frozen for storage.

Major Findings: The study has just begun and no significant results are available for assessment.

Significance to the Research Program of the Institute: Because of the ability with modern technology of limiting the effects of major bone marrow ablation, we now have the potential ability to salvage patients with bone marrow re-infusion from treatment which previously would have produced unacceptable hematopoetic toxicity. As these tumors are highly responsive to radiation therapy, TBI may prove to be an effective systemic treatment agent. We would then be able to incorporate high dose TBI in the primary treatment of patients who present with disease with bad prognostic features.

Proposed Course: Patient accrual will be continued until 14 patients have been accrued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06340-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Surgery and Intra-operative Radiation Therapy in Carcinoma of the Stomach

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joel E. Tepper, M.D.	Senior Investigator, ROB, NCI
	William Sindelar, M.D.	Senior Investigator, ROB, NCI
OTHER:	Eli Glatstein, M.D.	Chief, ROB, NCI
	Steven Rosenberg, M.D.	Chief, SB, NCI
	Richard Simon, Ph.D.	Chief, BRB, NCI
	Staff	ROB, NCI
	Staff	SB, NCI

COOPERATING UNITS (if any)

Surgery Branch, NCI
Biometrics Research Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1/2	1/4	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study intra-operative radiation therapy combined with radical surgical resection in controlling adenocarcinoma of the stomach. The plan is to take patients with carcinoma localized to the stomach and the regional lymph nodes and to treat all patients with a surgical resection. Patients will be randomized to receive either intra-operative radiation therapy which is delivered while the patient is still under general anesthesia and before the surgical incision is closed, versus receiving conventional post-operative irradiation. Patients will then be followed to determine the efficacy of this combined modality approach in improving the local control and thus the long term survival in patients with carcinoma of the stomach. Only one patient has been entered on the study to date, so no treatment results are as yet available.

Project Description:

Objectives: By assessing the survival, local recurrence rate, freedom from relapse, and toxicity after surgery, we hope to determine whether or not intra-operative radiation therapy when combined with radical surgery for carcinoma of the stomach is a more effective way of producing local control and long term disease free survival in this disease. We also hope to evaluate the toxicity of this combined modality approach to determine whether high dose intra-operative radiation therapy can be safely employed.

Methods Employed: Patients with previously untreated carcinoma of the stomach which is confined to the stomach and the local regional lymph nodes will be accepted on study. All patients will have full pre-operative evaluation. Patients who were thought to have tumor which is grossly resectable will then be randomized to receive either surgery combined with conventional external irradiation or surgery combined with intra-operative radiation therapy. Half of the patients will be randomized on each arm of the study. Intra-operative radiation therapy will be delivered immediately after the surgical resection and while the patient is still under general anesthesia and before the incision is closed. The hope is that with intra-operative radiation therapy we can decrease the amount of radiation delivered to the normal abdominal and retroperitoneal tissues and thus can deliver a higher dose to the tumor bed. Conventional irradiation will be delivered on a daily basis after the patient has convalesced from his surgical procedure. Patients will be followed for survival, evidence of local tumor recurrence, evidence of tumor recurrence outside of the radiation field, and treatment related toxicity.

Major Findings: Only one patient has been accepted on study and therefore no significant results are available.

Significance to the Research Program of the Institute: Although carcinoma of the stomach is decreasing in incidence, it is still a major cause of cancer mortality in the United States. Intra-operative radiation therapy represents a potentially valuable way to increase the dose to the area which is at risk for tumor involvement while minimizing the dose to the normal retroperitoneal and abdominal structures. This increased biological dose may thus allow us to increase the tumor control in this disease. If toxicity is minimal, we may also consider combining intra-operative radiation therapy with external beam or other chemotherapeutic agents.

Proposed Course: Patient accrual is continuing.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z0 1 CM 06341-01 RO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Intraoperative Radiation Therapy and Surgery for Pancreatic Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joel Tepper, M.D.	Senior Investigator, ROB, NCI
	William Sindelar, M.D.	Senior Investigator, SB, NCI
OTHER:	Eli Glatstein, M.D.	Chief, ROB, NCI
	Steven Rosenberg, M.D.	Chief, SB, NCI
	Richard Simon, Ph.D.	Chief, BRB, NCI
	Staff	ROB, NCI
	Staff	SB, NCI

COOPERATING UNITS (if any)

Surgery Branch, NCI
Biometrics Research Branch, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the efficacy of intra-operative radiation therapy combined with radical surgery in curing patients with adenocarcinoma of the pancreas. Patients are being treated who have disease which has not spread beyond the regional lymph nodes of the pancreas and who are thought to have a tumor which is grossly completely resectable. Patients are then randomized to receive surgery followed by conventional post-operative radiation therapy vs. surgery and radiation therapy delivered during the surgical procedure. With intra-operative therapy, we hope to be able to localize the area which is at risk for tumor involvement, and to irradiate the volume at risk while delivering minimal irradiation to the normal intra-abdominal and retroperitoneal tissues. By delivering high doses of radiation therapy by the intra-operative technique, we hope to improve the local control and thus to increase the cure rate. We will also be able to evaluate the toxicity of intra-operative radiation therapy when combined with radical surgery. To date, six patients have been treated on study and the toxicity of the intra-operative radiation therapy appears to be minimal.

Project Description:

Objectives: By assessing the survival, freedom from relapse, precentage of local recurrence, we hope to evaluate whether or not intra-operative radiation therapy is effective in augmenting surgical resection in patients with carcinoma of the pancreas. We also are assessing the toxicity which is caused by the combination of radical surgery and intra-operative radiation therapy or conventional radiation therapy in the treatment of this tumor.

Methods Employed: Patients with previously untreated carcinoma of the pancreas which is confined to the pancreas and to the regional lymph nodes without any evidence of distant metastatic disease receive a full pre-operative evaluation for extent of tumor. Patients are then randomized to receive intra-operative radiation therapy immediately following the surgical resection, and before the abdomen is closed. By this technique we hope to be able to move the normal abdominal and retroperitoneal structures out of the radiation field. After the completion of the intra-operative radiation therapy, the patient will be returned to the operating room where the surgical procedure will be completed. The rest of the patients will receive the surgical treatment alone to be followed in 1 to 2 months by post-operative radiation therapy in patients whose disease was not strictly limited to the pancreas.

Major Findings: The study has been in progress since May of 1980. Six patients have been accrued on study. At this point there does not appear to be significant toxicity related to the intra-operative radiation therapy or to the post-operative radiation therapy. Patients do have a prolonged post-operative recovery period regardless of whether or not intra-operative radiation therapy is employed. The study is too young to be able to make comments with regard to tumor sterilization and long term tumor cure.

Significance to the Research Program of the Institute: Intra-operative radiation therapy represents a potentially valuable way to deliver high radiation doses to the volume which is at risk for tumor recurrence while decreasing the potential morbidity from irradiation of abdominal structures. There is presently no treatment which has a high likelihood of success in treating this tumor which is presently the fourth largest cause of cancer death in the United States. Thus, if intra-operative radiation therapy proves to be a valuable adjuvant to surgery, and proves able to cure a significant percentage of patients with this disease, it will make a significant impact on cancer mortality.

Proposed Course: Patient accrual is continuing. Results will be evaluated at yearly intervals to determine if there is a survival advantage for either treatment arm. Toxicity will be evaluated routinely during the course of the study.

Publications: Tepper, J. and Sindelar, W.: Proceeding of the Conference on Intra-operative Radiation Therapy, Cancer Treatment Reports. To be published.

Tepper, J., Sindelar, W., Glatstein, E.: Phase I Study of Intraoperative Radiation Therapy Combined With Radical Surgery for Intra-abdominal Malignancies ASCO Proceedings, May 26-27, 1980; Vol. 21.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06342-01 RO

PERIOD COVERED

October 1, 1980 to September 1, 1981

TITLE OF PROJECT (80 characters or less)

Early and Late injury in mouse lung after neon peak irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Elizabeth L. Travis, Ph.D.	Cancer Expert	NCI ROB
	John F. Fowler, Ph.D.	Gray Laboratory	Northwood, England
	Stanley Curtis, Ph.D.	Lawrence Berkley Laboratory	Berkley, California
	Anne Marie De Luca, B.S.		NCI ROB

COOPERATING UNITS (if any)

Gray Laboratory, Northwood, England; Lawrence Berkely Laboratory, Berkely, CA

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heavy ions are being raised to treat some cancer patients. Some data is available on toxicity in a few normal tissues but only for early damage. Also, a potentiation of injury has been observed with fractionation of the neon beam in contrast to the well-known sparing effect obtained with fractionation of X-rays. This study will determine the repair or potentiation of both early and late damage in mouse lungs after fractionated doses of neon ions.

Project Description:

Objectives: To determine whether there is repair or potentiation of radiation damage in lungs of mice after fractionated doses of neon ions.

Methods Employed: Groups of mice were exposed to single doses, 4 fractions or 7 fractions of neon ions. Other groups of mice were exposed to single doses of 4 fractions or 7 fractions of X-rays. The assay of lung damage is breathing rate. Dose response curves are plotted and repair (or potentiation) determined at an isoeffect at 7 months and 12 months (corresponding to early and late damage respectively).

Major Findings: Data at 7 months indicated that there is no potentiation of early damage in mouse lung after 4 or 7 fractions of neon ions and that there is some repair between one and four fractions with no further repair between 4 and 7 fractions of neon ions. The repair values calculated from the X-ray data agree well with previous data, 50-60% of the dose is recovered between 400 and 600 rads/fraction.

Significance: The impact on radiotherapy would be to encourage the use of the neon ion beam to treat cancers which grow relatively fast, which are located close to slowly proliferating normal tissues.

Proposed Course: This experiment to be terminated between 12-18 months after initiation with a repeat experiment to be planned.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06343-01 RO

PERIOD COVERED

October 1, 1980 to Septmeber 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of the Radiosensitizer Bromodeoxyuridine (BUdR) NSC 38297

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: James G. Schwade, M.D. — Acting Head, Radiobiology Section ROB NCI
Others: E. Glatstein, M.D. — Chief, Radiation Oncology Branch NCI
S. Myers, M.D.
E. Quindlen, M.D.

COOPERATING UNITS (if any)

Surgical Neurology Branch NINCDS
Clinical Pharmacology Branch, COP, DCT, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Clinical Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bromodeoxyuridine is a radiation sensitizing drug which sensitizes rapidly dividing cells. Thymadine is replaced by the uridinedeoxyriboside compound predisposing DNA 2 single strand breaks. BUdR has been used in the past in head and neck lesions, osteosarcoma, and particulary in glioblastoma and other brain tumors, but was felt to need intra-arterial delivery to be effective.

Objectives: To assess the pharmacokinetics and toxicity with BUdR.

Methods Employed: Patients with brain metastases or primary malignant gliomas or glioblastoma are given BUdR intravenously for twelve hours every 24 hours. Patients with brain metastases are treated with 2000 rads in one week with a two week rest, followed by an additional 2000 rads in one week. BUdR is given on each day of treatment. Patients with malignant gliomas and glioblastoma are given BUdR for the first and last ten treatments of their 6000 rad/33 fraction schedule.

Major Findings: It would appear that in the seven patients currently evaluated, levels of BUdR comparable to those obtained with intra-arterial infusion can be obtained.

Significance of the Research Program to the Institute: The Radiation Oncology Branch has been deeply involved in the evaluation of new radiosensitizing compounds. BUdR represents a mode of radiosenitization different than the nitroimidazoles, a group of radiosensitizing compounds which sensitizes hypoxic cells. BUdR may be particulary useful in tumors which are dividing much more rapidly than the surrounding normal tissue, such as brain tumors and tumors in the lung.

Proposed Course: Patient accural continues

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06344-01 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Radiosensitizer Desmethylmisonidazole (NSC 261036)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James G. Schwade, M.D.	Acting Head, Radiobiology Section ROB	NCI
Others:	Eli Glatstein, M.D.	Chief, Radiation Oncology Branch	NCI
	J. Strong, Ph.D.		

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacy, DTP, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy Institute

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Radiation Oncology Branch is involved in the evaluation of numerous sensitizing compounds. Misonidazole has been evaluated by us in an intravenous form, and desmethylmisonidazole represents an improved compound. This compound is more hydrophilic, and has a shorter half-life, and will hopefully result in a significant decrease in neurotoxicity, the dose limiting toxicity seen with misonidazole.

Objectives: To assess the pharmacokinetics and toxicity of desmethylmisonidazole.

Methods Employed: Patients are given escalating doses of desmethylmisonidazole on a schedule prescribed in accordance with a national cooperative study being conducted by the Radiation Therapy Oncology Group.

Major Findings: Four patients have been entered from the Radiation Oncology Branch. The study has recently opened, and patient accural continues.

Significance of Research Program to the Institute: The Radiation Oncology Branch has been involved in the testing of radiosensitizing drugs, and this is a continuation of it's work in this very new and exciting field.

Proposed Course: Patient accural continues.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06345-01 RO

PERIOD COVERED
October 1, 1980 to Septmeber 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of radiosensitizer, Misonidazole (NSC 261037)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schwade, M.D.	Acting Head, Radiobiology Section ROB	NCI
Others:	E. Glatstein, M.D.	Chief, Radiation Oncology Branch	NCI
	J. Strong, Ph.D.		

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacy, DTP, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiation Therapy Institute

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacokinetic and toxicity studies have been performed on patients undergoing treatments with misonidazole, a nitroimidazole radiosensitizing agent. The studies have allowed better delineation of toxicity and pharmacology of these compounds, and was obtainable with the previously evaluated oral compounds.

Objectives: Assessment of the pharmacokinetics and toxicity of misonidazole.

Methods Employed: Patients receiving radiation therapy were treated with misonidazole in escalating doses twice weekly for five weeks.

Major Findings: Toxicity of misonidazole was found to correlate with area under the curve of concentration versus time. A dose of 1.5 gm/m^2 twice a week for five weeks was found to be a well-tolerated dose with only minimal peripheral neuropathy.

Significance of the Research Program to the Institute: Radiation sensitizing drugs are thought to increase the effectiveness of radiation by allowing an increased effect of radiation on hypoxic cells, while not effecting well-oxygenated cells. The Radiation Oncology Branch at NCI has been one of the leading groups involved in evaluating these very promising and innovative compounds.

Proposed Course: The Radiation Oncology Branch is currently evaluating other nitroimidazoles radiosensitizers, such as desmethylmisonidazole.

Publications: "I.V. Misonidazole (NSC 261037): Report of initial clinical experience" Cancer Clinical Trials

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06346-01 RO

PERIOD COVERED

October 1, 1980 - September 1, 1981

TITLE OF PROJECT (80 characters or less)

Proliferation in the lung after irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI
Others:	Margaret Ann Weston	Biologist	ROB	NCI
	Anne Marie De Luca	Biologist	ROB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

In vivo Radiobiology

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The extra dose needed to give an isoeffect in lung when doses are protracted over 14 days has been suggested to be due to a "slow repair" process rather than proliferation. Recent experiments with neutrons suggest that proliferation may be responsible for the dose sparing effect seen with protraction of time. The purpose of this project is to determine if proliferation occurs in the lungs between 1 and 14 days after irradiation.

Project Description

Objectives: To determine if proliferation occurs in the lungs between 1 and 14 days after irradiation.

Methods Employed: Groups of mice will be irradiated to the whole thorax. At 7 to 10 days after irradiation, one group of mice will be given a drug to arrest proliferating cells, and one group will be untreated. The lungs of both groups will then be irradiated with a second test dose of irradiation. Lung damage will be assayed by breathing rate.

Major Findings: This project has just begun and no data is available. Pilot experiments indicate that the arrest of proliferating cells can be accomplished with hydroxyurea.

Significance: Normal tissue toxicity is a limiting factor in radiation therapy. An understanding of the basic processes underlying the toxicity may lead to improved treatment by reducing normal tissue morbidity.

Proposed Course: To be continued

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06347-01 RO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

X-ray Sensitivity of Skin Fibroblast Cultures Ataxia Telangiectasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Timothy J. Kinsella, M.D.	Senior Investigator	ROB	NCI
PI: James B. Mitchell, Ph.D.	Senior Investigator	ROB	NCI
Other: Nirmolini Soares, M.S.	Biologist	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
ROB

SECTION
Radiobiology

INSTITUT AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ataxia telangiectasia is a human autosomal recessive disorder characterized by progressive degenerative changes in skin and the central nervous system. Affected persons have an increased risk of malignanc- and demonstrate marked sensitivity to X-rays when used in cancer treatment. Cultures of skin fibroblasts also show an increased sensitivity to X-rays compared to normal controls. This sensitivity is thought to represent a DNA-repair defect which may be related to their increased risk of cancer. Recently, presumed heterozygotes were reported to show increased sensitivity to X-rays under hypoxia. We propose to investigate the findings in homozygotes and heterozygotes of increased X-ray sensitivity.

Project Description

1) Objectives - To investigate the X-ray sensitivity of cultured skin fibroblasts from ataxia telangiectasia homozygotes and heterozygotes under oxic and hypoxic conditions.

2) Methods Employed - In vitro cell proliferation will be assayed by the stage cell plating technique. Cells will be exposed to X-ray under oxic and hypoxic conditions and oxygen enhancement ratios determined.

3) Major findings - The project is in the early stages of development.

4) Significance to the research program to the Institute - At heterozygotes are estimated to occur in up to 1% of the population. If the increased sensitivity of cultured skin fibroblasts to X-rays under hypoxia represents a DNA repair defect, then this population may be at a higher risk of induced malignancy by X-ray like carcinogens. This requires further study.

5) Proposed Course - Experiments are underway.

6) Publications - None

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1980 to September 30, 1981

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft-tissue sarcomas are exploring the role of adjuvant chemotherapy as well as the role of limb-sparing surgery. Active clinical protocols are in progress evaluating the effect of adjuvant chemo- and immunotherapy in the treatment of melanoma and colo-rectal cancers. New programs in the treatment of testicular cancer, esophageal cancer have been initiated as well as evaluations of the role of intraoperative radiation therapy in the treatment of cancer. Active laboratory research programs in tumor immunology and in tumor metabolism are also in progress.

Surgical procedures performed in the Surgery Branch from April 1, 1980 to March 31, 1981, are presented in Tables I to III.

TABLE I
Cases - General Surgery
April 1, 1980 - March 31, 1981

General:	Abdominal-perineal resection	2
	Aortocaval lymph node dissection and biopsy for ovarian cancer staging	18
	Colon/bowel resection	30
	Cholecystectomy	17
	Colonoscopy	13
	Electrofulguration	1
	Exploratory laparotomy	66
	Feeding jejunostomy	3
	Gastrectomy	3
	Gastroscopy	1
	Hepatic resection	12
	Herniorrhaphy/hernia repair	6
	Laparoscopy	2
	Major soft tissue or muscle group excision	43
	Pancreatectomy - partial/total	8
	Perirectal fistula repair	3
	Peritoneoscopy	4
	Revision of colostomy	3
	*Radiation therapy - intraoperative	10
	Splenectomy	7
	Staging laparotomy ± splenectomy for lymphoma	21
	Wound exploration/secondary closure	2
Surgery for Melanoma:	Wide excision with/without STSG	7
	Wide excision + radical neck LND	1
	Lymph node dissection: Axillary	2
	Superficial inguinal	2
	Excision solitary nodules/nevi	8
Head & Neck:	Radical neck + other resection	4
	Tracheotomy	16
Plastic:	Dermabrasion	1
	Skin grafting	3
GYN:	Hysterectomy	2
	Salpingo-oophorectomy	1
	Culdocentesis	1

* Actual operative procedure listed elsewhere

TABLE I (continued)

GU:	Aortocaval/retroperitoneal lymph node dissection	34
	Cystoscopy ± other procedures, biopsy	61
	Circumcision	2
	Debulking procedure	10
	Hemiscrotectomy	1
	Looposcopy	2
	Loop revision	3
	Meatoplasty	1
	Orchiectomy/orchiopexy	3
	Nephrostomy/nephrolithotomy/nephrectomy	2
	Penile prosthetic implant	2
	Prostatic biopsy	7
	Repair varicocele/hydrocele	3
	Transureteroureterostomy/ureterolithotomy	4
	Transureteroprostatectomy	4
	Testicular biopsy	9
	Urethral dilation	3
Breast:	Breast biopsy	75
	Simple mastectomy	2
	Modified radical mastectomy	22
	Axillary node dissection with biopsy	19
	Radical mastectomy	2
	Removal prosthesis	1
Orthopedics:	Below knee amputation	2
	Above knee amputation	8
	Hip disarticulation	6
	Hemipelvectomy	11
	Forearm amputation	2
	Forequarter amputation	6
	Claviculectomy	1
	Stump revision	1
	Bone biopsy	12
	Fasciotomy	2
Endocrine:	Parathyroidectomy	37
	Parathyroid implant/removal	6
	Adrenalectomy	11
	Thyroidectomy - complete/subtotal	24
	Radical neck LND - met. thyroid ca.	1
	Mediastinal exploration	3
	Excision pheochromocytoma	3
Vascular:	A-V shunt/shunt revision/shunt removal	21
	Broviac/Hickman catheter placement	29
	Subclavian CVP line placement	6
	Endarterectomy/embolectomy	6
	Arterial/venous bypass	1
	Ligation IVC	1
	Profundoplasty	0

TABLE I (continued)

Thoracic:	Unilateral thoracotomy	
	Biopsy nodules	55
	Chest wall resection	6
	Lobectomy	7
	Pneumonectomy	3
	Lingulectomy	1
	Drainage, empyema	2
	Bilateral thoracotomy/median sternotomy	21
	Tracheal dilation	5
	Bronchoscopy	28
	Mediastinoscopy	11
	Esophagoscopy/dilatation/± bronchoscopy	56
	Esophagogastrectomy	9
	Esophageal bypass/colon interposition	6
	Pericardectomy	1
	Omentoplasty	1
	Insertion peri cardial catheters	1
Minor:	Incision and drainage of abscess	26
	Biopsy: Node	94
	Tissue mass, NOS	67
	Insertion/removal abdominal catheter for chemotherapy	24
	Wound repacking/debridement	15

TABLE II
Consultants - Surgery
April 1, 1980 - March 31, 1981

Plastic Surgery:	Major skin grafting	5
	Myocutaneous skin flap transfer	4
	Mammoplasty	
	Augmentation after mastectomy	6
	Biopsy mass	2
	Epidermectomy	1
Vascular:	Creation A-V fistula	1
Thoracic:	Esophageal dilation and placement stint	2
Gynecological:	D&C	2
	Laporoscopy	5
	Total abdominal hysterectomy	1
	Salpingectomy	1
Orthopedic:	Bone biopsy	3
	Osteotomy	1
	Prosthetic limb replacement	1
	Muscle release	1
	Excision cartiledge	1
ENT:	Creation nasal antral window	4
	Laryngoscopy/pharyngoscopy/esophagoscopy/ bronchoscopy	6
	Myringotomy, bilateral	2
	Polypectomy	2
	Biopsy	6
	Septoplasty	2
	Tracheostomy	1
	Radical neck dissection	1
	Stapedectomy	1
	Palate resection	1
	Teflon vocal cord injection	1
	Mastoidectomy	1

TABLE III
STATISTICAL REPORT
SURGICAL SERVICES DEPARTMENT
April 1, 1980 - March 31, 1981

Patient		NCI	NHLBI	NINCDS		NIAMDD	NIAID	NIDR	NIMH	NICHD	NEI	CONSULTANTS	Total No. Pts. Operated	
				Neuro.	Muscle									
NCI	Major	819.5	2	10.5	-	-	-	-	-	-	2	43	877	1105
	Minor	219	-	1	-	-	-	-	-	-	-	8	228	
NHLBI	Major	43	287	2	-	-	-	2		2	-	4	340	373
	Minor	12	19	-	1	-	-	-	-	-	-	1	33	
NINCDS	Major	17	19	45	5	-	-	-	-	-	-	2	88	335
	Minor	4	-	6	232	-	-	-	-	-	1	4	247	
NIAMDD	Major	113	-	5	-	-	-	-	-	1	-	2	121	133
	Minor	11	-	-	1	-	-	-	-	-	-	-	12	
NIAID	Major	67	2	-	-	-	-	1	-	-	-	3	73	94
	Minor	16	-	-	4	-	-	-	-	-	-	1	21	
NIDR	Major	-	-	-	-	-	-	2	-	-	-	1	3	4
	Minor	-	-	-	-	-	-	-	-	-	-	1	1	
NIMH	Major	3	-	-	-	-	-	-	-	1	-	-	4	6
	Minor	2	-	-	-	-	-	-	-	-	-	-	2	
NICHD	Major	16	-	9	-	-	-	-	-	5	2	2	34	40
	Minor	2	-	-	2	-	-	-	-	-	-	2	6	
NEI	Major	-	-	-	-	-	-	-	-	-	11	-	11	13
	Minor	1	-	-	-	-	-	-	-	-	1	-	2	
MAJOR TOTAL		1078.5	310	71.5	5	0	0	5	0	9	15	57	1551	
MINOR TOTAL		267	19	7	240	0	0	0	0	0	2	17	552	
GRAND TOTAL		1345.5	329	78.5	245	0	0	5	0	9	17	74*	2103**	

*Includes 20 anesthesia procedures.

**Of these 2103 surgical procedures, 146 patients were on O.P.D. status and 29 procedures were performed on the nursing units. There were 202 emergency procedures and 134 nonemergency procedures added.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03800-11 SURG

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	G. D. Aurbach	Chief, Metabolic Dis. Br.	MD	NIAMDD
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	E. Glatstein	Chief, Rad. Oncol. Br.	RO	NCI
	J. Robbins	Chief, Clin. Endocrin. Br.	CE	NIAMDD
	J. Costa	Lab. of Pathology	LP	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	A. S. Levine	Chief, Ped. Oncol. Br.	PO	NCI
	J. Gardner	Chief, Digestive Dis. Br.	DD	NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts.

INTRODUCTION

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

Over 1000 consultations were received last year from other NCI Branches as well as other NIH Institutes.

Project Description: Selected projects are presented below to provide examples of the nature of Surgery Branch collaborative efforts.

Part I. Endocrine Surgery

Part II. Thoracic and Vascular Surgery

Part III. Nutritional Support

Part IV. Ovarian Cancer

Part V. Tenckhoff Catheters

Part VI. Vascular Access

Part I. Endocrine Surgery

All the endocrine surgery for the Clinical Center is provided by the Surgical Metabolism Section of the Surgery Branch.

A vigorous program of preoperative localization, intraoperative localization, assessment of adequacy of surgical resection, and human cryopreserved autografting in patients with parathyroid disease is being vigorously pursued both clinically and in the laboratory.

The remaining endocrine tumors tend to be dominated by the islet cell tumors of the pancreas, with adrenal tumors being less common but active surgical involvement in the management and treatment of phaeochromocytoma, adrenal Cushing's disease, hyperaldosteronism, and thyroid tumors has continued.

Part II. Thoracic and Vascular Surgery

Consultative services for thoracic and vascular surgical problems are handled through the Surgery Branch. Thirty-two major and fifty-two minor thoracic surgical procedures have been performed. Likewise, 4 major vascular procedures, between April 1980 and April 1981 have been performed along with 2 minor cases.

Part III. Nutritional Support

The Surgery Branch continues to mount a major effort in supporting patients nutritionally by intravenous feeding throughout the National Institutes of Health. The Surgery Branch has been responsible for 1623 days of total parenteral nutrition between the period of 2/1/80 and 2/1/81, involving 64 courses in 50 patients.

Part IV. Ovarian Cancer

Studies of ovarian carcinoma are undertaken in Medicine Branch protocols with the cooperation of the Surgery Branch. Adjuvant systemic melphalan chemotherapy is being compared with intraperitoneal radioactive phosphorus for high-risk patients following complete surgical tumor resections. Intraperitoneal chemotherapy is evaluated both as an adjuvant in early-stage patients and as definitive therapy in certain patients with advanced disease. Patients with incomplete surgical tumor resections or with disseminated disease are treated with various combinations of systemic chemotherapy. The Surgery Branch collaborates with the Medicine Branch in ovarian cancer studies by providing surgical evaluations and services, as well as performing definitive resections, staging laparotomies, explorations for complications or failures of treatment, and peritoneal catheter placements. During 1980, the Surgery Branch performed 40 operative procedures on patients in ovarian cancer protocols.

Part V. Tenckhoff Catheters

Tenckhoff catheters have been used for several years for peritoneal dialysis in patients with chronic renal failure. Using these catheters, direct administration of chemotherapeutic agents is possible into the peritoneal cavity. The conduct of these studies has been under the general direction of Dr. Charles Meyers, Chief of the Medical Pharmacology Branch. Phase I and Phase II trials of intraperitoneal 5-FU and adriamycin have been completed for ovarian cancer. An adjuvant 5-FU trial for poor risk patients who have had a resection for colon and rectal cancer is currently in progress. Phase I trials with intraperitoneal misonidozole for peritonial implants with ovarian or colorectal cancer have begun. The Surgery Branch is responsible for the insertion and removal of Tenckhoff catheters on protocol patients. Twenty-nine catheters were inserted 4/1/80-3/31/81.

Part VI. Vascular Access

The Surgery Branch accepts consults from the Clinical Center to provide means of vascular access. Renal dialysis, plasmaphoresis, drug and blood products infusion and blood withdrawal are some of the indications for vascular access procedures in selected patients. Between April, 1980 and April, 1981 8 arterio venous fistulae have been constructed, 5 Scribner shunts inserted, and 6 Hickman silastic catheters placed. Twelve other miscellaneous procedures have been performed.

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PERIOD COVERED
October 1, 1980, to September 1981

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cancer Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	J. Costa	Lab. of Pathology	LP	NCI
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	E. Glatstein	Chief, Rad. Oncol. Br.	RO	NCI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	A. Levine	Chief, Pediatric Oncol. Br.	PO	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.6	7.6	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, osteogenic sarcomas, and colorectal cancer. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

Project Description:

Part I.	Malignant Melanoma
Part II.	Soft Tissue Sarcomas
Part III.	Osteogenic Sarcoma
Part IV.	Testicular Cancer
Part V.	Colorectal Cancer
Part VI.	Total Parenteral Nutrition
Part VII.	Breast Cancer
Part VIII.	Endoscopy
Part IX.	Computer Applications
Part X.	Esophageal Cancer
Part XI.	Intraoperative Radiotherapy
Part XII.	Resection of Pulmonary Metastases

Part I. Malignant Melanoma

The Surgery Branch is involved in several studies designed to improve the treatment of patients with malignant melanoma. A prospective randomized study of the intralesional injection of BCG into primary cutaneous melanoma lesions was begun in 1975. Twenty-six patients were randomized in this study. A statistically significant improvement in disease-free survival ($p<.05$) is present in the patients treated with intralesional BCG compared to patients receiving standard surgical treatment alone. For the treatment of multiple cutaneous recurrences of melanoma, the Surgery Branch is investigating the use of BCG cell walls on oil droplets as an alternative to the use of viable BCG for the treatment of these lesions. In conjunction with the Immunology and Medical Oncology Branches, the Surgery Branch is conducting a prospective randomized trial of 3 adjuvant treatments in addition to surgery for the treatment of patients with Stage II melanoma. Approximately 180 patients have been randomized into these protocols. Preliminary analysis has revealed no significant differences among any of the treatment groups.

Part II. Soft Tissue Sarcomas

The Surgery Branch is conducting a variety of protocols evaluating the treatment of patients with soft tissue sarcomas. Forty-two patients have been included in randomized protocols evaluating the roles of amputative surgery compared to limb-sparing surgery plus radiation therapy in the treatment of patients with extremity sarcomas. While it appears that limb-sparing surgery may be effective in treating the majority of patients, certain locations such as the proximal

thigh appeared to be better treated with amputation. An initial evaluation of 55 patients treated with adjuvant chemotherapy in the Surgery Branch for the treatment of soft tissue sarcomas suggested that adjuvant chemotherapy might be of benefit in the treatment of these patients. A prospective randomized study evaluating the role of adjuvant chemotherapy is in progress and thus far 86 patients have been randomized into this protocol. Preliminary evaluation of this protocol suggests that adjuvant chemotherapy may be effective in improving the disease free survival in patients with extremity soft tissue sarcomas. This protocol is still in progress.

Part III. Osteogenic Sarcoma

Fifty-five patients have been entered into a study evaluating the use of high-dose methotrexate for the adjuvant treatment of patients with osteosarcomas. Continuous disease-free interval has improved from 20% in historical controls to 38% in adjuvant chemotherapy treated patients. The aggressive use of thoracotomy in these patients has led to a dramatic increase in disease-free survival. Currently 68% of patients are alive and NED following diagnosis of osteogenic sarcoma. This represents a substantial improvement over survival figures prior to the onset of this protocol.

A new protocol has been initiated comparing, in a prospective randomized fashion, adjuvant therapy with high-dose methotrexate compared to surgery alone in patients with osteogenic sarcoma. Twelve patients have been entered into this study.

Part IV. Testicular Cancer

Thirty-nine patients with stage III nonseminomatous testicular cancer were treated in a prospective randomized trial comparing cytoreductive surgery followed by cis-platinum containing combination chemotherapy regimen versus chemotherapy alone. All patients had one or more of the following poor prognostic signs: bulky retroperitoneal disease, liver involvement, invasion or obstruction of the inferior vena cava, or lung metastases > 2 cm in diameter. Cytoreductive surgery was technically feasible in this group of patients as assessed radiographically and by the decline in serum levels of alphafetoprotein and human chorionic gonadotropin following surgery. However, there was no statistically significant improvement in overall response rate (75% vs 74%), complete response rate (50% vs. 37%) or in overall survival between the two groups.

Since the overall complete response rate to chemotherapy in these 39 patients with bulky stage III disease was only 43%, alternate approaches, other than cytoreductive surgery, are necessary to improve the prognosis for this group of patients. A randomized protocol of ablative therapy utilizing platinum, vinblastine, bleomycin (PVB) and VP-16 vs, PVB alone is in preparation.

Part V. Colorectal Cancer

At the present time two protocols for the study of patients with colorectal cancer are operative. The first protocol concerns the postoperative management of patients who have had lymph node positive colon or rectal cancer removed. These patients are carefully followed with monthly CEA assays, 3-monthly

abdominal ultrasound and CAT scans, and monthly physical examination. By so doing we wish to pick up early recurrences for "second look surgery" as well as determine optimal follow-up regimens. In the second protocol the same group of patients are randomized to receive intravenous or intraperitoneal 5-FU. Twenty-five patients have been entered into these studies. In the second study patients with local or regional colon or rectal recurrence undergo second look surgery randomized to receive or not receive radiation therapy.

Part VI. Total Parenteral Nutrition

The Surgery Branch is involved in three prospective randomized studies of the effect of total parenteral nutrition in the management of malignancy. The first, the diffuse histiocytic lymphoma protocol, has been concluded and shows that total parenteral nutrition given in a random fashion regardless of preceding weight loss is not able to improve the tolerance to an aggressive chemotherapeutic regimen.

The use of total parenteral nutrition in ablative chemotherapy for recurrent metastatic soft tissue sarcomas continues its slow accrual with the Pediatric Oncology Service.

The recently instigated esophageal protocol which involved aggressive radiation with and without misonidazole preoperatively for carcinoma of the esophagus has included an intravenous nutritional support arm which is randomly allocated. The clinical protocol was terminated because of the high peri-operative mortality. Considerable metabolic information was obtained.

Part VII. Breast Cancer

Under protocol 79-C-111 which began accrual of patients in July 1979, 47 patients have been entered through April 1981. Patients with clinical Stage I or II unilateral breast cancer are randomly assigned to receive total mastectomy with axillary dissection, or excisional biopsy with axillary dissection followed by radiotherapy to the affected breast. Of the 47 patients, 23 have been randomized to the mastectomy group, and 24 to the radiotherapy group. With at least one positive axillary node, 21 patients (45%) have entered into adjuvant chemotherapy with IV Adriamycin and oral Cytoxan. With a median follow-up of 11 months there has been one recurrence. Patients who receive mastectomy will be given an opportunity to have reconstruction after 6 months. Three mastectomy patients have had reconstruction. An increased rate of accrual is hoped for during the next year.

Part VIII. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role for laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. During the past year, 41 patients have undergone laparoscopy on the NCI surgical service. Surgery Branch colon and rectal cancer protocols promise to increase our utilization of this endoscopic procedure in the coming year.

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both

the diagnostic and therapeutic point of view of colon lesions is well established. Twenty-four colonoscopic procedures were performed in the past year. These included 14 diagnostic examinations and 10 snare polypectomies. Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy. Premalignant lesions in patients with ulcerative colitis can be identified. Colonoscopy will play a role in the preoperative assessment of appropriate patients to undergo colon and rectal surgery.

Part IX. Computer Applications

Data for Surgery Branch research continued to be collected, stored, and reported for 3 primary systems: (a) Randomized Protocols, (b) Serum Inventory, and (c) Surgical Metabolism Studies. The basic objectives underlying the design of the Surgery Branch data systems are to: (1) ensure very high accuracy of the data and (2) permit information to be easily recorded, keyentered, verified, corrected, retrieved, and analyzed.

During the past year, however, much of the data previously maintained in the Surgery Branch Randomized Protocols System was transferred to a new generalized data management system developed for the Clinical Oncology Program called the Cancer Patient Research Information System (CAPRI).

These systems are maintained on computers at the Division of Computer Resources and Technology. On the IBM-370 system, the text-editor WYLBUR is used for data entry, program maintenance, and remote job submission. The Time Sharing Option is used for graphic displays and interactive computing. On the DECsystem-10 computer, various software packages are used for entry of laboratory data, graphic displays, and interactive computing. A high-speed Atlanthus T-1222 terminal is used for data entry and retrieval. A Tektronix 4012 terminal is used for data retrieval and graphic display of information. An MFE-5000 terminal is used to enter data which has been recorded on cassette tape by data loggers connected to automatic analyzers and radiation counters.

Data processing activity may be shown by the counts in the following table, reported as of May 1, 1981:

	System	No. of Subjects (6/75-5/81)	No. of Records 6/75-5/81)	No. of Retrievals (4/80-3/81)
(a)	Randomized + CAPRI	465	6,522	6
(b)	Serum Inventory	6,904	28,443	4
(c)	Surgical Metabolism	471	41,082	40
	Totals	7,840	76,047	50

In the Serum Inventory there are currently 46,880 vials of serum and 6,998 vials of lymphocytes collected in 21,414 drawings.

The processing of data by computer will play an increasingly significant role in assisting Surgery Branch investigators to define and describe the characteristics of protocol populations.

Part X. Esophageal Cancer

A study to determine the efficacy of a radiosensitizing drug, misonidizole, has been instituted in patients with esophageal carcinoma. In conjunction with the Radiation Oncology Branch, patients with squamous carcinoma of the mid or lower esophagus are radiated to 4000 rads with or without misonidizole. The patients then may undergo esophageal resection with reconstruction by exophagogastrotomy. Eleven esophagectomies, 4 bypass procedures and 22 other major operations have been performed in the past year. Minor procedures include 38 esophagoscopies, 13 dilations and 2 endoesophageal tube placements.

Part XI. Intraoperative Radiotherapy

The Surgery Branch has initiated investigations of the role of combined surgery and intraoperative radiation therapy for the local control of abdominal malignancies. A series of 7 pilot patients have been treated where the patients underwent surgical resection of a variety of abdominal tumors, were transported under anesthesia from the operating room to the radiotherapy treatment facility, were treated with electron radiation directly to the tumor bed having vital abdominal structures shielded from the radiation beam, and were returned to the operating room for completion of surgery. No technical difficulties were encountered in the pilot series, and therefore investigative prospective randomized protocols were initiated during 1980 to evaluate combined surgical resection and intraoperative radiation in certain poor-prognosis malignancies. Protocols for the treatment of gastric and pancreatic cancers were established, comparing intraoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative low-dose external beam radiation with conventional high-dose postoperative radiotherapy in patients with surgically resectable tumors; in addition, the use of adjuvant chemotherapy is evaluated in patients treated for retroperitoneal sarcomas. Protocol for the treatment of bony sarcomas of the pelvis was initiated, treating patients with combined surgical resection and intraoperative radiation therapy to the tumor bed, comparing disease control in protocol patients with historical experience. As of 4/15/81, accrual status of the various intraoperative radiotherapy studies was: gastric carcinoma 5 evaluated, 3 randomized, 2 on study; pancreatic carcinoma 20 evaluated, 13 randomized, 6 on study; retroperitoneal sarcoma 20 evaluated, 9 randomized, 9 on study; pelvic bony sarcoma 3 evaluated, 2 on study.

Part XII. Resection of Pulmonary Metastases

The Surgery Branch continues its protocol for the aggressive removal of metastatic sarcoma to the lung. After a thorough work-up to rule out metastatic disease at other sites, patients with locally controlled osteo or soft-tissue sarcoma are subjected to pulmonary resection. An attempt is made to remove all gross evidence of tumor while preserving as much pulmonary parenchyma as possible. Multiple procedures are often required. Patients with unilateral pulmonary metastases from melonoma are also considered for resection. These patients undergo formal mediastinal lymph node dissections in conjunction with

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03811-07 SURG

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Immunotherapy of Animal and Human Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery	SURG NCI
Other:	T. Sharp	Clinical Associate	SURG NCI
	A. Mazumder	Clinical Associate	SURG NCI
	Ben Kim	Clinical Associate	SURG NCI
	T. Eberlein	Clinical Associate	SURG NCI
	M. Rosenstein	Research Fellow	SURG NCI
	J. Brown	Staff Fellow	SURG NCI
	I. Suslov	Expert	SURG NCI
	S. Schwarz	Biologist	SURG NCI
	H. Wexler	Biologist	SURG NCI
	P. Spiess	Biologist	SURG NCI
	C. Hyatt	Biologist	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 8.5	PROFESSIONAL: 5.0	OTHER: 3.5

CHECK APPROPRIATE BOX(ES)
X
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Fetal antigens have been identified on human osteogenic sarcomas and monoclonal antibodies are being developed using hybridoma techniques. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells grown in long term culture in T cell growth factor. Techniques for the prolonged growth of cytotoxic T cells have been developed. These cells have been shown to mediate immunologic rejection of allografts and syngeneic tumors and attempts to use these cells for the adoptive immunotherapy of mouse and human tumors are in progress.

Publications:

Levine, A.M. and Rosenberg, S.A.: Alkaline phosphatase levels in osteosarcoma tissue are related to prognosis. Cancer 44:2291-2293, 1979.

Rosenberg, S.A., Parker, G. and Thorpe, W.P.: Serologic studies of murine and human sarcomas. In Sela, M. (ed.): Pontificiae Academiae Scientiarvm Scripta Varia. Ex Aedibvs Academicis in Civitate Vaticana, 1979, pp. 93-119, Rome, Italy.

Rosenberg, S.A., Brown, J.M., Thorpe, W.P., Hyatt, C., Shoffner, P., Tondreau, S.: Serologic studies of the antigens on human osteogenic sarcoma. In Human Tumor Antigens. Current Status and Compendium of Methods, (ed.) S.A. Rosenberg, Academic Press, New York, pp. 93-116, 1980.

Rosenberg, S.A., Schwarz, S., Spiess, P.J. and Brown, J.M.: In vitro growth of murine T cells. III. Partial characterization of T cell growth factor (TCGF) and analysis of its activity. J. Immunol. Meth., 33:337-350, 1980.

Rosenberg, S.A., Spiess, P.J. and Schwarz, S.: In vitro growth of murine T cells. IV. Use of T cell growth factor (TCGF) to clone lymphoid cells. Cellular Immunol., 54:293-306, 1980.

Mathisen, D.J. and Rosenberg, S.A.: Comparison of in vivo cell distribution following either intraperitoneal or intravenous injection of lymphoid cells. Transplantation 29:347-349, 1980.

Catapano, M.S., Brown, J.M., and Rosenberg, S.A.: Levels of cytotoxic reactivity to cultured normal skin fibroblasts in sera from normal and sarcoma-bearing patients. Cancer Res. 40:979-983, 1980.

Yron, I., Wood, T.A., Spiess, P., and Rosenberg, S.A.: In vitro growth of murine T cells: V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J. Immunol. 125:238-245, 1980.

Lotze, M.T., Strausser, J.L. and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. J. Immunol. 124:2972-2978, 1980.

Mathisen, D.J., and Rosenberg, S.A.: The in vivo distribution of adoptively transferred syngeneic, allogeneic, and xenogeneic lymphoid cells: Implication for the adoptive immunotherapy of tumors. J. Immunol. 124:2295-2300, 1980.

Austin, E., Brennan, M.F., and Rosenberg, S.A.: The effects of protein calorie restriction on the immune response to skin allografts in the rat. Transplantation 30:219-225, 1980.

Lotze, M.T., Line, B.R., Mathisen, D.J., and Rosenberg, S.A.: The in vivo distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J. Immunol. 125:1487-1493, 1980.

Lotze, M.T., Strausser, J.L., Line, B.R. and Rosenberg, S.A.: Tumor lysis by human T lymphocytes in long term culture and their distribution in vivo: Implications for immunotherapy. Surg. Forum 31:404-406, 1980.

Levine, A.M., Triche, T. and Rosenberg, S.A.: Osteosarcoma cells in tissue culture: II. Characterization and localization of alkaline phosphatase activity. Clin. Ortho. 146:259-268, 1980.

Spiess, P.J. and Rosenberg, S.A.: A simplified method for the production of murine T cell growth factor free of lectin. J. Immunol. Meth. 42:213-222, 1981.

Brown, J.M. and Rosenberg, S.A.: Serologic Analysis of Human Solid Tumor Antigens. In Serologic Analysis of Human Cancer Antigens, in press.

Lotze, M.T., Strausser, J.L. and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. III. Lysis of fresh and cultured autologous tumor by lymphocytes cultured in T cell growth factor (TCGF). J. Immunol., in press.

Lotze, M.T. and Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. IV. The preparation of lectin free T cell growth factor (TCGF) and an analysis of its activity. J. Immunol., in press.

Strausser, J.L., Lotze, M.T. and Rosenberg, S.A.: Lysis of human solid tumors by autologous cells sensitized in vitro to alloantigens. J. Immunol., in press.

Rosenstein, M., Eberlein, T., Kemeny, M.M., Sugarbaker, P.H. and Rosenberg, S.A.: In vitro growth of murine T cells: VI. Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J. Immunol., in press.

Brown, J.M., Shoffner, P.C., Tondreau, S.P., Matthews, E.J., Terry, W.D. and Rosenberg, S.A.: Cytotoxic reactivity in the sera of melanoma patients to paired autologous and allogeneic cultured tumor and skin fibroblasts. Cancer Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03826-07 SURG

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biologic Tumor Markers in the Cells and Sera of Patients with Urologic Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: N. Javadpour Senior Investigator SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques have been developed to localize and quantitate biologic tumor markers in the cells and sera of patients with urologic cancers. These markers include human chorionic gonadotropin, alphafetoprotein, pregnancy specific β_1 glycoprotein (SP_1), different isoenzymes of lactic dehydrogenase (LDH), ABO(H) antigens, and steroid receptors. These markers have been correlated with the histology, grade, stage, and management of patients with urologic cancers. Experimental animal models of cancer induction for the kidney, bladder, prostate, and testis have also been developed. These animal models are being utilized to study the role of cytoreductive surgery, chemotherapy, and immunotherapy of these urologic cancers. Athymic mice have been utilized for xenografts of testicular tumor that produce markers. By utilizing specific antibody labeled with ^{131}I, the role of radioimmunodetection and radioimmunotherapy is being investigated in testicular and prostatic cancer.

1. Bergman, S., and Javadpour, N.: The cell surface antigen A, B, or O(H) as an indicator of malignant potential in Stage A bladder carcinoma. J. Urol. 119:49, 1978.

2. Javadpour, N., Hyatt, C.L., and Barone, J.J.: Common antigens found on fetal cells and viral transformed adult prostatic tissue. J. Surg. Oncol. 10:245, 1978.

3. Javadpour, N., McIntire, K.R., and Waldmann, T.A.: HCG and AFP in sera and tumor cells of patients with seminoma. J. Urol. December, 1978.

4. Javadpour, N.: The value of biologic markers in diagnosis and treatment of testicular cancer. Sem. in Oncol. 6:37, 1979.

5. Javadpour, N., Bagley, D.H., and Zbar, B.: Failure of cryosurgical treatment of experimental intradermal tumors to eradicate microscopic lymph node metastases in guinea pig. J. Natl. Cancer Inst. 62:1479, 1979.

6. Woltering, E.A., Emmott, R.C., Javadpour, N., Marx, J., and Brennan, M.F.: ABO(H) cell surface antigens in parathyroid adenoma and hyperplasia. Surg. Forum 30:107-109, 1979.

7. Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of HCG and AFP. Invest. Urol. 18:11, 1980.

8. Javadpour, N.: Immunocytochemical discordance in localization of pregnancy specific β_1 glycoprotein (SP_1), alphafetoprotein (AFP) and human chorionic gonadotropin in certain testicular cancer. J. Urol. 124:615, 1980.

9. Javadpour, N.: Radioimmunoassay and immunoperoxidase of pregnancy specific β_1 glycoprotein (SP_1) in certain testicular cancer. J. Urol. in press (April 1980).

10. Johnson, H., Flye, M.W., and Javadpour, N.: Serum β_2 microglobulin levels in patients with testicular cancer. Urology, 15:522, 1980.

11. Danforth, D., and Javadpour, N.: Pressure effects of urinary reflux studies with renal autotransplantation and pyelocystostomy. Urology, (January 1980).

12. Woltering, E.A., Knox, R.D., Javadpour, N.: Detection of human chorionic gonadotropin in fresh and formalin-fixed testicular tumor tissue: a comparison of immunoperoxidase to radioimmunoassay. Urology, 16:215, 1980.

13. Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of human chorionic gonadotropin and alphafetoprotein in testicular cancer. J. Urol. 18: 11, 1980.

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16. Javadpour, N.: Significance of elevated serum alphafetoprotein in seminoma cancer. September 1980.

17. The role of biologic tumor markers in testicular cancer. Cancer, 45: 1755, 1980.

18. Javadpour, N., Utz, M., and Soares, T.: Immunocytochemical discordance in localization of pregnancy specific β_1 glycoprotein (SP_1), AFP and HCG in certain testicular cancers. J. Urol.

19. Emmott, R., Droller, M.J., and Javadpour, N.: The ABO(H) cell surface antigens in carcinoma in situ and nonmalignant lesions of the bladder. J. Urol. 125:32, 1981.

20. Burt, M., and Javadpour, N.: Germ cell tumors in patients with apparently normal testes. Cancer, 47:191, 1981.

21. Javadpour, N., Goldenberg, D.M., and Kim, E.: Radioimmunodetection of metastatic testicular cancer with radioactive antibodies to human chorionic gonadotropin and alphafetoprotein. JAMA in press.

22. Woltering, E.A., Knox, R.D., and Javadpour, N.: Detection of HCG in fresh and formaldehyde fixed testicular tissue: The sensitivity of immunoperoxidase vs. RIA. Urology.

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25. Shull, J.H., Javadpour, N., and deMoss, E.V.: The ABO(H) cell surface antigens in carcinoma and benign lesions of the breast. J. Surgical Oncol. in press.

26. Javadpour, N., Soares, T., and Princler, G.: In vitro synthesis of AFP and HCG in nonseminomeatous testicular cancer. Cancer, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06652-05 SURG

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Immune Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI::	P. H. Sugarbaker	Senior Investigator	SURG NCI
	W. Matthews, Jr.	Chemist	SURG NCI
	Y. Roth	Investigator	SURG NCI
	M. Kemeny	Medical Staff Fellow	SURG NCI
	C. McCullough	Clinical Associate	SURG NCI
	F. Gianola	Physician's Assistant	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The work in this laboratory includes three major projects: (1) Studies involve an assessment of cell-mediated immune responses after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. The significance of this work comes from attempts to understand regulatory mechanisms of cellular immune responses. This work has resulted in the development of a new mechanism of T cell control - the alloantigen elimination hypothesis. (2) Studies on the use of activated killer cells in the destruction of tumor cells have been undertaken. The receptor on tumor cells for killer lymphocytes is the subject of current investigation. (3) Studies of local immunotherapy have begun; allogenic and xenogenic systems have been characterized. An adjuvant immunotherapy attack on cancer cells remaining after surgery is planned.

1) Chang, A.E., and Sugarbaker, P.H.: Abrogation of T cell homing by allo-antibody, a new mechanism of immunologic enhancement. Transplantation 29:127-132, 1980.

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3) Sugarbaker, P.H., Sugarbaker, S.P., and Pun, P.P.: Characterization of in vivo suppression of syngeneic tumor by allogeneic effector cells. J. Surg. Oncol., 15:297-308, 1980.

4) Sugarbaker, P.H.: Diminished cellular cytotoxicity in mice hyperimmunized prior to skin grafting. Cell. Immunol., 57:99-113, 1981.

5) Sugarbaker, P.H., and Matthews, W.: Early antigen elimination by cytotoxic T cells results in diminished cellular immune response of allogeneic tumor. Cell. Immunol., 57:114-123, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06653-05 SURG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolic Studies in Tumor-Bearing Patients and Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

M.F. Brennan	Head, Surg. Metab. Sec.	SURG NCI
M.E. Burt	Expert	SURG NCI
J. Arbeit	Medical Staff Fellow	SURG NCI
M. Maher	Clinical Nurse	SURG NCI
D. White	Computer Analyst	SURG NCI
C. Gorschboth	Medical Technologist	SURG NCI
M.L. Peters	Clinical Associate	SURG NCI
A. Kirkemo	Medical Staff Fellow	SURG NCI
A. Saxe	IPA, Univ. of California	SURG NCI
L. Wagman	Clinical Associate	SURG NCI
S.L. Chen	Chemist	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Surgical Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
00.0	0.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch is vigorously involved in the assessment of the efficacy of total parenteral nutrition as a means of nutritional support of the cancer-bearing host. These studies utilize methodologies employing stable isotopes and labelled substrates to examine glucose and amino acid metabolism. Animal models are utilized for examination of gluconeogenesis and glucose kinetics. Particular efforts are directed at examining methods of host preservation and metabolic cost of antineoplastic therapy.

Continued effort is being made in the elucidation of function of fresh and cryo-preserved parathyroid and other endocrine tissues.

1. Burt, M.E., Norton, J.A. and Brennan, M.F.: The human tumor-bearing limb: An ex vivo model. Surgery 87:128-132, 1980.

2. Brennan, M.F.: Prehepatic hyperalimentation. Editorial, Surgery 87:355-356, 1980.

3. Burt, M.E., Arbeit, J. and Brennan, M.F.: Chronic arterial and venous access in the unrestrained rat. J. Appl. Physiol. 238:H599-603, 1980.

4. Devereux, D.F., Triche, T., Webber, B. Thibault, L. and Brennan, M.F.: Etiology of Adriamycin wound breaking strength in rats - An evaluation by light and electron microscopy, induction of collagen maturation and hydroxyproline assay. Cancer 45:2811-2815, 1980.

5. Norton, J.A., Burt, M.E. and Brennan, M.F.: In vivo utilization of substrate by human sarcoma bearing limbs. Cancer 45:2934-2939, 1980.

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8. Devereux, D.F., Thibault, L. and Brennan, M.F.: The effects of tumor removal on wound breaking strength in the rat. Surgical Forum 31:234-236, 1980.

9. Austin, E.H., Brennan, M.F. and Rosenberg, S.A.: The effects of protein depletion on the immune response to skin allografts in the rat. Transplantation 30:219-225, 1980.

10. Lowry, S.F., Burt, M.E. and Brennan, M.F.: Glucose turnover and gluconeogenesis in a patient with a somatostatinoma. Surgery 89:309-313, 1981.

11. Burt, M.E., Lowry, S.F., Gorschboth, C. and Brennan, M.F.: Metabolic alterations in a non-cachectic animal tumor system. Cancer (in press).

12. Devereux, D.F., Kent, H. and Brennan, M.F.: Time dependent effects of Adriamycin and x-ray therapy on wound healing in the rat. Cancer (in press).

13. Brennan, M.F. and Burt, M.E.: Nitrogen metabolism in cancer. Cancer Treat. Rep. (in press).

14. Popp, M.B., Morrison, S.D. and Brennan, M.F.: Total parenteral nutrition in a methylcholanthrene-induced rat sarcoma model. Cancer Treat. Rep. (in press).

15. Shamberger, R.C., Devereux, D.F. and Brennan, M.F.: The effect of chemotherapeutic agents on wound healing. Int. Adv. in Surg. Oncol. Ed. Gerald P. Murphy, publisher, Alan R. Liss, Inc. (in press).

16. Lowry, S.F., Norton, J.A., Foster, D., Berman, M. and Brennan, M.F.: Glucose disposal and gluconeogenesis from alanine in tumor-bearing rats. J. Natl. Cancer Inst. (in press).

17. Sloan, G.M., Harrison, L.C., Underhill, L.H. and Brennan, M.F.: Inhibition of tumor size by streptozotocin-induced diabetes mellitus. J. Surg. Res. (in press).

18. Norton, J.A., Shamberger, R.C., Stein, T.P., Milne, G.W.A. and Brennan, M.F.: The influence of tumor-bearing on protein metabolism in the rat. J. Surg. Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06654-04 SURG

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Malignant Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Sindelar	Senior Investigator	SURG NCI
Other:	C. Kurman	Microbiologist	SURG NCI
	C. Hyatt	Biologist	SURG NCI
	A. R. Dresdale	Clinical Associate	SURG NCI
	Y. Skornick	Visting Fellow	SURG NCI
	J. Tepper	Senior Investigator	ROB NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	3.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with sarcomas are studied for evidence of serum reactivity against tumor-associated determinants expressed on both fresh and cultured syngeneic and allogeneic tumor cells using immunofluorescence and immunoperoxidase staining techniques. An experimental model of pancreatic carcinoma has been developed in hamsters. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and are being investigated for possible applications to immunotherapy or methods of immunodiagnosis. Tissue-specific antigens have been isolated and are being investigated for possible use in immunotherapy of pancreatic carcinoma. Intraoperative radiotherapy is evaluated in dogs to determine responses of both normal and surgically-manipulated tissues to direct single-dose electron-beam irradiation in approaches to adapt operative radiotherapy to the treatment of human abdominal malignancies.

1) Auda, S.P., Sindelar, W.F.: Bipedicle transverse abdominal cutaneo-fascial flap for reconstruction of lwoer thoracic wall defects. J. Surg. Oncol., 15:195-199, 1980.

2) Rosenberg, S.A., Sindelar, W.F.: Surgery and Adjuvant Chemo-Immuno-therapy in Osteosarcomas: Review of Treatment of the National Cancer Institute. In van Oosterom, A.T., Muggia, F.M., Cleton, F.J. (Eds.): Therapeutic Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas. Martinus Nijhoff, Boston, pp. 301-316, 1980.

3) Rosenberg, S.A., Sindelar, W.F.: Surgery and Adjuvant Radiation-Chemo-Immunotherapy in Soft Tissue Sarcomas: Result of Treatment of the National Cancer Institute. In van Oosterom, A.T., Muggia, F.M., Cleton, F.J. (Eds.): Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas. Martinus Nijhoff, Boston, pp. 397-412, 1980.

4) Tepper, J., Sindelar, W.F., Glatstein, E.: Phase I study of intra-operative radiation therapy combined with radical surgery for intra-abdominal malignancies. Proc. Am. Soc. Clin. Oncol. 21:395, 1980.

5) Sindelar, W.F.: Cancer of the Small Intestine. In DeVita, V.T., Hellman, S., Rosenberg, S.A. Principles and Practice of Oncology. Lippincott, Philadelphia, in press.

6) Sindelar, W.F., Javadpour, N., Bagley, D.H.: Histological and ultra-structural changes in rat kidney after cryosurgery. J. Surg. Oncol., in press.

7) Tepper, J., Sindelar, W.F.: Summary of the workshop on intra-operative radiation therapy. Cancer Treat. Rep., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06655-01 SURG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Analyses of Factors Influencing Host Cellular and Humoral Immune Responses to Neoplasia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. A. Roth Senior Investigator SURG NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our laboratory has focused on factors that influence host responses to tumors and may adversely influence responses to immunotherapy. We have identified an immunoregulatory factor produced by a variety of human tumors that profoundly inhibits in vitro cell mediated immune responses. We are in the process of characterizing and purifying this factor and also determining its role in vivo. We are developing sensitive new techniques to measure cellular and humoral immune responses to human tumor-associated antigens. These inclued an automated micro slide leukocyte adherence inhibition test utilizing cryopreserved lymphocytes and an enzyme-linked immunabsorbant solid-phase assay using soluble human tumor-associated antigens. We have collected over 25 extracts from human primary sarcomas as well as metastases. Using autologous lymphocyte and sera, we are analyzing the distribution of tumor associated antigens on primary tumors and their metastases. Concurrently, we have established a murine melanoma model with cloned primary and metastatic cell lines to test various therapeutic options.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 through September 30, 1981

INTRODUCTION

The Biological Response Modifiers Program (BRMP) is a comprehensive program of the Division of Cancer Treatment, National Cancer Institute (NCI), involved in clinical and laboratory research with both extramural and intramural components to investigate, develop and bring to clinical trials potential therapeutic agents which may alter biological responses important in the biology of cancer growth and metastasis. This program was conceived as a focused approach in DCT to support further basic research in biological response modfiers and to rapidly apply potential leads from that research to the treatment of cancer in man. The classes of agents to be investigated in this program include immunoaugmenting, immunomodulating and immunorestorative agents, interferons and interferon inducers, lymphokines, cytokines, antigrowth factors, thymic factors, tumor antigens and modifiers of tumor antigen cell surface components, antitumor antibodies, antitumor cells, and maturation and differentiation factors. It is recognized that considerable research is underway in each of these areas but a focused, coordinated approach by the NCI may result in the rapid acquisition of knowledge and a more rapid application of information to the treatment of cancer.

BACKGROUND

The National Cancer Institute has been evaluating the potential role of biological response modifying agents in the treatment of cancer over the past several years. In March, 1975, Dr. Frank Rauscher, then director of the NCI, established the interferon working group to "monitor developments in interferon." The following November, the National Cancer Advisory Board recommended that the NCI purchase interferon for basic clinical studies. In November, 1975, a report to the DCT Board of Scientific Counselors recommended that interferon be further investigated as an antitumor agent. The Board recognized the emerging importance of biological response modifying agents and directed the staff of DCT, NCI to carefully monitor this important new field.

In mid 1976, the NCI purchased human leukocyte, lymphoblastoid and fibroblast interferons through the Division of Cancer Biology and Diagnosis (DCBD). Six investigators were given interferons for clinical trials and 33 investigators received interferon for basic laboratory research. The DCT Board of Scientific Counselors was again presented with information on the clinical use of interferon as an anticancer agent in October, 1976, with a review of the Karolinska Institute trials.

The increase in availability of interferon and the recognition that it might be a useful antitumor agent prompted the NCI to ask the Board of Scientific Counselors of the Division of Cancer Treatment to review the data on interferon as well as other biologicals that might have some influence on tumor growth and metastasis. In October of 1978, the Board recommended that a more concerted biological response modifiers program be developed within the Division of Cancer Treatment (DCT). The BRMP began with the appointment of the Subcommittee on Biological Response Modifiers (BRM Subcommittee) by the Board of Scientific Counselors, DCT in October, 1978. This Subcommittee was established because of the recognition that BRM were destined to play an increasing role in the treatment and understanding of cancer. The initial charge of this Subcommittee was to review existing clinical and laboratory data and to review ongoing investigations to develop guidelines for a focused program within DCT which could subsequently encourage, support and direct the NCI effort in this area.

This Subcommittee completed the very difficult task of reviewing the BRM background information and made programmatic recommendations in their interim report of September, 1979.

Through a series of meetings among the Subcommittee members, workshops organized by the Subcommittee, informal discussions with scientists conducting research in the area, extensive travel to and attendance at scientific meetings on BRM and through extensive contacts with consultants with expertise in the laboratory and clinic relevant to these agents, the Subcommittee completed an interim report for the Board of Scientific Counselors. This interim report defined BRM as those agents or approaches which modify the relationship between tumor and host by modifying the host's biological response to tumor cells with resultant therapeutic effects. Included in this definition were several approaches:

- to increase the host's antitumor responses through augmentation and/or restoration of effector mechanisms or decrease that component of the host reaction which may be deliterious;
- to increase host defenses by the administration of natural or synthetic effectors or mediators;
- to augment host responses to modified tumor cells which might stimulate a greater host response or increase tumor cell sensitivity to an existing response;
- to decrease the transformation and/or increase a differentiation (maturation) of tumor cells;
- to increase the ability of the host to tolerate damage by cytotoxic modalities of cancer treatment.

The interim report of the Subcommittee consisted of two documents. The Appendix to the interim report reviewed the pertinent literature and provided scientific background on BRM. This selective review attempted to identify promising approaches from previous studies with these agents. The interim report described the Subcommittee's view of how the BRMP should be organized, initiated and administered.

As the Subcommittee deliberated, DCT began to identify resources to support laboratory research and clinical trials of BRM. An interim administrative program was established in the Office of the Director, DCT and a search for the Program Director was initiated. The Subcommittee was consulted frequently by DCT Program Staff for assistance in decision making relative to the initial organization of the BRMP and relative to the design and initial organization of BRM research contracts, grants and clinical trials. As a result of these deliberations, DCT and the NCI made available 13.5 million dollars in fiscal year FY 80 to establish the BRMP and to begin initial work under this Program. Initially, there was a strong emphasis on interferon and interferon related research. There were lesser, though substantial, resources applied to other biological response modifiers.

The Program Director for the BRMP began working at the NCI in October, 1980 and the semifinal report by the BRM Subcommittee was presented to the Board in the same month. In consultation with DCT and those program officials involved in the initial organization of the BRMP, the Program Director set up and organized the BRMP according to the guidelines established by the BRM Subcommittee. This report will describe the progress to date and will describe future initiatives currently under consideration at the NCI.

PROGRAM OBJECTIVES

- Establish a well focused program within DCT to promote laboratory and clinical research in biological response modifiers.

- To support both basic and clinical research in the extramural community through a balanced program of grants and contracts. Requests for Applications (RFA), Program Announcements (PA) and Requests for Proposals (RFP) will all be used to support these efforts.

- Establish the BRMP as a resource for both the extramural and intramural community to provide certain BRM for laboratory and clinical use in further investigations as to mechanism of action and therapeutic efficacy.

- Establish a screening program and a BRM development network similar to that now in effect for drug development in DCT. Both a broad based screening program to identify potentially active agents (common track) and assays of more specific BRM (specific tracks) will be developed to assist the BRMP Decision Network Committee in making judgments about which BRM should be pursued through further preclinical work and formulated for clinical trials.

- Establish an intramural laboratory and clinical program to pursue investigations on basic mechanisms of action of BRM in animal tumor models, in in vitro assays and in man. Produce BRM through the genetic engineering and fermentation resources of the BRMP for potential use in clinical trials in man.

INITIAL PROGRAM DEVELOPMENT

The BRMP was initially set up in the Office of the Director, DCT, in 1980. The Associate Director for the BRMP was identified in 1980 and officially came on board October 20, 1980. Much of the initial ground work, personnel actions, equipment purchases and other arrangements to begin the program were under way at the time of the announcement by Health and Human Services Secretary, Richard Schweiker of the official establishment of the BRMP in April, 1981. During the initial year, the BRMP has grown to program status and now over 40 individuals including more than 14 scientists are currently working on BRMP projects. The laboratories are located in approximately 6,000 square feet of interim space as the BRMP building is being renovated. The clinical program has been established at the Frederick Memorial Hospital as will be described below. Most of the sections and a majority of the activities planned for the BRMP were operational by spring, 1981. At the current rate of development, it is anticipated that the BRMP will be at full strength and fully operational by early 1982.

SPECIFIC ACTIVITIES OF THE ASSOCIATE DIRECTOR, BRMP

A major focus in FY 81 has been the development of the BRMP as a DCT program. The majority of the objectives for program development including personnel acquisition, equipment purchases, space acquisition and program development have been accomplished. The BRM screening program has been established and currently represents an evolving system to evaluate experimental model systems the therapeutic efficacy of biological response modifiers. A total of six workshops were sponsored by the BRMP in FY 81. The topics covered were as follows:

- Augmenting Agents in Cancer Therapy
- Workshop on Growth and Maturation Factors
- Role of NK, ADCC and Macrophages in Tumor Rejection and as Indicators of BRM Activity
- BRMP Hybridoma Monoclonal Antibody Workshop
- Potential Utilization of Lymphokines in Cancer Therapy
- Potential Role of T-cell Subpopulation and Their Modulation in the Therapy of Cancer

In addition to initial program development and workshop coordination, the office of Associate Director has been heavily involved with establishing the clinical trials of the BRMP. Our clinical program has been functional since April, 1981 with an inpatient unit, an outpatient unit and the appropriate supporting services to conduct clinical experimental research with BRM at Frederick Memorial Hospital. This clincial unit represents the sole clinical outlet for the Frederick Cancer Research Center (FCRC) and is the major thrust of the BRMP. Finally, the extramural program has been established and is fully functional. A balanced program of grants and contracts is in place and further initiatives are currently being developed to further expand the scope of work being supported by the BRMP. Although many biological response modifiers are being investigated, a major thrust for FY 81 has been the use of interferon in therapeutic trials. The major thrust for FY 82 will be in the development of monoclonal antibody as an anticancer agent. The major focus in this initial year of the BRMP has been to establish the program as a working unit. This has been accomplished and within the next six months a completed program will be developed which should be able to approach most the priorities established by the Division of Cancer Treatment and the Board of Scientific Counselors of DCT.

SUMMARY REPORT

BIOLOGICAL RESOURCES BRANCH

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 through September 30, 1981

INTRODUCTION

The Biological Resources Branch (BRB), located in the Landow Building, Bethesda, Maryland administers all extramural activities of the BRMP. The responsibilities of this branch include:

- Supporting both basic and clinical BRM research in the extramural community through a balanced program of grants and contracts.
- Providing a BRM distribution resource to both the extramural and intramural community to supply BRM for laboratory and clinical use in further investigations as to mechanism of action and therapeutic efficacy.
- Establishing a preclinical screening program for the selection and preclinical assessment of efficacy of BRM.
- Monitoring DCT supported clinical studies utilizing BRM to assess biological effects in patients and to correlate changes in immunological functions with antitumor activity.

The BRM is organized into two sections: 1) Procurement, Formulation and Preclinical Trials Section (PFPTS), 2) Clinical Trials Section (CTS)

PERSONNEL

During FY 81 the following BRB personnel were recruited to staff the branch office:

1. W. John Martin, M.D., Ph.D.- Chief, BRB
2. Cedric W. Long, Ph.D. - Head, PFPTS
3. To be appointed - Head, CTS
4. Ann Peale - Biologist
5. Phyllis Gordon - Secretary to the Chief

SUMMARY OF FY 81 ACTIVITIES

A major focus of the BRB since its inception this year has been the development of a system of grant and contract administration.

Grants Administration: Following discussions with the directors of related extramural programs and officials of the Division of Research Grants, precise referral guidelines have been defined for grants to be administered by the BRB. The FY 81 level of R01, R23 and P01 grant support is in excess of $10 million. A listing of these grant areas is as follows:

GRANT CATEGORIZATION - R01s, R23s and P01s

INTERFERONS

- Therapeutic investigations of interferon's antitumor action.
- Pharmacology of interferon in its relation to antitumor activity.
- Development of reference Type II interferon (mouse and human)

THYMIC FACTORS

- Study of thymic polypeptides and factors for their selective antitumor action in therapeutic studies.
- In vitro assay(s) of monitoring the correlates of antitumor activity of thymic factors in man.

ANTIBODIES AND TUMOR ASSOCIATED/SPECIFIC ANTIGENS (TAA)

- Preparation of therapeutic antibodies as immune modulators or direct anticancer agents.
- Support of monoclonal antibody as carrier of specific drugs, toxins and radioisotopes.
- Production and modification of TAAs for the purpose of preparing specific vaccines.

RETINOIDS AND MATURATION FACTORS

- Therapeutic applications of retinoids alone and in combination with other biological response modifiers.
- Use of maturation factors as anticancer agents.

OTHER AREAS OF INTEREST

- o In vitro antitumor activity of biological response modifiers in man.
- o Study of biological response modifiers in combination with chemotherapy, radiotherapy and combined modality treatment.
- o Studies of the effects of chemotherapy and radiotherapy on biological response modification.
- o Preparation of specifically educated lymphoid cells for cellular therapy; exploration of continuous lines or clones in cellular therapy.
- o Fraction and isolation of purified components of microbial adjuvants.
- o In vitro and in vivo monitoring of biological response modification and antitumor activity by biological response modifiers in therapeutic studies.
- o Study of delivery and targeting systems for biological response modifiers.
- o Production of lymphokines for therapeutic purposes.

GRANT CATEGORIZATION - P01s

ANTIBODIES AND TUMOR ASSOCIATED/SPECIFIC ANTIGENS (TAA)

- o Preparation of therapeutic antibodies as immune modulators or direct anticancer agents.

OTHER AREAS OF INTEREST

- o Study of biological response modifiers in combination with chemotherapy, radiotherapy and combined modality treatment.

Contract Administration: The BRB has assumed responsibility for seven contracts transferred from the Immunology Program, DCBD and one contract transferred from the Developmental Therapeutics Program, DCT, which are as follows:

- o Kolling Institute of Medical Research, NO1-CB-84251
  CHARACTERIZATION OF FACTORS CAUSING INHIBITION OF MACROPHAGE FUNCTION OR INFLAMMATORY RESPONSES

- Harvard College, NO1-CB-64001
  IMMUNOTHERAPY OF OUTBRED CAT LYMPHOMA

- Karolinska Institutet, NO1-CB-74144
  IN VITRO IMMUNIZATION WITH AUTOCHTHONOUS HUMAN TUMOR

- Sloan-Kettering Institute for Cancer Research, NO1-CB-74145
  IMMUNIZATION WITH BCG AND ALLOGENEIC RENAL CANCER CELLS IN PATIENTS WITH RENAL CELL CANCER

- University of Washington, NO1-CB-84247
  ADOPTIVE CELLULAR IMMUNOTHERAPY OF MURINE TUMORS

- University of California/San Diego, NO1-CB-84250
  PRODUCTION OF MONCLONAL ANTIBODIES AGAINST HUMAN MALIGNANT LYMPHOMA AND LEUKEMIA TUMOR ASSOCIATED ANTIGENS

- University of Texas, NO1-CB-84248
  INTRALESIONAL IMMUNOTHERAPY PRIOR TO SURGERY IN THE TREATMENT OF CANINE BREAST CARCINOMA

- Sloan-Kettering Institute, NO1-CM-07385
  TUMOR NECROSIS FACTOR

Four new contracts will be awarded in FY 81. Two of these contracts will provide direct support to the administration of the BRB. One contract will assist information collection and organization on BRM and transfer of pertinent information to a computer based data retrieval system. The other contract will provide for the collection, storage, quality assurance and distribution to qualified investigators of BRM. The other two contracts will be for the procurement of human immune interferon and Type I and II mouse interferon.

Task Order Administration: Under terms of a master contract mechanism established by the BRMP, 27 institutions have been identified as suitable for performing clinical studies using BRM. Specifically, a total of 14 task orders for testing of interferons, thymosin and MVE-2 have been awarded under this master contract (Table I). These studies are being monitored by the BRB to assess significance of changes in immunological parameters observed in patients under treatment with the BRM. Four new task orders are being prepared for release subsequently in FY 81 or at the beginning of FY 82.

Other accomplishments during FY 81 include the development of a screening program to detect active biologicals in the treatment of cancer. A screening program has been formulated based on the drug screening program and on the recommendations of the BRM Subcommittee, as well as the recommendations of many experts in the field. The BRM screen (common track) will be done under contract by Dr. Isaiah Fidler of the Cancer Biology Program of the Frederick Cancer Research Center (FCRC). The common track screen capable of identifying immunomodulating activity in assays of T cell, B cell, NK cell and macrophage activity, is now operational. Specific tracks for the analysis of BRM, such as monoclonal antibody, are being developed as well.

The BRMP Decision Network Committee (BRMPDNC) was organized in FY 81 according to the recommendations of the DCT Board of Scientific Counselors Subcommitte. The BRMPDNC is chaired by Dr. W. John Martin. It has a broad based constitution with representatives from many areas of expertise relative to the BRMP and representatives from across the National Cancer Institute, as well as other Institutes in the National Institutes of Health. Consultants will be utilized when needed by the BRMPDNC.

The procedure has been established whereby compounds are selected for entry into the screen. BRM yet to be entered into the screen have been categorized and prioritized on the basis of review by the BRMP Operating Committee (BRMPOC) and BRMPDNC for subsequent consideration by these committees for entry into either the common track or a specific track of the screen.

BRM PROCUREMENT, FORMULATION AND PRECLINICAL TRIALS SECTION

Cedric Long, Ph.D. assumed the role of Section Chief for this section of the BRB in September, 1980. This section is responsible for the identification of BRM of interest to the BRMP through literature reviews in addition to coordinating information access in such a manner that relevant information on potential agents is directed toward appropriate program personnel and working groups. This section also assists in the development of BRM through animal toxicology and therapeutic trials and serves as a liaison for this activity in the Developmental Therapeutics Program (DTP). Other responsibilities include the pursuit of development of appropriate experimental systems for detection and evaluation of potential BRM and the coordination, planning and monitoring of detailed evaluations of biological response modifiers in relevant systems.

Considerable progress has been made in the identification of currently available compounds showing BRM activity. Using the BRM subcommittee report as a guide, and through literature review and discussions with officials within the Developmental Therapeutic Program of DCT, a listing of presently available compounds showing BRM activity was compiled and categorized according to the presumed mode of action.

This listing of potential agents and approaches was initially presented to the BRMPOC. Subsequently, the listing was presented to the BRMPDNC and accepted. The BRMP listing of potential agents and approaches is shown in Table II.

Other activities of this section relative to the operation of the BRMPOC and the BRMPDNC include the preparation of monthly presentations to both committees. Detailed synopses of data pretaining to NED-137, MVE-2, azimexon, thymosin, MDP, tuftsin and genetically engineered interferon were prepared for presentation to the BRMPOC. Each of these compounds was recommended by the BRMPOC for submission to the BRMPDNC and was approved at the DNC meetings for entry into the BRM common track screen. This section has arranged for the procurement of several of these BRM for the purpose of screening their biological activity.

BRM CLINICAL TRIALS SECTION

This section will monitor clinical trials involving the use of BRM (Table I) and will administer clinical grants. At present, these functions are being performed by the Branch Chief. An important aspect of this function is the close liaison with the Biological Evaluation Branch of CTEP in assessing correlations between changes in immunological reactivity and clinical efficacy and toxicity in these studies.

NEW INITIATIVES

New task orders have been prepared for FY 81 (Table III). In addition, several grant program announcements (Table IV), RFAs (Table V), and RFPs (Table VI) have been prepared, concept reviewed by the DCT Board of Scientific Counselors and may be available in FY 82. These new initiatives, in addition to those already in the BRMP, give the program the balanced approach recommended by the BRM Subcommitee of the Board of Scientific Couselors. During FY 82 a program of resource collection, storage, as well as quality assurance and distribution of BRM to qualified investigators, will be established and supported by the contract mechanisms currently being competed.

Information pertinent to the evaluation of BRM for potential preclinical and clinical trials will be collected on over 100 BRM and made readily available to the BRB through a computer based data retrieval system supported by another contract currently being competed.

Liaisons have been established with other programs in the NCI, such as the Immunology Program and the Tumor Biology Program in the Division of Cancer Biology and Diagnosis, to minimize overlap in the grant and contract area. A regular working relationship has been established with the Developmental Therapeutics Program of the Division of Cancer Treatment so that ongoing evaluations in the preclinical area as to efficacy and toxicology of BRM, can be jointly appreciated. Finally, a cooperative clinical evaluation system has been established with the Cancer Therapy Evaluation Program of DCT for the ongoing BRM clinical trials. Additional workshops are being planned and it is anticipated that at least one BRM workshop per year will be supported. The current and future initiatives of the extramural program should establish the Biological Response Modifiers Program, National Cancer Institute as a major support mechanism for preclinical and clinical research with biological response modifiers.

PUBLICATIONS/MAJOR PRESENTATIONS

Dr. W. John Martin presented a paper entitled "Role of Histocompatibility Antigen Expression in Determining Nature and Specificity of Anti-Tumor Immune Response" at the Armand Hammer Symposium on Class Regulation of the Immune Response, Salk Institute, La Jolla, California, June 1-6, 1981.

Table I

CLINICAL TASK ORDERS - FY 81

Institution	Agent		Approximate No. of Patients	
Georgetown U.	Leukocyte		Phase I - 30	Phase II - 30
Sidney Farber	Leukocyte		Phase I - 30	Phase II - 30
N. Cal. Can. Pro.	Leukocyte		Phase I - 30	
Sloan-Kettering	Fibroblast		Phase I - 30	Phase II - 50
U. of Wisconsin	Fibroblast		Phase I - 30	Phase II - 30
UCLA	Lymphoblast		Phase I - 30	Phase II - 30
Duke U.	Lymphoblast		Phase I - 30	Phase II - 60
	Subtotal	$1,396,519		
U. Cal./San Diego	Thymosin		Phase I - 80	
Fred Hutchinson	Thymosin		Phase I - 80	
George Washington	Thymosin		Phase I - 40	
Sloan-Kettering	Thymosin		Phase I - 40	
N. Cal. Can. Pro.	Thymosin		Phase I - 40	
	Subtotal	$969,466		
Vanderbilt Univ.	MVE-2		Phase I - 35	
Ohio State Univ.	MVE-2		Phase I - 35	
	Subtotal	$192,673		
	TOTAL	$2,558,658	TOTAL 560	TOTAL 230

Table II

BIOLOGICAL RESPONSE MODIFIERS PROGRAM DECISION NETWORK COMMITTEE

LISTING OF POTENTIAL AGENTS AND APPROACHES

A. Immunomodulator Agents

Azimexon
Cimetidine
Therafectin (SM1213)
DTC (Sodium diethyldithiocarbamate)
Bestatin
NED-137
Krestin
Picibanil
Thiobenzimidazole
Alkyl lysophospholipids (ALP)
BAI 7433
CP 46665
Pretazetin
Thiazolidine-14-carboxylic acid (thioproline "Norgamem")
Isoprinosine
NPT 15392
Russian glucomannian
Amphotericin B

B. Immunostimulator Agents

MDP
Tuftsin
Lentinan
Glucan (soluble)
Mannozyme
Levan
Tilorones

C. Immunorestorative Agents

Levamisole
Thiobendazole

D. Miscellaneous Chemicals

6-Ayrlpyrimidinoles
C353
Substituted pyrimidine from Upjohn Company
CL-246,738 (Cyanamid)
Prostaglandin Inhibitors (Aspirin, Indomethacin)

BRMPDNC - Listing of Potential Agents and Approaches

J. Antibody

Monoclonal antibodies against:
- Anti-T-cell
- Anti-T supressor cell
- Anti-tumor Ab
- Antibody to lymphokines, cytokines, etc.

Cytophilic antibody

K. Antigens

Tumor Associated Antigens

L. Alloantigen Effector cells

Effector cells - T-cell clones
T-helper cells

* M. Growth/Maturation Factors

Chalones
Antigrowth Factors
Maturation Factors
Growth Factors

N. Miscellaneous Approaches

Bone Marrow Transplantation and Reconstitution
Plasmapheresis
Allogenic Immunization
Virus Infection of Cells
Vaccinia

NOTE: Agents are listed in order of priority under each category. Categories are not in any order of priority.

* Revised 4/22/81 per BRMPDNC

E. Miscellaneous Bacterial Extracts or Bacteria

BCG, (P3, CWS)
Nocardia rubra CWS
Staph. phage lysate
Pseudogen
Brucella abortus (Bru-pel)
C. Parvum
Aerobacteria polysaccharide
Corynebacterium equi.
Microbicyclic peptide (Pseudomona)
Mixed Bacterial Vaccines

F. Miscellaneous Biologicals

Tumor necrosis factor
Endotoxin
"Immune" RNAs

G. Interferons and Interferon Inducers

Interferons
MVE-2
Poly IC/LC
Bru Pel
Tilorones
Levan
Blue tongue virus
Mumps Virus

H. Thymic Factors

Thymosins - $\alpha 1$
Thymosins - F-5
Other thymic factors

I. Lymphokines - Cytokines

Macrophage activation factor/MAF
Lymphocyte activation factor (LAF)
Cytotoxic factor (lymphotoxin - LT)
Transfer factor
Colony stimulating factor
T helper cell replacing factor (TRF)
Macrophage chemotactic factor
T-cell growth factor (TCGF)
Thymocyte mitogenic factor (TMF)
Macrophage inhibitory factor (MIF)

Table III

NEW TASK ORDERS - FY 81

- Monoclonal Anti T-Cell Antibody in T-Cell Malignancies
- Clinical Trial of Lymphokines in Human Cancer
- Purified Microbial Adjuvants in Cancer Therapy
- Anti-Suppressor Cell Monoclonal Antibody Therapy

TOTAL $1,750,000

Table IV

GRANT PROGRAM ANNOUNCEMENTS FY 81 - 82

- Development of Genetically Engineered Biological Response Modifiers
- Development of Cell Lines Producing Biological Response Modifiers
- Effect of Growth Factors and Anti-Growth Factor on Cancer

Table V

RFAs - FY 82

- Monclonal Antibody in Animal Tumor Models
- Monoclonal Antibody in Cancer Therapy
- Immunization of Purified Tumor Associated Antigens
- Sensitized T-Cell Lines in Adoptive Immunotherapy
- Therapeutic Approaches to Lymphokine Dependent Lymphoid Malignancies
- Therapeutic Efficacy of Adoptively Transferred Lymphoid Subpopulations in Tumor-Bearing Hosts
- Animal Tumor Models for Antipeptide Growth Factor and Maturation Factor Therapy
- Therapeutic Use of Lymphokines in Cancer

TOTAL $5,100,000

Table VI

RFPs FY 81 - 82

- Procurement of Human Immune Interferon
- Procurement of Mouse Interferon
- Review and Evaluation of Biological Response Modifiers
- Collection, Storage, Qualify Assurance and Distribution of BRM
- Chemical Coupling of Cytotoxic Agents to Tumor Reactive Monoclonal Antibody
- Production of Human Lymphokines and Cytokines and Assessment of Purity and Toxicity
- Studies on the Immunogenicity of Human Cytokines in the Mouse and the Production of Hybridomas Secreting Antibodies Reactive Specifically with the Cytokine
- Effect of Monoclonal Tumor Reactive Antibody on Bone Marrow Tumor Cells and Stem Cells

TOTAL $2,250,000

SUMMARY REPORT

BIOLOGICAL DEVELOPMENT BRANCH

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER TREATMENT

October 1, 1980 through September 30, 1981

The Biological Development Branch (BDB) is located at the Frederick Cancer Research Research Center (FCRC). Within the goals of the BRMP, the BDB is responsible for all intramural laboratory activities as well as clinical approaches in pursuing the development of appropriate experimental systems for the detection and evaluation of biological response modifiers. This effort involves coordinating the testing of new BRM and the detailed evaluation of BRM in relevant experimental systems. Dr. Robert K. Oldham is currently the Acting Branch Chief.

The BDB is comprised of six sections; Clinical Investigations, Monoclonal Antibody - Hybridoma, Tumor Antigens, Lymphokines, Basic Mechanisms and Genetic Engineering and Fermentation. Research efforts in each of these sections is directed by an M.D. or Ph.D. level NCI scientist.

The BDB was located at the FCRC for several reasons. Available laboratory space and a clinical facility were major considerations for placing this NCI program in Frederick. The "critical mass" of research and biologicals was already present at the FCRC and repesented a major consideration in this respect. The association of a genetic engineering capability and the use of this capability through the fermentation pilot plan with the translation of the material produced into the clinical trials was a major consideratin in the decision to locate the BRMP at the FCRC. As currently constituted, the six sections of the BDB have established strong collaborative ties with other scientists, both contractor and government, to pursue appropriate leads in BRM research. The clear and evident interprogram working relationship that has been established at the FCRC should be a real strength for the BDB and allow the program to grow without recreating capabilities within the BRMP which already exist at the FCRC. In a complimentary way, the BRMP now represents the clinical outlet as well as a research program focused at therapy for the FCRC laboratories. It is expected that this working relationship should be a major strength to the National Cancer Institute and should heighten the ability of the NCI to develop effective biologicals for treatment in the coming years.

CLINICAL INVESTIGATIONS SECTION

The Clinical Investigations Section (CIS) of the Biological Development Branch is responsible for the investigation of the therapeutic efficiency of BRM and the analysis of biologic response modification and toxicity of BRM. This section of the BRMP was established to facilitate the early clinical trials of biologic products with potential as anticancer agents. Agents being tested initially include interferons, lymphokines, immunomodulators, and monoclonal antibodies. The Clinical Investigations Section is particularly concerned with in-depth Phase I and II trials of biological response modifiers involving small numbers of patients. Optimal immunomodulatory doses as well as maximum tolerated doses of these new agents are being determined.

During the year, considerable effort has been devoted to the organization and development of the Clinical Investigations Section. A contract was negotiated between the NCI - Frederick Cancer Research Center (FCRC) and Frederick Memorial Hospital which described the clinical research facilities and the support services to be provided by the hospital. Recruitment of nursing personnel to staff the inpatient and outpatient units was successfully completed. Liaison between physicians of the Clinical Investigations Section and the physicians of the Frederick Memorial Hospital Staff were formed. Working relationships with various support services at the hospital including radiology, pathology, clinical laboratory and pharmacy were easily established. Where appropriate, mechanisms for further suport from the NIH Clinical Center facilities were arranged. In addition, a Biological Response Modifiers Intramural Trials Committee was established to foster collaborative interactions between the BRMP Clinical Investigations Section and other NCI intramural groups.

Although the FCRC had an active Investigational Review Board (IRB) with General Assurance status from the OPRR prior to the development of the BRM Clinical Program, it was necessry to expand the IRB with members from FMH. This was done under the auspices of the cooperative activities clause of the Code of Federal Regulations for Protection of Human Subjects. The expanded FCRC IRB received subsequent approval for this action from OPRR.

The facilities of the Clinical Investigations Section include a four-bed inpatient unit at Frederick Memorial Hospital and a ten-bed outpatient unit located in the Medical Pavilion adjacent to the hospital. The well-equipped outpatient unit has greatly expanded the patient treatment capacity of the Clinical Section. In addition, a leukapheresis unit is being established in the outpatient unit to permit development of this treatment modality in cancer therapy. Finally, a computerized axial tomography scanner will be installed at Frederick Memorial Hospital to provide optimal diagnostic radiology support for the Clinical Section.

The personnel of the Clinical Investigations Section include the following physicians all of whom are medical oncologists:

Stephen A. Sherwin, M.D., Acting Chief
James A. Knost, M.D., Expert
Paul G. Abrams, M.D., Expert
(Staff Physician, TBA)
Jeffrey Ochs, M.D., Clinical Associate
(Clinical Associate, TBA)

Two additional medical oncologists on the BRMP staff who are not part of this section also participate in the day-to-day management of patients (Robert K. Oldham, M.D., Director, BRMP and Kenneth A. Foon, M.D., Chief, Monoclonal Antibody Section). The above-named physicians share in on-call responsibilities and round twice weekly as a group. The Inpatient Unit is staffed by a head nurse and six registered nurses of the Frederick Memorial Hospital staff, of whom the majority have extensive experience in oncology research. The Outpatient Unit is staffed by a head nurse, two staff nurses with experience in oncology and leukapheresis respectively, and a medical unit coordinator.

Patients entered into trials of the Clinical Investigations Section have been largely referred by their primary physician (generally a medical oncologist) with the understanding that they are to be referred back to this physician upon completion of their treatment. No attempt is made to provide multimodality, ongoing cancer care to these patients beyond the experimental treatment period. The referred patients have disseminated malignancies for which no effective curative therapy exists and yet are relatively early in their disease course with good performance status. A considerable effort in terms of correspondence and speaking engagements has been devoted to generating referals from local and regional oncologist with considerable sucess. In addition, a Special Ambulatory Care Program (SACP) was also established to permit referal of patients from greater distances when otherwise appropriate.

The Clinical Investigations Section Inpatient Unit opened on April 20, 1981 and the Outpatient Unit opened one week later. The initial clinical protocols were multidose Phase I trials of recombinant leukocyte interferon (supplied by Hoffmann-La Roche) in patients with disseminated cancer. These studies represent the first multidose Phase I trials of genetically engineered inteferon in humans and include a major emphasis on the careful monitoring of immune parameters in order to assess an optimal immunomodulatory dose for this substance. A complete pharmokinetic study is also being done in each patient several times during the treatment. In the first 6 weeks of operation of the Clinical Investigations Section, more than twenty patients were entered into this Phase I trial. Two additional studies scheduled to begin within the next 2-4 months include a Phase I trial of intravenously-administered lymphoblastoid interferon and a Phase I trial of an antimelanoma monoclonal antibody developed and produced by BRMP intramural investigators. Future trials involving other lymphokines and monoclonal antibodies are currently in the planning stages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09200-01 BDB

PERIOD COVERED

February 2, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Phase I Trial of Recombinant Leukocyte Interferon in Patients with Disseminated Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert K. Oldham	Associate Director	BRMP	NCI
Other:	Stephen A. Sherwin	Acting Chief, Clin. Invest. Section	BDB	NCI
	James Knost	Expert, Clin. Invest. Section	BDB	NCI
	Paul Abrams	Expert, Clin. Invest. Section	BDB	NCI
	Richard Leavitt	Investigator	BCRP	NCI
	Ronald Herberman	Chief	LID	NCI

COOPERATING UNITS (if any)

Hoffmann-La Roche, Nutley, New Jersey

LAB/BRANCH

Biological Development Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	3.0	5.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with disseminated malignancies for which no curative therapy exists or who have failed standard therapy have been entered into a Phase I Trial of human leukoycte interferon prepared by recombinant DNA technology and supplied to the NCI by Hoffmann-La Roche. This trial is designed to determine the maximum tolerated and optimal immunomodulatory doses of this interferon preparation in cancer patients, as well as to study its pharmacokinetics and antitumor effect. Requirements for entry include good performance status and normal hematologic, hepatic, and renal function as judged by routine clinical tests. Different patients receive increasing doses (one dose/five patients) of interferon by intramuscular injection for 28 days on a twice daily schedule (BRMP Protocol # 81-01/Roche Protocol #2328) or three times weekly schedule (BRMP Protocol #8102/ Roche Protocol #2329). To date approximately 20 patients have been entered on each of the two treatment schedules. Objective antitumor responses have been seen in a limited number of patients. Toxicities observed include fever, fatigue, anorexia and neutropenia. The study continues

MONOCLONAL ANTIBODY-HYBRIDOMA SECTION

The Monoclonal Antibody-Hybridoma (MoAb) Section (MAHS) investigates and develops new hybridomas producing monoclonal antibody via cell hybridization with the aim of selecting MoAb with specificity for tumor-associated antigens and biological response modifiers. This laboratory also pursues investigations into the use of monoclonal antibody as therapeutic agents either as antibodies per se or carriers for other toxic substances directed at the tumor cell or to favorably affect the relevant component of the immune response.

Personnel recruited during the year for this section includes the following scientists:

Kenneth A. Foon, M.D., Section Chief
Michael I. Bernhard, Ph.D., Expert

In December 1980, Dr. Michael Bernhard arrived at FCRC and initiated research in the Monoclonal Antibody Hybridoma Section. The Hybridoma Laboratory was developed to give the BRMP the capacity to develop monoclonal antibodies in animal and human tumor systems for in vitro and in vivo trials in laboratory animals and in man. The long range plans encompass the use of monoclonal antibodies as tumor-specific conjugated toxin carriers or as antibody in immunotherapeutic regimens to treat various types of cancer.

During the year, commencing with December 15, 1980, considerable progress has been made in the development of the MoAb Laboratory. Initially, tissue culture operations were established, provisions were made for animal maintenance and technical support personnel were recruited and oriented to their respective scope of work. A computerized inventory system has been established for the liquid nitrogen frozen cell bank. This system will also computerize the laboratory-generated data e.g. gamma counts by a Delta Data Systems 7000 CRT Terminal, a model compatible with the NIH system.

Eleven fusions to produce MoAb have been performed. Three mouse myeloma cell lines have been used for fusions (p3x 63-NS-1, P3x63 Ag 8-653 and P3 Ag8:NP-3) as well as 1 human myeloma line (Sultan). The human myeloma line SK-007 (Kaplan) and 4672 (derived from GM-1500 by Croce) will also be utilized.

Techniques which have been established for characterizing MoAb include immunodiffusion and quantitative radial immunodiffusions, indirect immunofluorescence, fluorescent activated cell sorting (using a Becton-Dickinson FACS IV). Cr-release and trypan blue cytotoxicity, iodinated protein A (IPA) radioimmunoassays (RIA) using live cells, glutaraldehyde-fixed cells, soluble antigens and membrane preparations. These techniques are currently under evaluation to determine the most effective techniques for specific applications. An ELISA assay utilizating biotin - avidin conjugated reagents has been developed and is being evaluated in comparison to several of the IPA-RIA's listed above. Several different techniques for the preparation of stable membrane antigen preparations are also being evaluated.

Ammonium sulfate and sodium sulfate precipatations as well as ultrafiltration have been used to concentrate ascites fluids. Protein A-Sepharose has been used to separate mouse IgG1, IgG_{2a}, IgG2b, IgG_3 and IgM immunoglobulin isotypes. Characterization of essential reagents (rabbit α-mouse serum, rabbit α-mouse immunoglobulin isotype sera, antihuman, etc.) isotopes, is underway and anti-mouse reagents have been assayed for specificity and titer. Additional assays for characterization and analysis of MoAb are currently under development.

Collaborative studies have been initiated with various investigators. We are working with Dr. Charles Hoover of Johns Hopkins University to produce MoAb to human colon cancers by immunization of mice with human tumor cells to produce mouse x mouse hyridomas as well as fusion of draining lymph node cells from these patients with mouse and/or human myelomas. Similar collaborations with surgeons at Frederick Memorial Hospital are also underway, providing us with tumor and normal cells, involved lymph nodes and peripheral blood (PB). PB lymphocytes (PBL) purified by Ficoll-Hypaque and frozen for autologous and allogenic testing for HLA reactivities in the MoAb produced. Some PBL will be Epstein-Barr virus transformed to produced cell lines for further specifiity testing of MoAb.

A collaborative investigation of the guinea pig line 10 hepatoma model system with Dr. M. G. Hanna of FCRC is underway. Further, an intra-BDB collaboration was begun with Dr. John Pearson of the Basic Mechanisms Section, BDB; we are preparing hybridomas to Dr. Pearson's spontaneous guinea pig mammary carcinoma model, and plan to use this as a model system to assay the effects of MoAb in a solid tumor. This model system will also be used to assess the in vivo toxicity and efficacy of various drugs and toxins coupled to MoAb by several different techniques. Dr. Gerald Putterman of the Biological Markers Program, FCRC, will perform the biochemical conjugations.

Other immediate goals for the MoAb Section have been identified. These include the development of MoAb to human lymphoblast interferon to be used in interferon purification and assays. MoAb to various other human lymphokines and biological response modifiers will also be initiated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09201-01 BDB

PERIOD COVERED
January 15, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Monoclonal Antibodies to Human Tumor Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael I. Bernhard	Expert, MoAb Section	BDB	NCI
Other:	Robert K. Oldham	Associate Director	BRMP	NCI

COOPERATING UNITS (if any)
John Hopkins University, Baltimore, Md
FCRC Cancer Metastasis and Treatment Laboratory
Frederick Memorial Hospital, Frederick, Md

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

MoAb are produced to fresh human tumors. Cooperating surgeons at John Hopkins and Frederick Memorial Hospital are providing fresh tumor, matched normal tissue, involved lymph nodes and peripheral blood. Mice are immunized for fusion with mouse myeloma cells and tissue culture lines established from mechanically disaggregated tumor cells. When possible tissue lines will also be established from normal tissues. Normal tissues (as mechanically disaggregated cells) will also be frozen. Lymphocytes from involved lymph nodes are fused with mouse and human myeloma lines, and peripheral blood lymphocytes (PBL) are Ficoll-Hypaque purified and frozen. Mouse/mouse, human/mouse + human/human hybridomas are then assayed vs. autologous tumor and normal cells & PBL. Wells producing antibodies of appropriate specificity are minicloned, assayed again (including allogenic tumor and normal cells, cell lines, PBL Raji cells and human fetal cell lines), and selected wells are single-cell cloned. Hybridoma cells are frozen at all stages of MoAb production. Resulting MoAb will be extensively assayed and characterized. Promising MoAb will be used in immunodiagnostic & therapeutic studies.

Five patient samples have been processed to date. Involved lymph nodes derived lymphocytes from a lymphokines patient with adenocarcinoma of the lung were fused with human & mouse myeloma cells. No hybridomas grew. Similar cells from another patient with colon cancer were fused with mouse myeloma cells with no hybridoma growth.

Mouse x mouse hybridomas to a colon cancer are now growing; tumor cells are also growing in primary culture. Tumor cells from another patient with colon cancer were used to immunize BALB/c mice. Fusion of mouse spleen cells with the mouse NS-1 myeloma has produced hybridoma growth BALB/c mice were immunized with tumor cells from a patient with melanoma. Fusion with NS-1 resulted in growth in 100 of 192 wells. Initial screening vs. autologous tumor cells, PBL & RAJI cells identified 45 wells producing antibodies of appropriate specificity. Additional screening vs. live M14 melanoma cells (grown in serum-free chemically-defined medium) and tissue culture supernatants from M14 cells (grown in serum-free chemically defined medium) identified 19 wells producing antibody to cells and not supernatants, supernatants and not cells, or both. Fourteen wells were selected for minicloning & 5 were frozen for later study. In addition, involved lymph node lymphocytes were fused directly with NS-1 cells. Growth was observed in 7 of 10 wells. One of these showed antibody reactivity to autologous tumor and not to Raji cells. Another well showed the opposite reactivity. Both have been minicloned and are producing human IgG.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09202-01 BDB

PERIOD COVERED
May 12, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Monoclonal Antibodies to Human Lymphoblast Interferon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael I. Bernhard Expert, MoAb Section BDB NCI

COOPERATING UNITS (if any)
FCRC Fermentation Program
FCRC Cell Culture Laboratory

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human lymphoblastoid interferon is being produced from mass cultures of Namalva cells and purified with a small Celltech MoAb affinity column. This purified interferon will be used to immunize BALB/c mice using a modification of the procedure of Staehelin et. al. (PNAS 78, 1848, 1981). In addition, in vitro immunizations will be attempted. Growing hybridomas will be screened by inhibition of interferon effects in a cytopathic assay and by the Celltech RIA. The aim of this project is to produce MoAb to be used by the FCRC Fermentation Program for large scale purification and assay of interferons for the BRMP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09203-01 BDB

PERIOD COVERED
January 12, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Guinea Pig Mammary Carcinoma Monoclonal Antibodies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael I. Bernhard	Expert, MoAb Section	BDB	NCI
Other:	John W. Pearson	Microbiologist, Basic Mechanisms Sect.	BDB	NCI

COOPERATING UNITS (if any)
FCRC Biological Markers Program

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A spontaneous transplantable mammary carcinomia was discovered in guinea pigs. Hybridomas have been produced (by immunizing BALB/c mice) which react in a livecell RIA to the tumor cells and not to syngeneic guinea pig spleen and liver cells.

After extensive characterization by numerous normal & tumor-derived guinea pig cells and by immunochemical techniques, the selected MoAbs will be used directly in immunotherapy trials and after conjugation to various drugs and toxins (i.e. daunomycin, ricin, etc),

Attempts are underway to put this tumor into culture. Spontaneous metastases formed in guinea pigs and MoAb to tumor metastasis will be developed and may prove to be more useful than MoAb to the primary tumor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09204-01 BDB

PERIOD COVERED
February 3, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Guinea Pig Line 10 Monoclonal Antibodies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael I. Bernhard	Expert, MoAb Section	BDB	NCI
Other:	Robert K. Oldham	Associate Director	BRMP	NCI

COOPERATING UNITS (if any)

FCRC Cancer Metastasis and Treatment Laboratory

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal Antibodies (MoAb) to the guinea pig line 10 hepatoma have been produced. Two clones were selected and put into ascites in mice. The original ascites fluid produced in BALB/c mice from clone C-2 titered to $1/1x10^6$ vs. line 10 hepatoma cells with minimal cross-reactivity to guinea pig line 1 and normal liver & spleen cells by live cell radioimmunoassays (RIA) utilizing iodinated protein A. By immunofluorescence (IF) and cytotoxicity (CTX) assays the cross-reactivity was as high as 30%. Isotyping showed the MoAb to be an IgG_1. Frozen C-2 hybridoma cells have been put back into tissue culture and are no longer producing immunoglobulins. No further work with the C-2 clone is anticipated. Clone D-3 has been put in tissue culture and supernatant fluids show very high titer ($1/5x10^4$) vs. line 10 and no cross-reactivity with line 1 by RIA. Preliminary isotyping indicates a probable I_gG_1. The clone has been put into mice for ascites production, and complete characterization by RIA, IF and CTX will be done to determine specificity. Fluorescent activated cell sorting (FACS) analysis of specificity is underway. In vivo therapy experiments with the monoclonal antibody in this guinea pig needed will follow.

Immunotherapy protocols in guinea pigs are anticipated using modified Winn-type assays as well as attempts to cure pre-existing tumors, paying special attention to the probable development of host antibodies to mouse immunoglobulin in the latter case, and the effect of these antibodies in immunotherapy. This may prove to be a useful model system for human tumor immunotherapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09205-01 BDB

PERIOD COVERED
December 22, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
MCF-7 Monoclonal Antibody

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael I. Bernhard	Expert, MoAb Section	BDB	NCI
Other:	Robert K. Oldham	Associate Director	BRMP	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hybridomas (mouse x mouse) have been prepared to the human breast cancer cell line MCF-7. Clones producing specific antibody have been selected and will be screened vs. live MCF-7 and numerous other normal and tumorogenic human cell lines.

Selected clones will be put into BALB/c mice for ascites production. Isotyping, immunofluorescence, cytotoxic and immunoperoxidase will be performed on tumor and normal tissue sections as well as two-dimensional SDS-page analysis to fully characterize the MoAb.

Cross-reactivity with other human cells (normal adult & normal fetal, tumor) will be assessed. Diagnostic and immunotherapeutic potential will be determined. This should provide a useful model system for MoAb immunotherapy by transplanting tumors to nude mice. Immunoperoxidase staining of tumor and normal tissue sections will provide further information.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09206-01 BDB

PERIOD COVERED

May, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Interspecies Monoclonal Antibodies Against Human Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Abrams	Expert, Clinic. Investig. Section	BDB	NCI
Other:	Kenneth Foon	Chief, MoAb Section	BDB	NCI
	Michael Bernhard	Expert, MoAb Section	BDB	NCI
	Jeffrey Ochs	Medical Staff Fellow	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Development Branch

SECTION

Monoclonal Antibody Section

INSTITUTE AND LOCATION

NCI, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal antibodies selective against various human malignancies would be powerful diagnostic and possibly therapeutic tools. The ability to raise interspecies antibodies against the desired, but unknown, antigens depends upon the strategies employed. The purpose of this study is to concentrate on a few common human tumors and to use a variety of strategies with regard to preparation of the immunogen, mode of immunization, schedule of immunization and strains of laboratory animals to develop a panel of monoclonal antibodies against adenocarcinoma and epidermoid carcinoma of the lung, and by mass screening, to select those with the greatest potential for use in clinical practice. Human adenocarcinoma and epidermoid carcinoma of the lung lines have been obtained and are growing well in standard tissue culture. Antigens from antigen-antibody complexes of these tumors have also been developed and are currently frozen. Immunizations shall commence as soon as sufficient assay systems and the personnel to use them are in place.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09207-01 BDB

PERIOD COVERED
May, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Characterization of Mouse Monoclonal Antibodies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Abrams	Expert, Clinic. Investig. Section	BDB	NCI
Other:	Michael Bernhard	Expert, MoAb Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two mouse monoclonal antibodies, IgG2a and IgG1 respectively, were produced by the principal investigator by fusing splenocytes from mice immunized with an adenocarcinoma of the lung cell line and screened against a large battery of human tumors and normal tissues and found to react with non-small cell lung cancer lines but not small cell lung cancer, with a variety of other human tumors, skin fibroblasts cultured in fetal calf serum but not B-cells, T-cells or normal autopsy tissues. The objectives of this study is to further characterize the range of reactivity of these antibodies, and grow them into mass production in ascites for possible use in monitoring and/or treatment of appropriate human malignancies. The antibody-producing cells have been grown in tissue culture and are nearly ready to be grown in mouse ascites. Plans are to test the cells for their ability to precipitate proteins from malignant cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09208-01 BDB

PERIOD COVERED
May, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Human Monoclonal Antibodies against Human Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Abrams	Expert, Clinic. Investig. Section	BDB	NCI
Other:	Michael Bernhard	Expert, MoAb Section	BDB	NCI
	Jeffrey Ochs	Medical Staff Fellow	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Monoclonal Antibody Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal antibodies offer the potential of developing reagents which are specific to human tumors. The "traditional" approach has been to prepare rodent antibodies against human tumors. While promising, these have potential disadvantages: selecting the correct immunogen, and eventually administering rodent serum repeatedly to human subjects. The advent of continuously growing, HGPRT negative human myeloma cell lines may circumvent these problems: human lymphocytes from patients, which would theoretically recognize only aberrant antigens, could be fused to produce relatively stable clones of human antibodies, which may also have less chance of causing serum sickness reactions when administered repeatedly to patients. The objective is to use lines developed by others and one by the principal investigator to fuse with human lymphocytes to form human-human hybrids producing tumor specific human antibodies against various human malignancies. The laboratory has only recently opened; so far fusions with a lymph node suspension from a patient with colon carcinoma, and lymphocytes from the pleural effusion of patient with small cell lung cancer have been performed, and the initial screening of the hybrids is being done.

TUMOR ANTIGENS SECTION

The Tumor Antigens Section (TAS) was established to investigate tumor associated antigens and/or tumor specific antigens as therapeutic reagents and biological response modifiers in neoplastic disorders. The advent of monoclonal antibodies as effective reagents in identifying and separating tumor associated antigens (TAA), was a major reason for establishing this section within the BRMP. The objectives of this section are to identify, characterize and finally purify tumor associated membrane antigens, both as therapeutic agents and as biologic probes, to better understand the nature of the cancer cell membrane. Immunochemical methods and monoclonal antibody will be used in a complimentary way to identify and purify these antigens.

Dr. Alton C. Morgan, Jr. was recruited from Dr. Ralph Reisfeld's laboratory because of his extensive background in the use of monoclonal antibody in purifying membrane associated antigens. He has been involved with the development of two monoclonal antibodies which have potential therapeutic impact on melanoma and breast cancer. In addition, another monoclonal antibody may have therapeutic importance as a broad based therapeutic agent in cancer. His strong immunological background and his immunochemical capabilities should allow this section to approach its objectives during FY 82.
Dr. Edward S. Kimball also joined the program in FY 81 and has a strong background in immunochemical purification of DR antigens from cell membranes. His capabilities in this area and his interest in approaching the immunochemical isolation in both tumor associated antigens and of lymphokines will lend considerable strength to the research capabilities of this section.

Although the projects of this section are detailed in the research project to follow, it is important to note that the antimelanoma monoclonal antibody developed by Dr. Morgan is already being produced in large quantities in anticipation of a clinical trial. Dr. Kimball is currently pursuing the isolation of macrophage activating factor, a lymphokine soon to be utilized in clinical trials. Thus, the research activities of this section are already having an impact on the development of reagents for clinical trial.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09209-01 BDB

PERIOD COVERED
June 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
In Vivo Human Lymphoid and Tumor Cell Interactions: Studies in Human Lymphoid-Nude Mouse Chimeras

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Alton C. Morgan, Jr. Expert, Tumor Antigens Section BDB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Tumor Antigens Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In Vivo Human Lymphoid and Tumor Cell Interactions: Studies in Human Lymphoid-Nude Mouse Chimeras.

Nude mice, which are congenitally athymic, will not reject xenografts of human normal or neoplastic tissues including normal lymphoid tissues. It should thus be possible to create chimeric nude mice whose lymphoid system has been replaced by human cells. This will be accomplished by total body irradiation of nude mice (400-800 R) followed by reconstitution with adult human bone previously depleted of T-cells. T-cell depletion will be done by incubation of bone marrow cells with guinea pig or rabbit complement and monoclonal antibody to pan-T-cell determinants (anti-Leu-1 or OKT3). These antibodies have been shown to inhibit MLC, an in vitro corollary of graft versus host disease, and thus T-cell depletion should lead to the establishment of stable chimeras. Reconstitution with grafted cells will be monitored by histology of lymph nodes and spleens and by the number of cells reactive with monoclonal antibody to HLA-A,B,C (W6/32). Establishment of stable chimeras will then allow lateral

grafting of chimeric spleen cells to other nude mice. These animals can then be utilized in a variety of studies to: 1) determine the effect of thymic reconstitution on the chimeric state, (a) induction of tolerance to preexisting xenografts and (b) reconstitution of T-dependent responses; 2) determine whether tumor antigens defined by monoclonal antibodies can function as transplantation rejection antigens; and 3) develop human-human monoclonal antibodies to tumor associated antigens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09210-01 BDB

PERIOD COVERED
June 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Heterogenity of Human Melanoma Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alton C. Morgan, Jr.	Expert, Tumor Antigens Section	BDB	NCI
Other:	Edward S. Kimball	Sr. Staff Fellow, Tumor Antigens Section	BDB	NCI

COOPERATING UNITS (if any)

Scripps Clinic and Research Foundation, La Jolla, California

LAB/BRANCH
Biological Development Branch

SECTION
Tumor Antigens Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular Heterogenity of Human Melanoma Associated Antigens

Recent studies have described a 250,000 M.W. glycoprotein that is associated with proteoglycan, and seemingly expressed only by human melanoma cells. With only rare exceptions the glycoprotein can be found on the cell surface and in spent culture medium of melanoma of ocular or skin origin but not normal melanocytes both in vitro and in vivo. Preliminary evidence indicates that the glycoprotein is also expressed by gliomas and tumorgenic somatic hybrids of malignant and normal cells. Thus far, antigen, isolated from these varied sources utilizing monoclonal antibody, shows only slight variation in molecular weight by SDS-PAGE analysis and highly variable association with proteoglycan. The object of these studies is to determine if the molecular structure of this tumor associated antigen is conserved when isolated from different cellular sources. This will be accomplished with tryptic peptide mappling by reverse phase HPLC and limited N-terminal amino acid sequencing. Special emphasis will be placed on comparing glycoprotein isolated from multiple melanoma

cell lines derived from the same patient. These studies should give insight into the functional significance of this human melanoma associated antigen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09211-01 BDB

PERIOD COVERED
April 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Characterization of Circulating Immune Complexes with Monoclonal Antibody to Human Melanoma Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Alton C. Morgan, Jr. Expert Tumor Antigens Section BDB NCI

COOPERATING UNITS (if any)
Baylor College of Medicine and Veterans Administration Medical Center, Houston, Texas

LAB/BRANCH
Biological Development Branch

SECTION
Tumor Antigens Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Characterization of Circulating Immune Complexes with Monoclonal Antibody to Human Melanoma Antigens.

Circulating immune complexes have been detected in cancer patient sera utilizing antigen non-specific assays. The etiology and molecular nature of these complexes is not well understood. Methods of immune complex detection, utilizing monoclonal antibody containing tumor associated antigens, should reduce the complexity of interpretation of assay results and provide an unequivocal answer as to whether immune complexes containing tumor antigens play a role in the neoplastic disease process. Utilizing HPLC gel filtration and solid phase ELISA technology and monoclonal antibodies raised to human melanoma antigens from spent culture medium, tumor antigen containing complexes will be detected and the components characterized. The characterization will include: 1) molecular ratios of antigen to antibody; 2) the class of patient antibody; 3) detection and specificity of antiglobulin; and 4) molecular nature of tumor antigen as compared to the form in spent culture medium. A variety of solid

phase reagents for affinity isolation of tumor antigen containing immune complexes will be tested. These include soluble protein A, purified human Clq and monoclonal antibody to C3b, IgM, IgG, and melanoma associated antigens. Sera utilized for isolation and characterization of tumor antigen containing complexes will be pretested by four antigen non-specific assays for immune complexes, including the Clq binding and Raji cell radioimmunoassay.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09212-01 BDB

PERIOD COVERED
February 2, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies on Macrophage Activating Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edward Kimball	Senior Staff Fellow, Tumor Antigens Sec.	BDB	NCI
Other:	Gary B. Thurman	Acting Chief, Lymphokines Section	BDB	NCI
	Henry C. Stevenson	Chief, Basic Mechanism Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Tumor Antigens Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Peripheral blood cells and certain lymphoblastoid tissue culture cell lines secrete a variety of soluble substances called lymphokines which in turn activate other cells in the body. One of these lymphokines, Macrophage Activation Factor (MAF) has the ability to induce resting macrophages to become cytotoxic to tumor cells. There has not been any clinical trials of MAF due to difficulties in obtaining this material in large enough amounts and of high enough purity. Production of human MAF of high quality, would allow one to study the primary amino acid sequence structure of MAF its subunit organization, and delineate the active binding site(s) in the molecule. MAF has been isolated from a Burkitt's lymphoma cell line, Namalva, by standard proteins chemistry techniques. These include G-100 chromatography and affinity chromatography over mixed brais gangliosides. We have currently made arrangements to obtain the culture supernatant from 500 liters of Namalva cells (approx. 10^{12} cells) and to begin various tractionations procedures which will include concentration by TCA precipitation biological activity for MAF will be assayed by measuring cytotoxicity of tumor cell targets culture with MAF-treated macrophages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09213-01 BDB

PERIOD COVERED
February 2, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Characterization of Tumor Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edward Kimball	Senior Staff Fellow, Tumor Antigens Sec.	BDB	NCI
Other:	Alton C. Morgan	Expert, Tumor Antigens Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Tumor Antigens Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tumor Antigens, or molecules unique to the membrane of a tumor cell, as such, have yet to be systematically studied on a tumor-by-tumor basis. It is our intention to obtain tumors of all types and routinely prepare isolated membranes from them for future studies. As part of these studies, the constituents of the tumor cell membranes will be solubilized and fractionated according to solubilities in a variety of solvent systems. These include n-BuOH, 4M Urea and detergents. The antigens thus dissolved will be further characterized by size exclusions, differential lectin affinity, and by hydrophobic chromatography and will be examined for reactivity with existing monoclonal antibodies as well as be used for generating monoclonal antibodies to novel antigens. Ultimately, we hope to examine size and subunit structure, isoelectric focusing patterns, extent of glycosylation, amino acid composition and sequence, cross reactivity with antibodies to other tumor antigens as well as antigens on normal tissues and possibly examine degrees of relatedness by peptide mapping techniques.

LYMPHOKINES SECTION

The Lymphokines Section of the BDB investigates the mechanisms of action and therapeutic usefulness of lymphokines and other cellular products in the treatment of cancer. The three major immediate goals of this section are: (1) The development of sources of lymphokines by the evaluation of cell lines for the constitutive or inducible production of lymphokines, and the development of prototypes for large scale production and purification of lymphokines; (2) The evaluation of the immunological and biochemical effects of lymphokine treatment on mononuclear cells; and (3) The development and testing of tumor models capable of evaluating the use of lymphokines or lymphokine-treated cells for immunotherapy of cancer.

The senior personnel recruited for this section includes the following scientists:

Gary B. Thurman, Ph.D., Acting Chief
Luigi Varesio, Ph.D., Visiting Associate

During the year, the Lymphokines Section has developed methodology for the rapid evaluation of cell-lines for their production of factors such as Interleukin 1 (IL1, Lymphocyte Activation Factor), Interleukin 2 (IL2, T cell Growth Factor), macrophage Migration Inhibition Factor (MIF) and Macrophage Activation Factor (MAF). A method for rapid and accurate measurement of endotoxin contamination of cell line supernatants has been adapted to our protocols and will detect endotoxin levels as low as 0.1 ng/ml. Methods for endotoxin removal from endotoxin-bearing samples are being evaluated and methods for eliminating the lymphokine-mimicing effects of endotoxin are being developed. As first reported at the BRMP Lymphokine Workshop in March, 1981, at FCRC, a thymosin polypeptide has been found to have MIF activity at nanomolar concentrations. This polypeptide, thymosin B_4, is one of a family of thymic polypeptides and the involvement of thymic hormones in the generation of lymphokine-producing cells is being studied. Thymosin B_4 is the first thymic polypeptide with demonstrated lymphokine activity and other purified thymosin polypeptides are being evaluated for lymphokine activity in collaboration with Drs. A.L. Goldstein and T.L.K. Low at George Washington University.

The effects of lymphokines on macromolecular synthesis of protein or RNA in lymphocytes and macrophages is being actively pursued. It has been found that macrophages responding to MAF have a marked decrease in RNA synthesis and that activated macrophages have a suppressive effect on lymphocyte protein synthesis and lymphokine production. We are extending these observations to determine the role these macromolecular events play in tumor cell killing by activated macrophages. Actinomycin D inhibition of RNA synthesis has also been shown to induce the cytolytic capacity of non-activated macrophages. The role of activated macrophages in the regulation or suppression of lymphokine production is being studied to determine the macromolecular method whereby suppressor macrophage act to suppress lymphocyte reactivity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 09214-01 BDB

PERIOD COVERED
July 1, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Roles of Thymic Hormones in the Maturation of Lymphokine Producing Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Gary B. Thurman Acting Chief, Lymphokines Section BDB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Lymphokines Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many lymphokines of particular interest in tumor immunology are secreted by lymphocytes that have trafficed through the thymus gland. The exact nature of maturational steps and the thymic polypeptides that control these steps are not known. Although much progress has been made in the separation and purification of thymic polypeptides, little is still known about which of these thymic polypeptides act on lymphocyte maturation, act on lymphokine production or are lymphokines themselves. Two thymosin polypeptides have shown distinct properties in the production or action of lymphokines. Thymosin α_1 (28 amino acids, mol. wt. of 3108 and a pI of 4.2) has been shown to induce the capability of antigenreactive peripheral blood lymphocytes of thymectomized guinea pigs to produce macrophage Migration Inhibition Factor (MIF). This has been shown both by the agarose droplet MIF assay and by the agarose droplet MIF assay. Another polypeptide, thymosin β_4 (43 amino acids, mol. wt. of 4982 and a pI of 5.1) mimics MIF or is the active component of MIF in that it inhibits macrophage migration itself in a non-cytotoxic manner.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09215-01 BDB

PERIOD COVERED
January 1, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Lymphokine Production and Analysis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gary B. Thurman	Acting Chief, Lymphokines Section	BDB	NCI
Other:	Luigi Varesio	Visiting Assoc., Lymphokines Sect.	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Lymphokines Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland, 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lymphokines are involved in the cell-to-cell communication of immunologically reactive cells. Lymphocytes, when stimulated, release products such as T Cell Growth Factor (TCGF), Macrophage Migration Inhibition Factor (MIF), and Macrophage Activation Factor (MAF). TCGF (also known as Interleukin 2) is being studied for its ability to perpetuate cytotic T-Lymphocytes which can be cloned for cytotoxic capability against specific tumor cells. Using TCGF, cytotoxic cells generated in vitro are being tested for their capability of influencing tumor growth in vivo. MIF is being studied for its ability to localize tumor-reactive macrophages at tumor sites. MAF is being studied for its macromolecular effects on macrophages and for its ability to activate macrophages to kill neoplastic cells. Novel approaches for the production and purification of lymphokines are being evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09216-01 BDB

PERIOD COVERED
March 1, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Macromolecular Events in Lymphokine Production and Utilization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Luigi Varesio	Visiting Associate, Lymphokines Section	BDB	NCI
Other:	Gary B. Thurman	Acting Chief, Lymphokines Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Lymphokines Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lymphocytes undergoing reactions to tumor cells release a variety of factors that influence the reactions of other lymphocytes and macrophages. One factor, called Macrophage Activation Factor (MAF) induces a series of biochemical events to occur in macrophages that enables them to gain cytotoxic potential for neoplastic cells. A definite reduction of RNA synthesis has been documented and it has been shown that this reduction is directly involved in cytotoxic potential development. Using radioisotopic methodologies of determining cell viability, it has been determined that blocking of RNA synthesis artificially with actinomycin D leads to macrophage activation and the development of cytotoxicity for tumor cells. It has also been determined that macrophages can have a definite effect on the production of lymphokines and that suppressor macrophages from tumor bearing animals suppress the production of lymphokines. The possibility that immunosuppression in tumor-bearing hosts occurs via this mechanism is being investigated.

THE BASIC MECHANISMS SECTION

The Basic Mechanisms Section investigates the mechanism of action of biologic response modifiers at the cellular, membrane and molecular level. The section interacts with the other sections of the BRMP by identifying new biologic response modifiers (BRM) in the course of basic cancer immunology research and also by providing laboratory systems with which identified biologic response modifiers can be further characterized.

During the FY 81 period, personnel were recruited to staff the Basic Mechanisms Section of the BDB, BRMP. Henry C. Stevenson, M.D. was named section chief in November 1980. Other senior personnel recruited during FY 81 are as follows:

John W. Pearson, Ph.D., Research Microbiologist
Eugenie S. Kleinerman, M.D., Senior Investigator
Craig W. Reynolds, Ph.D., Staff Fellow

This section also provides laboratory space to BRMP clinicians with research interests in basic mechanisms. These investigators are Dr. Stephen Sherwin and Dr. James Knost of the Clinical Investigations Section.

Initial startup activities for this section, like the other BDB Sections, have focused on operational development, support personnel recruitment and equipment procurement.

Considerable progress has been made in the initiation of projects relevant to this section.

Cytotoxicity assays for NK cells and macrophages have been developed using chromium-labelled targets. The discriminatory way in which chemotherapeutic agents affect human monocyte cytotoxicity has been assessed. The biochemical basis for the human monocyte cytotoxic event is being investigated. We have developed assays of human B cell function utilizing an ELISA automated assay system sensitive to 10 ng of secreted immunoglobulin per ml of cell culture supernatant. With the elutriator we are capable of isolating in very high purity up to 1 billion monocytes from a single normal human donor. These cells can be assayed for purity with battery of assays consisting of cell sizing, phagocytosis, estorase staining, Wright's stainig and Fc receptor function. (Additional assays now in use include assaying for surface immunoglobulin and sheep erythrocyte receptors.) Human monocytes are obtained from the elutriator in suspension and can be placed in suspension culture to allow the normal monocytes to mature into macrophages. Up to 5 billion normal human monocytes can be effectively monocyte-depleted with the elutriator. This number of lymphocytes can be rapidly T cell enriched over the course of two hours with a newly-developed AET rosetting technique. In addition, experiments to purify normal human NK cells and B cells with elutriation technology are underway.

A series of mouse-hybrid cell lines secreting anti-human monocyte antiodies have been established. These hybridomas are currently being characterizied as to growth and antibody secreting capacities.

Other activities include the development of a spontaneous mammary carcinoma model in guinea pigs. Monoclonal antibodies against this tumor are being devloped.

A rat model for in vivo assessment of NK activity has been established. This system allows for the reconstitution of immunosuppressed animals with in vitro purified NK cells. Natural killer cell clones from this animal system are being developed. The functional characteristics of a human monocyte cell line, V937, are being investigated, including the effect of BRMs as modulators of the cytotoxic functins of this cell.

A cytapheresis unit is being developed at the BRMP Outpatient Unit. Henry C. Stevenson will be the physician-in-charge of the cytapheresis operations. Pending renovations, the unit will be operational in late FY 81 or early FY 82. A head nurse has been recruited to supervise the daily functions of the cytapheresis unit.

The cytapheresis unit be utilized for highly experimental research applications. Patients with ciruclating tumor antigens or immune complexes will be plasmapheresed against affinity columns prior to the administration of tumor specific monoclonal antibodies. Very large numbers of monocytes, NK cells and/or cytolytic T cells will be removed for in vitro augmentation of function prior to re-administration to the cancer patient. A mechanism for pre-testing these experimental systems in animals has been established. The cytapheresis unit will also harvest large numbers of normal human B cells, NK cells, T cells and monocytes for cryopreservation as standards for a number of immunologic assays including BRM screening.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09217-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Role of Monocytes of the Human B Cell Activation Process

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Henry C. Stevenson Chief, Basic Mechanisms Section BDB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human monocytes are critical cells in the initiation of the B cell activation process to antigens and certain mitogens. A very sensitive ELISA assay system has been developed which allows for the measurement of human B cell function in vitro. Elutriation technology will allow us to separate monocytes and evaluate the nature of their role in B cell activation. This system will also be utilized to localize the site(s) of BRM's activity in the cell-to-cell interactions operative in the human B cell activation process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09218-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Long Term Culture of Human Monocytes Grown in Suspension

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Henry C. Stevenson Chief, Basic Mechanisms Section BDB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanism Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Up to 1 billion normal human monocytes from a single donar can be purified in suspension by elutriation. These negatively selected monocytes have been grown in suspension cultures and allowed to mature into macrophages. The changes in monocyte functions that occur as monocytes mature into macrophages are in the process of being assessed. This system is also being utilized for the screening of monocyte growth factor activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09219-01 BDB

PERIOD COVERED
November, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Role of Liposomes in Human Monocyte Cytotoxicity Activation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Henry C. Stevenson Chief, Basic Mechanisms Section BDB NCI

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, FCRC

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human elutriator monocytes can be obtained in suspension in large numbers. Their spontaneous cytotoxicity against several tumor targets has been assessed. The types of liposomes that best incorporate into human monocytes has been investigated. The ability of BRMs to incorporate into liposomes and augment human monocyte cytotoxicity is being assessed. The ultrastructural findings associated with liposome-augmented human monocyte cytotoxicity is being assessed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09220-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Elutriation of Human Mononuclear Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Henry C. Stevenson	Chief, Basic Mechanisms Section	BDB	NCI
Other:	Robert K. Oldham	Associate Director, BRMP	BDB	NCI
	Craig W. Reynolds	Staff Fellow, Basic Mechanisms Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Leukapheresis and elutriation have been utilized in a tandem fashion to obtain up to 1 billion purified monocytes (in suspension) and 6 billion monocyte-depleted lymphocytes from a single normal human donor. Innovative elutriation techniques are now being developed to allow the purification of very large numbers of natural killer cells (NK), B cells, and T cell subsets (helper, suppressor and cytotoxic) for subsequent research investigations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09221-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Applications of Leukapheresis to Patients with Malignancy in an Attempt to Augment Their Immune System Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Henry C. Stevenson	Chief, Basic Mechanisms Section	BDB	NCI
Other:	Robert K. Oldham	Associate Director, BRMP	BDB	NCI
	Alton C. Morgan	Expert, Tumor Antigens Section	BDB	NCI
	Stephen A. Sherwin	Acting Chief, Clin. Invest. Sect.	BDB	NCI

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, FCRC

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Leukapheresis allows for the removal of very large numbers of human mononuclear cells (the major effector cells of the immune system) and/or plasma. This technology is being applied to cancer patients with tumor antigens found in their peripheral blood to remove soluble antigen prior to monoclonal antibody (directed against their tumor) treatment. This system will also be directed to the leukapheresis removal of large numbers of NK cells, monocytes and/or cytolytic T-cells for in vitro augmentation prior to re-infusion back into the patient. A mechanism for dog testing of these experimental systems is being developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09222-01 BDB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Various Chemotherapeutic Agents on Monocyte-Mediated Cytotoxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eugenie S. Kleinerman	Sr. Invest., Basic Mechanisms Sect.	BDB NCI
Other:	Leonard A. Zwelling	Cancer Expert, Lab. of Mole. Pharma.	DTPB NCI
	Andrew V. Muchmore	Sr. Invest., Matabolism Branch	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Development Branch

SECTION

Basic Mechanisms Section

INSTITUTE AND LOCATION

NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

When peripheral blood monocytes are cultured in vitro they spontaneously develop a cytotoxic ability and will lyse a variety of target cells including red blood cell and tumor targets. The effect of various cancer chemotherapeutic agents, including L-phenylalanine mustard (L-PAM), cis-DDP; Adriamycin (ADR) and Actinomycin D (Act.D.) on this cytotoxic function was investigated. Using ^{51}Cr-labeled chicken red cells (CRC) as targets, we found that L-PAM, ADR, and cis-DDP all enhanced monocyte-mediated cytotoxicity (MMC). While cis-DDP and ADR enhanced cytotoxicity by direct stimulation of the killer monocyte; L-PAM affect both the killer monocyte and the suppressor lymphocyte. By contrast Actinomycin D suppressed cytotoxic function. We would like to propose that chemotherapeutic agents may in addition to causing direct tumor cytotoxicity also enhance normal immune function and therefore aid in host tumor rejection.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09223-01 BDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Development of Cytotoxic Function in a Human Monocyte Cell Line

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eugenie S. Kleinerman	Sr. Invest., Basic Mechanisms Sect.	BDB	NCI
Other:	Ronald H. Schwartz	Sr. Invest., LI,		NIAID

COOPERATING UNITS (if any)

Department of Medicine, Div. of Rheumatology, Duke Med. Cntr., Durham, NC

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A human monocyte-like cell line, U937, can be stimulated to differentiate into macrophages in vitro in the presence of lymphokines. These U937 cells do not kill in vitro spontaneously even after activation. However, when co-cultured for 7 days with normal human lymphocytes these U937 cell will show cytotoxic function. It is felt that a helper lymphocyte may be necessary in the development of monocyte cytotoxicity and it is hoped that through studying these and other monocyte cell lines that the mechanism of monocyte activation can be more clearly defined.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09224-01 BDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effect of AMSA on Monocyte Cytotoxicity In Vitro and In Vivo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eugenie S. Kleinerman	Sr. Invest., Basic Mechanism Sect.	BDB	NCI
Other:	Ken Micetich	Clinical Associate,	MD	NCI
	Leonard A. Zwelling	Cancer Expert, Lab. of Mole Pharma.	DTPB	NCI
	Robert C. Young	Chief,	MD	NCI
	Kurt Kohn	Chief, Lab. of Mole. Pharma.	DTPB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of m-AMSA, an effective chemotherapeutic agent used in the treatment of leukemia, on spontaneous monocyte-mediated cytotoxicity (SMMC) was investigated. Using the cytotoxicity assay previously described, it was found that pulsing peripheral MNLs with m-AMSA for 15 minutes had no effect on SMMC. However, when doses of 0.1-1 M were left in vitro throughout the culture period, AMSA increased SMMC 2-4 fold. Using a 72 hour continuous infusion protocol to give m-AMSA IV to patients with various malignancies, we are presently studying the effect of this agent in vivo on SMMC.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09225-01 BDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Mechanism of Spontaneous Monocyte-Mediated Killing

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eugenie S. Kleinerman	Sr. Invest., Basic Mechanisms Sect.	BDB	NCI
Other:	Robert Hall	Clinical Associate	MB	NCI

COOPERATING UNITS (if any)
Div. of Rheumatology, Dept. of Med., Duke Medical Center, Durham, NC
Clinical Pharmacology Branch, NCI

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of monocyte-mediated killing is poorly understood. We have been investigating the mechanism of killing through various biochemical pathways. While SOD, and catalase inhibit killing, direct measurement of superoxide production shows that both killer cells and fresh cells which do not kill produce equal amounts of superoxide. Likewise prostaglandin biosynthesis has no effect on killer function. Since S-adenosyl-L-methionine methylation is important in monocyte functions, we will investigate the effects of inhibiting methylation with competitive inhibitors. In addition, the lipid content of killer and non-killer cells is being analyzed to see if the biosynthesis of macromolecules changes when these cells differentiate into killer cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09226-01 BDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effect of Biologic Response Modifiers (BRMs) of Guinea Pig Spontaneous Mammary Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John W. Pearson	Research Microbiologist	BDB	NCI
Other:	Robert K. Oldham	Associate Director, BRMP	BDB	NCI

COOPERATING UNITS (if any)

Cancer Metastasis and Treatment Laboratory, FCRC

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A spontaneous guinea pig mammary carcinoma in vivo system has been developed and is in the process of being clinically characterized. The baseline immunologic parameters of the normal and tumor-bearing animal are being assessed. A monoclonal antibody against the tumor cell surface antigens is being developed. This animal system will be utilized to assess the effect of BRMs on the immunologic function of animals with spontaneously occurring tumors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09227-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Role of Biological Response Modifiers in the Augmentation of Human NK Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Craig W. Reynolds	Staff Fellow, Basic Mechanisms Sect.	BDB	NCI
Other:	Henry C. Stevenson	Chief, Basic Mechanisms Sect.	BDB	NCI
	Robert K. Oldham	Associate Director, BRMP		NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The augmentation and suppression of natural killer (NK) activity in humans will be studied. This will include experiments to determine the effect of biological response modifiers (BRMs) on both in vivo and in vitro NK activity. In addition, in vitro studies will examine the functional changes which occur in NK cells during their activation or suppression.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09228-01 BDB

PERIOD COVERED
November 1, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
NK Activity in the Rat: The In Vivo Role in the Immunosurveillence of Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Craig W. Reynolds	Staff Fellow	BDB	NCI
Other:	Ronald B. Herberman	Chief, Laboratory of Immunodiagnosis		NCI
	Robert K. Oldham	Associate Director	BRMP	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Natural killer (NK) cells will be isolated from rats and their role in the in vivo protection to transplantable and chemically induced tumors will be investigated. These studies will involve the reconstitution of immunosuppressed animals with purified populations of rat NK cells and the suppression of NK activity in vivo by the injection of specific monoclonal antibodies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09229-01 BDB

PERIOD COVERED
November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Establishment of IL-2 Dependent Cytotoxic Lymphocytes in the Rat: In Vitro Cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Craig W. Reynolds Staff Fellow, Basic Mechanisms Section BDB NCI

COOPERATING UNITS (if any)

University of Helsinki, Department of Pathology, Finland

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies will involve the establishement of cytotoxic lymphocyte cell lines in the rat. In particular, we are interested in establishing cell lines from natural killer cells and additional cell lines with specific cytotoxicity for transplantable tumors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09230-01 BDB

PERIOD COVERED
February 2, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Relationship of Peptide Growth Factors to Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stephen A. Sherwin	Acting Chief, Clin. Invest. Section	BDB	NCI
Other:	Daniel R. Twardzik	Biochemist	LVC	NCI
	George J. Todaro	Chief	LVC	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	None

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied the relationship of peptide growth factors to various types of human malignancy in vitro A series of well-characterized human lung cancer cell lines were tested for the expression of growth factors receptors and the production of soft agar growth factors. Small cell and non-small cell lung cancer cells were found to differ in growth factor receptor expression but both types of lung cancer cells were capable of producing soft agar growth factors. A human mesothelioma cell line was studied in detail and found to produce an EGF-related soft agar growth factor similar to the previously described transforming growth factor (TGF). In addition, since these growth factors are acid stable and of low molecular weight, the urine of cancer patients was tested and found to contain soft agar growth factor activity. Preliminary results indicate the presence of unique molecular species of this activity not found in normal controls. The potential clinical applicability of these observations is being explored.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09231-01 BDB

PERIOD COVERED
February 2, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Screening Biological Response Modifiers with the Soft Agar System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: James A. Knost Expert, Clinical Investigations Section BDB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Development Branch

SECTION
Basic Mechanisms Section

INSTITUTE AND LOCATION
NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A soft agar assay system to screen both fresh human tumors and established cell lines for the effects of various biological response modifiers has been established. Modifications are currently being made to convert this system to a micro-assay procedure for the semi-automated evaluation of BRMs on tumor cells grown in the clonagenic assay system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09232-01 BDB

PERIOD COVERED

November 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Monoclonal Antibodies to Human Monocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jean-Yves Doulliard	Visiting Fellow, Lab. of Immunodiagnosis		NCI
Other:	Thomas M. Hoffman	Sr. Investigator, Lab. of Immunodiagnosis		NCI
	Ronald B. Herberman	Chief, Lab. of Immunodiagnosis		NCI
	Robert K. Oldham	Associate Director, BRMP	BDB	NCI
	Henry C. Stevenson	Chief, Basic Mechanisms Section	BDB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Development Branch

SECTION

Basic Mechanisms Section

INSTITUTE AND LOCATION

NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Numerous mice have been hyperimmunized with elutriator-purified human monocytes. After screening the fusion hybrid cell lines with 3 different assays, it appears that the antibody product of 2 lines recognizes antigens found only on monocytes, 3 lines recognize antigens found on monocytes and lymphocytes, and 2 lines recognize only lymphocytes. These monoclonal antibodies and the antigens they recognize will be further characterized. Monocytic leukemias will be screened for these antigens. The ability of the antibodies to modulate monocyte function will be assayed. Subsets of monocytes will be sought.

GENETIC ENGINEERING AND FERMENTATION SECTION

The Genetic Engineering and Fermentation Section was established as part of the BRMP to capitalize on the genetic engineering capability as the FCRC and to assist in the application of that capability toward the development of effective biologicals for treatment. Already, genetically engineered interferon fromm Hoffmann - La Roche is being utilized in clinical trials by the BRMP Clinical Investigations Section. This illustrates the rapidity with which developments in this area are occurring. There is a clear need for the effective application of genetic engineering principals to the development of therapeutic biologicals. Two major components of that development work include mass cell culture and the insertion of genetic messages into bacteria.

The fermentation pilot plant at the FCRC has been a Division of Cancer Treatment resource for many years. Previously heavily involved in the development of chemotherapeutic agents, this fermentation plant was ideally suited for mass cell cultures so necessary to the BRMP. With the advent of the BRMP, a portion of this pilot plant was dedicated to develop biologicals through mass cell culture. The fermentation pilot plant has actively produced interferon over the past year as has been described in the Developmental Therapeutics Program Progress Report. More recent developments have included the use of the pilot plant to grow cells producing interleukin 3, a biologic response modifier that induces helper cells and stimulates their division in vitro. The fermentation plant has also mass cultured cells as research reagents for various investigators around the NCI and in the extramural community. In FY 81 a committee was established to make decisions about the use of the fermentation pilot plant as a DCT resourse. It is anticipated that the future production of biologicals through mass cell culture will continue with a gradual shift toward the production of some of these biologicals through program bacteria in the future. The Genetic Engineering and Fermentation Section is currently recruiting two staff scientists to develop the BRMP capability in this area. One of these individuals has been identified and should be with the program by summer, 1981. As the personnel begin to work in this section, the BRMP expects to select a genetic material coding for biologicals such as lymphokines and other smaller molecular weight biologicals so that programmed bacteria for the production of these materials in highly purified form can be accomplished. It is expected that this section will be fully staffed and functioning by early 1982.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BALTIMORE CANCER RESEARCH PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 through September 30, 1981

The Baltimore Cancer Research Program has completed its 6th year at the University of Maryland Hospital. The Clinical Branch has provided for approximately 12,000 inpatient days and 16,000 outpatient visits.

The Clinical Branch continues to pursue Phase I and II drug testing. New drugs, such as AMSA, dihydroxyanthracenedione, aclacinomycin, vindesine and ADC have been studied in patients.

Phase III studies of hyperthermia and chemotherapy for patients with metastatic sarcoma and lung cancer are in progress. A major objective of these studies is to determine whether hyperthermia can alter efficacy on toxicity of cancer chemotherapeutic agents to the advantage of the patient.

Phase III studies of daunorubicin or adriamycin and cytosine arabinoside in adult acute nonlymphocytic leukemia have demonstrated a 70% complete response rate. Current studies in adult acute leukemia are evaluating various maintenance schemes, including late intensification therapy, splenectomy, and immunotherapy with neuraminadase-treated leukemic cells. Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage IIIA or with early stages associated with "E" substage of the lung. Evaluation of a recently completed Hodgkin's disease study comparing chemotherapy with radiotherapy plus chemotherapy shows that MOPP chemotherapy and radiotherapy are equally effective in producing complete responses in patients with stage II-IIIA Hodgkin's disease. These results have led to a joint study with other DCT clinical branches of early Hodgkin's disease which is testing the relative merits of radiotherapy, chemotherapy, and combined modality treatment. In addition, we have initiated a combination chemotherapy study of advanced Hodgkin's disease involving all DCT clinical branches.

Evaluation of over 100 patients with oat cell carcinoma of the lung who have received combination chemotherapy alone has revealed interesting results. The combination of cyclophosphamide, adriamycin, and VP16-213 appears to yield results comparable to other studies in which chemotherapy and radiotherapy were used together. A small fraction of patients treated on our chemotherapy alone studies are disease free and off all treatment for 6 to 18 months thus far. Prophylactic cranial irradiation is of value only when given after the achievement of complete remission.

Several active new drug combinations have been identified for metastatic breast, ovarian, cervical and bladder cancer.

Programs of the Section of Infection Research and Research Microbiology have continued in five major areas, namely surveillance, epidemiology, diagnosis,

treatment, and prevention. This work has led to the identification of refined empiric antibiotic combinations with which to treat febrile granulocytopenic cancer patients more and more successfully.

Studies with autologous frozen platelets in acute leukemia patients without compatible donors have shown them to be life saving. This study plus the prophylactic granulocyte transfusion study represent major contributions from our Cell Component Therapy Unit to our program and to the treatment of leukemia and cancer patients at large.

The Laboratory of Clinical Biochemistry general areas of investigation include studies of drug free radical pharmacology, the study of human metabolic pathways for new chemotherapeutic agents, the study of metal-anthracycline interaction and its relationship to anthracycline-related cardiotoxicity and anthracycline mechanism of action, the study of cellular control mechanisms that affect cell growth and differentiation, the study and identification of new biologic response modifiers, and, finally, certain immunological studies in conjunction with the Clinical Oncology Branch. All new drug clinical evaluations performed by that branch are correlated with LCB laboratory investigations. Clinical evaluation of new modalities of treatment, such as hyperthermia, is done in conjunction with the LCB. The laboratory is responsible for defining certain drug metabolic alterations that result from such new treatment modalities. Thus, the LCB performs basic scientific work related to drug pharmacology and metabolism in addition to sharing in clinical investigations with the COB.

A major reorganization of scientific priorities of the Laboratory of Molecular Biology has resulted in that laboratory assuming a larger share of the BCRP mission within the scope of the DCT program. Recently activated studies of this laboratory are designed to shed light on the mechanism of action of interferon and several important observations concerning interferon and nuclease interactions have been made. Technology developed in this laboratory has allowed for the identification of new nuclease species which may be disease-specific. Studies are underway in collaboration with the Clinical Oncology Branch to determine the significance of these enzymes and their potential usefulness as biomarkers. Studies of functions of viral proteins have uncovered new data potentially useful in understanding viral oncogenesis. Various interactions between Rauscher leukemia virus reverse transcriptase and P30 and P12 protein have been identified which may be important in determining the fidelity of transcription of viral DNA and retroviral replication. A new oncogenic virus has been isolated from AKR mice which appears to be a recombinant between ectotropic and xenotropic viruses. The presence of portions of the herpes simplex virus type 2 gene has been detected in leukemic cells from some patients and in tumor cells from patients with cervical carcinoma. These exciting studies are continuing in collaboration with the Clinical Oncology Branch. New studies investigating the mechanisms by which human leukemic cells differentiate when exposed to certain stimuli in vitro are under investigation in collaboration with the Clinical Oncology Branch.

Close scientific interaction among the various DCT branches in Bethesda, Baltimore, and Frederick continues. To date, this cooperation has led to joint lymphoma and leukemia protocols and to joint Phase I studies of drugs considered of prime importance to the DCT, including interferon. This cooperation has led to better utilization of clinical material on both campuses and to faster clinical results derived from larger numbers of patients that previously were not possible for the DCT intramural program.

SUMMARY REPORT

CLINICAL ONCOLOGY BRANCH

BALTIMORE CANCER RESEARCH PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1980 through September 30, 1981

The Clinical Oncology Branch has had its most productive year to date. Phase I studies of aclacinomycin and dihydroanthracenedione have been completed, and Phase I studies of ADC and interferon have been started. Phase II studies completed or nearing completion include AMSA in renal cell, ovarian cancer, and melanoma; methyl GAG in renal cell cancer, vindesine in lung cancer; and IV thioguanine in metastatic colon cancer. A Phase II study of thymidine and cytosine arabinoside for relapsed adult acute nonlymphocytic leukemia patients has yielded a 50 percent response rate. Phase III studies of anthracycline antibiotics in conjunction with cytosine arabinoside in acute nonlymphocytic leukemia have continued to yield complete response rates of approximately 70% and a 4 year disease free survival rate of 25%. Allogeneic neuraminadase-treated leukemic blast cells are under investigation as immunotherapy in these patients. Early results suggest remission duration prolongation with this treatment. The interferon inducer Poly IC:LC is under investigation in relapsed acute leukemia adult patients, and antileukemic effect has been demonstrated.

Hodgkin's disease treatment research continues to be a major interest of the Branch. Phase IV combined modality studies continue to show significant advantage over radiotherapy in patients with extensive Stage IIIA disease, and in patients with E substage disease (lung). Some of this work has recently been confirmed by a joint BCRP, Harvard, Vanderbilt, and University of Chicago study which has just been published. New Hodgkin's disease studies undertaken jointly by the Clinical Oncology Branch, BCRP and the Radiation Oncology and Medicine Branches, COP are well underway, and are designed to further define the optimal therapy for patients with Hodgkin's disease of all stages. Several more years of patient accrual will be necessary before meaningful results are obtained.

Studies of cyclophosphamide, adriamycin, and VP16-213 in oat cell carcinoma of the lung have resulted in a 90% response rate. Approximately 15 percent of patients so treated remain disease free off all therapy for 6-18 months after treatment thus far. It is hoped that some of these patients may actually be cured of their disease with chemotherapy alone.

Phase I and II studies in gynecological malignancies have recently been possible due to collaboration of the Department of Obstetrics and Gynecology of the University of Maryland Hospital with the Branch. A Phase II study of spirogermanium in gynecological cancer is nearing completion.

A Phase III study of vinblastine and Mitomycin C in advanced refractory metastatic carcinoma of the breast has shown that treatment to be highly active. An

international study of advanced breast cancer conducted under the auspices of Cancer and Leukemia Group B and chaired by members of the Branch has demonstrated the benefit of the addition of adriamycin to conventional treatment.

Phase III studies of hyperthermia and chemotherapy for lung cancer and metastatic sarcoma are underway. Twenty patients have been treated and pharmacokinetic data have been collected, but it is too early to fully evaluate the results. A study of hyperthermia alone for relapsed acute leukemia has been initiated.

In the Cell Component Therapy Section, studies of frozen autologous platelet transfusions for acute leukemia patients continue to be rewarding. We have transfused platelets, frozen at liquid nitrogen temperature for more than 5 years, back to the donor at a time when he was thrombocytopenic due to leukemic relapse. The transfusions have yielded increments suggesting approximately a 50% recovery rate of frozen platelets. A prophylactic granulocyte transfusion study has been completed. Newer methods of cryopreservation are under study. Indium radiolabelled granulocytes have been utilized in an effort to determine the fate of transfused granulocytes. This Section continues to supply human leukemic cells for study by several DCT laboratory branches in Baltimore, Bethesda, and Frederick.

Programs of the Section of Infection Research and Research Microbiology involve five major areas: surveillance, epidemiology, diagnosis, treatment, and prevention. Treatment studies have included evaluations of new antimicrobials, both in the laboratory and in the clinic. New drugs, including piperacillin, mezlocillin and trimethoprim, have been evaluated *in vitro* and in the clinic. Infection prevention has emphasized refinements of antimicrobial prophylaxis. The opening of a new 4 bed laminar air flow unit has allowed studies of relapsed leukemia patients, and patients over the age of 65 in this setting. Early results suggest significant infection prevention by these units in poor risk leukemia patients.

Biologic markers of malignant disease such as terminal transferase and reverse transcriptase have been studied in some detail and the data correlated with the type and extent of hematologic malignancy. New, previously unreported biomarkers are under study in oat cell carcinoma jointly by the Branch and the Frederick Cancer Research Center.

The massive research data accumulated by the Branch have been prepared for analysis on the PROMIS System. Physicians, nurses, pharmacists, and other related health care personnel take part in the input and the retrieval of this data. This system is undergoing major expansion as a result of the arrival of our own computer and terminal equipment.

Participation in oncology programs at the two medical schools in Baltimore and at other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community, the impact of the therapeutic advances resulting from the COB program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06259 08 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Extension of the Computerized PROMIS System into the Cancer Treatment Area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Charles A. Schiffer, M.D.	Head, Cell Component Therapy Section	COB,NCI
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	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
PROMIS Laboratory, Department of Medicine, University of Vermont, Burlington, Vermont
National Center for Health Services Research, OASH (NCHSR)

LAB/BRANCH
Clinical Oncology Branch

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Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

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9.0	9.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Cancer Research Program (BCRP) has developed cancer guidance displays (treatment protocols) on the computerized Problem Oriented Medical Information System (PROMIS) in Burlington, Vermont at the University of Vermont School of Medicine, with the aid of a communication linkage between BCRP and PROMIS. The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the phases of medical action of the Problem Oriented Medical Record. The problem-defining action determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. It is through the use of these explicit information and directional displays that the health care provider can couple learning with doing while gathering information and treating patients' cancer problems. The entire systems package has been shipped to BCRP and testing and debugging is underway in conjunction with the Interagency Agreement with the National Center for Health Services Research (Y01 CM 80109). This project ends on May 31, 1981.

PROJECT DESCRIPTION Z01 CM 06259 08 COB

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (cancer treatment research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device similar to those in use in Burlington, Vermont, where the computer is located. BCRP has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRP are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, touch screen and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

Results:

1. The BCRP staff has developed antineoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. The drug information sequences have been updated and new sequences have been added. Platinum drug displays have been added. Update and revison of the pyrimidine antagonists are completed. Reference citations using new citation tables are complete for alkylating agents and for antimetabolites. Monitoring information for all antineoplastic agents is continuing. All alkylating agents and antimetabolites have been audited and updated. Drug dosage information structures were expanded to include 1) general information for planning drug dosages; 2) technique to prepare drugs and 3) technique to administer drugs.

2. Problem formulation sequences for all hematologic and solid cancers have been completed and expanded to include histologic types and staging as documented in the Cancer Staging Manual.

3. Problem specific plans for small cell carcinoma of the lung have been completed including a new protocol (BCRP #8020). A BCRP protocol for acute lymphocytic leukemia has been added to the system. In addition, phase II and phase I studies have also been added. The facility for adding additional protocols does not require any additional programming effort.

4. Bone marrow reporting displays have been completed.

5. The entire systems package has been shipped to BCRP and testing and debugging is underway in conjunction with the Intragency Agreement with the National Center for Health Services Research (Y01 CM 80109).

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem formulation sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific antineoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user is provided guidance while gathering information and treating patients' cancer problems.

PUBLICATIONS:

1. Esterhay RJ Jr, Walton ES: Cancer Treatment Research Protocols and PROMIS. Proceeding of the Fourth Annual Symposium on Computer Applications in Medical Care, November 1980.

2. Foy JL, Esterhay RJ Jr: Flowgraph: The Display of Clinical Data and Events in Time-Oriented Graphic Form. Proceedings of the Fourth Annual Symposium on Computer Applications in Medical Care, November 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06277-10 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemotherapy of Adult Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Stephen C. Schimpff, M.D.	Head, Section of Infection and Microbiological Research	COB,NCI
	Charles A. Schiffer, M.D.	Head, Cell Component Therapy Section	COB NCI
	Joseph Aisner, M.D.	Head, Section of Medical Oncology	COB NCI
	David E. Van Echo, M.D.	Senior Investigator	COB NCI
	Kerry Van Sloten, R.N.	Chemotherapy Nurse	COB NCI
	Patricia Duffy, R.N.	Chemotherapy Nurse	COB NCI
	Susan Markus, R.N.	Chemotherapy Nurse	COB NCI
	Theodore Breitman, Ph.D.	Senior Investigator	LTCBNCI

COOPERATING UNITS (if any)
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Laboratory of Clinical Biochemistry, BCRP, NCI

LAB/BRANCH
Clinical Oncology Branch

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Office of the Chief

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A 7-day continuous intravenous infusion of cytosine arabinoside plus a 3-day schedule of intermittent daily doses of Adriamycin has been shown to be as effective chemotherapy for the induction of remission in adults with acute nonlymphocytic leukemia as the combination of cytosine arabinoside with daunorubicin. A complete remission rate of 65% has been obtained with either combination. However, the adriamycin combination has been shown to be more toxic and to require more careful dosage adjustment and monitoring of blood levels. The addition of either neuraminadase-treated allogeneic myeloblasts or splenectomy to chemotherapy during remission maintenance treatment enhances the duration of remission and survival of patients. Reverse transcriptase has been identified in the bone marrow cells of leukemia patients prior to treatment and in morphologically normal peripheral cells during complete remission. The leukemic cells of patients with acute progranulocytic leukemia have been shown to differentiate in vitro with cis-retenoic acid. Treatment of those patients with cis-retenoic acid has not produced differentiation in vivo, however.

I. Therapy:

A. Remission Induction.

Our previous investigation demonstrated that a 7-day continuous intravenous infusion of cytosine arabinoside plus a 3-day schedule of intermittent daily doses of daunorubicin is a highly effective chemotherapy for induction of remission in adults with acute non-lymphocytic leukemia. A complete remission rate of 65% was obtained with a median remission duration of 6 months and a median survival from the initiation of therapy of 12 months. Analysis of factors which may have affected the duration of remission demonstrated that younger patients, patients who were uninfected at the time of admission, and female patients had significantly higher remission induction rates and overall survival than did their counterparts. Half of the patients in this study were also treated with an immuno-reconstitutive agent, levamisole, which did not appear to augment either remission induction rate or remission duration. Although survival after relapse in patients who received levamisole was somewhat longer than survival in the group of patients not receiving levamisole, the reason for this effect has not been completely elucidated.

Seventy-five patients have been entered into a new study utilizing a 7-day continuous infusion of cytosine arabinoside with a 3-day intermittent dosage regimen using Adriamycin instead of daunorubicin for induction therapy. Of 44 evaluable patients, 65% have achieved complete remission with a median duration of remission of 11 months. Toxicity with this induction regimen appears to be greater than that seen with cytosine arabinoside plus daunorubicin. For this reason, daunorubicin has been substituted for adriamycin in our current study. Sixty-five patients have accrued to the current study and a 65% complete response rate has been observed. The purpose of the current study is to provide an adequate number of complete responders for a 3 arm randomized study of maintenance therapy.

B. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

Following the attainment of remission, patients are currently being randomly assigned to receive either cytosine arabinoside and 6-thioguanine given to hypoplasia intermittently, the same chemotherapy with the addition of neuraminadase-treated allogeneic myeloblasts, or the same chemotherapy plus splenectomy. In our previous study, 7 patients received the late intensification phase of maintenance with cytosine arabinoside and thioguanine. Although 4 of these patients have now relapsed from 2 to 4 years after remission induction, 3 patients continue in their first complete remission more than 4 years since the initiation of treatment. Because of this observation, we are currently using intensive intermittent courses of cytosine arabinoside and thioguanine as the control arm of this study. Another third of the patients who achieve remission are assigned to receive this same chemotherapy plus neuraminadase-treated myeloblasts in an attempt

to immunologically stimulate patients who have achieved complete remission to destroy or suppress the development of additional leukemic blasts. This study design is based on the successful trials of this type of immune stimulation in delaying the development of spontaneous AKR lymphoma in mice, as well as a prospectively randomized trial of immunotherapy begun in 1973 by Bekesi and Holland which suggests that there is a highly significant prolongation of both median remission duration and survival in a group of patients receiving neuraminadase-treated cells as immunotherapy in addition to their chemotherapy. Sixteen patients are currently under treatment with this regimen. Another third of the patients who achieve complete remission undergo splenectomy after remission attainment following which they are also treated with chemotherapy identical to that of the patients above. This is based on reports that splenectomy significantly delays the onset of leukemia in the AKR mouse model, and also on anecdotal reports of long-term survival in splenectomized adult patients with acute nonlymphocytic leukemia. An additional study done by Fleming and associates also suggested that there was an enhanced remission duration and survival in children with acute myelogenous leukemia who underwent splenectomy plus chemotherapy during maintenance compared with children who are not splenectomized. In 12 of the 14 patients, there was also histologic evidence of residual leukemia in the spleens which were removed. Seventeen patients have been randomized to this arm.

With 15 patients in each maintenance arm, remission duration is already significantly greater in the patients who received neuraminadase-treated cells. A trend toward greater remission duration also exists in the splenectomy arm of the study.

II. Biomedical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia:

A. Serum and Urine Muramidase Studies

We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute nonlymphocytic leukmia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer complete remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen

We have observed that approximately 30% of adult acute non-lymphocytic leukamia or acute lymphocytic leukemia patients have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse the presence or absence of CEA in their plasma at that time does not correlate with

findings at the time of presentation. CEA levels in these patients fluctuate with hepatic function.

C. Serum Immunoglobulin Concentrations in Patients with Acute Nonlymphocytic Leukemia

We have mesured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG and elevated levels of IgM in their serum. When complete remission is achieved these levels become normal.

D. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia

Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 98 patients by recall-antigen skin testing and 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis in terms of attainment of complete remission of his leukemia, duration of remission and survival does not correlate with the pre-therapy demonstration of intact delayed cutaneous hypersensitivity.

E. Deoxynucleotide Terminal Transferase Studies

This DNA polymerase, originally found in normal thymic cells, has subsequently been identified in relatively high concentrations in the blast cells of most patients with childhood and adult acute lymphocytic leukemia. It has also been reported to be present in high concentrations in blasts from a large minority of patients with chronic myelogenous leukemia in blast crisis, as well as a small fraction of patients with otherwise morphologically classified acute non-lymphocytic leukemia in whom histochemistry and Romanowsky staining are equivocal. Because of the suggestion that this enzyme may provide a means for classifying leukemic cells (and hence stratifying treatment for patients) in addition to the classical morphologic and clinical criteria, blast cells of all new patients with acute leukemia are being assayed for their terminal transferase activity in addition to more routine histologic studies.

Publications:

1. Daly, P.A., Schiffer, C.A., and Wiernik, P.H.: Acute promyelocytic leukemia - Clinical management of 15 patients. Amer. J. Hematol. 8: 347-359, 1980.

2. Freireich, E.J., Bennett, J.M, Vogler, R.W., and Wiernik, P.H.: Adult acute leukemia. In Hoogstraten, B. (Ed.): Cancer Research, Impact of the Cooperative Groups, Masson Pub., U.S.A., New York, pp. 25-38, 1980.

3. Van Echo, D.A., Wiernik, P.H., and Aisner, J.: High dose VP16-213 (NSC-141540) for the treatment of patients with previously treated acute leuekmia. Cancer Clin. Trials 3: 325-328, 1980.

4. Van Echo, D.A., Chiuten, D.F., Markus, S., and Wiernik, P.H.: A clinical trial of pyrazofurin in combination with 5-azacytidine in acute adult non-lymphocytic leukemia. Cancer Clin. Trials 4: 129-133, 1981.

5. Newman, K.A., Schimpff, S.C., Young, V.M., and Wiernik, P.H.: Lessons learned from surveillance cultures in patients with acute nonlymphocytic leukemia. Amer. J. Med. 70: 423-431, 1981.

6. Wade, J.C., Schimpff, S.C., Hargadon, M.T., Fortner, C.L., Young, V.M., and Wiernik, P.H.: A comparison of trimethoprim/sulfamethoxazole plus nystatin with gentamicin plus nystatin in the prevention of infection in acute leukemia. N. Engl. J. Med. 304: 1057-1062, 1981.

7. Wade, J.C., Newman, K.A., Schimpff, S.C., Van Echo, D.A., Gelber, R.A., Reed, W.P., and Wiernik, P.H.: Two methods for improved venous access in acute leukemia patients. J. Amer. Med. Assn. in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06293 09 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Teaching and Consultation Service in Medical Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Other:	Joseph Aisner, M.D.	Head, Section of Medical Oncology	COB NCI
	Stephen C. Schimpff, M.D.	Head, Section of Infection and Microbiological Research	COB NCI
	Charles A. Schiffer, M.D.	Head, Cell Component Therapy Section	COB NCI
	David A. Van Echo, M.D.	Senior Investigator	COB NCI
	Robert J. Esterhay, M.D.	Senior Investigator	COB NCI
	Richard M. Kaplan, M.D.	Senior Investigator	COB NCI
	Richard D. Leavitt, M.D.	Senior Investigator	COB NCI
	Arlene Forastiere	Senior Investigator	COB NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Oncology Branch

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INSTITUTE AND LOCATION

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3	3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to improve the clinical approach to various cancers, dissemination of information concerning the latest techniques, diagnosis and therapy is of paramount importance. By providing exposure and training of potential physicians early in the course of their education, such as during their student or house officer years, such information will be brought to the community in an orderly fashion and will preclude the formation of prejudicial opinions about the treatment of malignancies. With the provision of a consultative service to the various cooperating units, we have the opportunity to educate the patients' physicians regarding modern techniques of management of the various neoplastic diseases. These consultative and teaching functions also elevate the level of care available to the patient with cancer in the metropolitan area.

The objective of this project is to acquaint physicians in training (medical students and house officers) with the theory and pratice of medical oncology and how to apply the modalities of chemotherapy, radiotherapy and surgery in the management of neoplastic diseases. In addition, the object of this project is to provide consultative services for difficult patients with neoplastic diseases for other physicians in the Baltimore metropolitan area.

Medical students from the University of Maryland are assigned to this service during their second year on a regular basis for physical diagnosis. In addition, students from the University of Maryland as well as Johns Hopkins rotate through the BCRP during elective periods for a clinical clerkship in medical oncology. In addition, house officers from the Greater Baltimore Medical Center and Good Samaritan Hospital have been assigned here for elective periods of their house officer years. Assistant residents in medicine from the University of Maryland rotate through the Clinical Oncology Branch on a regular basis. In addition, physicians from our Program provide lectures and discussions of difficult cases at many of the cooperating units.

Physicians in the community refer patients here for outpatient consultation. If the patient meets the qualifications for a BCRP protocol study, he or she is accepted and offered the opportunity to be admitted to our Program. If not, the patient is completely evaluated as an outpatient and returned to his physician with specific recommendations for further therapy.

The rotation through the BCRP for medical students and house officers has resulted in a wider acceptace of medical oncology as a sub-specialty of internal medicine at the University of Maryland and elsewhere in the Baltimore community. Because of the contact with house officers and local physicians it has become more accepted that patients with neoplastic diseases require special care based on knowledge derived from specialty training. Some local private physicians have suggested that the general care of patients with neoplastic disease in the community has advacned because of our consultative services.

It is of paramount importance that information concerning the latest techniques in the diagnosis and treatment of cancer patients be disseminated to the medical community. By training potential physicians early during their student to house officer years, such information will be brought to the community in a more orderly and useful way and will preclude the formation of unfounded prejudices. By seeing patients in consultation, we have the opportunity to educate the patient's physician regarding modern techniques of management for any given neoplastic disease. By providing these teaching functions, we engage in and are of significant import in elevating the level of care available to patients with cancer in this area.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06295 08 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Census: A Remote Access Patient Information Storage and Retrieval System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other: Nancy S. Brandt Computer Operator
Brian K. Duvall Computer Programmer

COOPERATING UNITS (if any)
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Section of Microbiology, Clinical Research Pharmacy Section,
Administrative Office, BCRP, NCI, NIH

LAB/BRANCH
Clinical Oncology Branch

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Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH

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9.0	9.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The CENSUS system is a set of computer programs which enables the storage and retrieval of a data base of information on cancer patients treated at the Baltimore Cancer Research Program. The computer programs and the data base are accessed from remote terminals connected to a commercial time-sharing computer facility. Structured data input programs facilitate the addition of new information to the data base. A flexible retrieval program produces patient listing sorted by patient name, cancer diagnosis, attending physician, and/or status. A data processor updates the data base daily and then generates a CENSUS report which lists all patients currently on the Clinical Oncology inpatient service and Clinical Oncology clinical visits made that day. The system has been in operation since August of 1971, and the data base currently contains records of over 2400 cancer patients. This system has been converted to run on the PROMIS system in conjunction with an Interagency Agreement with the National Center for Health Services Research (Y01 CM 80109). The CENSUS system will, in the future, operate as a subset of the PROMIS system.

OBJECTIVES:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible and maintained by a single non-technical individual.

2. To generate a daily report on all inpatients and their locations, along with their vital statistics and current therapy data to be distributed to all involved in the tasks of clinical research, patient care and administration.

3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

METHODS EMPLOYED:

1. Information is compiled and recorded daily in a data input log by a data processor.

2. A computer program accepts the input datum from the secretary, checks for a logical content and accuracy, and records it on the data base.

3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.

4. The report is then distributed to the scientific and professional staff, remote contracting laboratories and administrative office.

5. The programs and data base are executed and stored on a commercial time-sharing service.

6. Mass memory disc files and remote terminals form the storage and access mediums.

The Daily Census Report has been utilized by all sections and services of the Cancer Program.

1. Admissions Officer: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.

2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.

3. Clinical Oncology Branch: the master retrieval programs are most useful to the physician performing a chart review to evalute treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, etc.

4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.

5. Sections of Microbiology and Infection Research: provides the laboratory staff with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemological tool in detecting the spread of infectious organisms.

6. A general purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.

7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

8. The entire CENSUS system including the data base of 2400 patients has been converted to run on the PROMIS system. This new software is currently being tested in parallel on the BCRP in-house PROMIS system via an Interagency Agreement with the National Center for Health Services Research (Y01 CM 80109). It is anticipated that the remote commercial-time sharing computer facility will no longer be needed to support the CENSUS system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Program with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Nine years of continuous operation of the Census System have provided BCRP with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by the PROMIS system at the BCRP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06917 08 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
CVP Versus CAVP For Disseminated Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
Other: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, M.D. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ninety-three patients with previously untreated pathologically documented stage III or IV non-Hodgkin's lymphomas were randomized to receive either CVP (high dose cytoxan plus vincristine and prednisone) or CAVP (adding Adriamycin with a somewhat lower dose of cytoxan) every three weeks. The median followup is 3.5 years. The histologies of all patients have been reviewed and classified according to both the Rappaport and new International schemes.

The CR rate was 51% in both arms. Remission duration and survival were also similar. However, in diffuse histiocytic lymphoma and in the comparable category G (diffuse large cell) of the new formulation, there was a distinct advantage to CAVP which gave a 60% CR rate in DHL and 67% CR in category G. CVP produced only 10% CR in these categories (p=.057). Eighty-six percent of CR's remained free of disease at a median of 3.75 years. Thus, a survival advantage was seen for CAVP in DHL. For the other diffuse histologies and all nodular histologies, there was no significant difference between the regimens, although there was a trend in favor of CVP for nodular patients.

Patients who were found to have advanced non-Hodgkin's lymphomas, either stage III or stage IV on the basis of clinical and pathological staging procedures were randomily assigned to receive therapy with either cyclophosphamide 1500 mg/m^2 IV day 1, vincristine 1.4 mg/m^2 on days 1 and 8 and prednisone 40 mg/m^2 orally on days 1 through 10 (CVP) or cyclophosphamide 1000 mg/m^2 IV on day 1, Adriamycin 45 mg/m^2 IV on day 1 and vincristine and prednisone as per the other regimen (CAVP). Patients who achieved pathologically documented complete remissions then underwent 3 courses of consolidation therapy with CCNU, bleomycin and prednisone.

Ninety-three patients were randomized and the median followup is 3.5 years. The histologies of all patients have been reviewed and classified according to both the International formulation and the Rappaport classification. Nine patients were inevaluable for response because of inadequate documentation but were included in all survival analyses.

Overall the CR rate was identical (51%) on the two regimens. The CR plus PR rates were not significantly different (CVP 68%, CAVP 83%).

In Rappaport diffuse histiocytic lymphoma (DHL), CAVP produced 60% CR (6/10) and CVP produced only 10% (1/10) CR (p=0.057). CR's were durable with 86% of CR's continuing free of disease for a median of 3.75 years. Thus, there is a survival advantage for CAVP therapy in DHL. The situation was similar with the diffuse large cell (category G) subtype in the International formulation. This group closely corresponds with the Rappaport DHL and the results were essentially identical. In diffuse lymphocytic and all nodular histologies, no significant advantage was seen in either treatment, though in nodular PDL there is a trend in favor of the CVP compared to CAVP. For nodular histologies there is a trend toward increased survival with achievement of CR (p=0.09).

Proposed Course: The project is now terminated and new protocols for favorable and unfavorable histologies will be undertaken. In favorable histologies, we will continue to accrue to a joint Bethesda-Baltimore protocol (MB-110) for assessment of conservative "watch and wait" management versus aggressive chemotherapy. For the aggressive histologies, we are developing a new protocol which compares adriamycin plus infusion of Ara-C to the successful CAVP regimen.

Publications

1. Diggs CH, Wiernik PH and Ostrow SS: Nodular lymphoma: Prolongation of survival by complete remission. Cancer Clin Trials (in press), 1981.

2. Bishop JF, Wiernik PH, Kaplan RS, Wesley M, Diggs CH, Sutherland JC, Marcus SD: High dose cyclophosphamide, vincristine and prednisone plus or minus Adriamycin (CAVP versus CVP) in advanced poorly differentiated lymphocytic and lymphocytic-histiocytic non-Hodgkin's lymphoma (NHL) in a randomized trial. Proc Am Assoc Cancer Res and ASCO 22: 518, 1981.

3. Bishop JF, Schimpff SC, Wiernik PH: Infections in non-Hodgkin's lymphoma (NHL) patients undergoing intensive chemotherapy. Proc Am Assoc Cancer Res and ASCO 22: 417, 1981.

4. Fuks JZ, Aisner J, Ostrow SS, Wiernik PH: Re-staging laparotomy in the management of the non-Hodgkin's lymphomas. Proc Am Assoc Cancer Res and ASCO 22: 511, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06939 05 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Methotrexate, Oncovin, Asparaginase and Dexamethasone Therapy for Adults With Acute Lymphocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert J. Esterhay, M.D.	Senior Investigator	COB,NCI
Other: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to improve the remission induction rate of acute lymphocytic leukemia in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by asparaginase. A second objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration. The third objective is to determine the efficacy of high dose methotrexate in preventing meningeal leukemia and to correlate the clinical effectiveness with the cerebrospinal fluid methotrexate levels. To date 38 patients have been entered onto this study. Twenty-four patients had no prior treatment, 14 had prior treatment. For the no prior treatment patients, complete remissions have been achieved in 18 of 24 (75%) patients. For the prior treatment patients, 11 of 14 (79%) achieved complete remission. The median survival for complete remission patients (no prior and prior treatment) is 18.1+ and 17.2+ months and for all patients 17.0+ and 11.2+ months respectively. Only 2 of 24 previously untreated patients (8.3%) developed CNS leukemia at 3.3 and 42.7 months from the start of therapy.

The purpose of this project is to improve the remission induction rate of Acute Lymphocytic Leukemia (ALL) in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by asparaginase. The second objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration in a significant percentage of adult patients with ALL. The third objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate clinical effectiveness with the cerebrospinal fluid methotrexate levels.

Patients 15 years of age and over with a diagnosis of ALL are eligible. The induction regimen consists of methotrexate (M) 100/mg/m^2 on day 1, vincristine (O) 2.0 mg and asparaginase (A) 500 IU/kg on day 2, and dexamethasone (D) 6 mg/m^2 on days 1-10. Courses of MOAD are repeated every 10 days if possible. After complete remission is obtained, the same methotrexate and asparaginase dose and schedule (without vincristine and dexamethasone) is repeated every 10 days if possible. After complete remission is obtained, the same methotrexate and L-asparaginase dose and schedule (without vincristine and dexamethasone) is repeated every 10 days for 6 courses of consolidation therapy. This is followed by 12 monthly cytoreduction courses of the same vincristine dose and methotrexate 100 mg/kg on day 1, followed by citrovorum factor for 72 hours, and the same dexamethasone dose on days 2-6. Maintenance therapy consisting of monthly vincristine, weekly methotrexate 15 mg/m^2 PO, daily 6-mercaptopurine 100 mg/m^2 PO, and dexamethasone 6 mg/m^2, days 1-5 of each month is then given until relapse.

To date 24 previously untreated patients with ALL (median age 31, range 15-60) have entered the study and 18 (75%) have achieved complete remission. The median duration of complete remission is 11.1+ months, with a range of 0.7+ to 55.9+ months and a median survival for previously untreated, complete remission patients of 18.1+ months and 17.0+ months for all previously untreated patients. Eight of 18 patients who achieved complete remission have relapsed, 2 with meningeal leukemia. Three died of infection in complete remission during consolidation and seven remain in complete remission from 0.7+ to 55.9+ months. During high-dose methotrexate treatment therapeutic CSF levels of methotrexate were achieved. Eleven of 14 (79%) previously treated patients (median age 30, range 15-52) also achieved complete remission. The median duration of complete remission is 7.5+ months and median survival from the start of MOAD therapy is 17.2+ months for previously treated, complete remission patients and 11.2+ months for all previously treated patients. Six of 11 patients who achieved complete remission had marrow relapses. One patient died of a pulmonary embolus in complete remission and four remain in complete remission from 2.6+ to 55.3+ months. The major toxicity was an allergic reaction to the E. coli strain of asparaginase, necessitating subsequent use of the Erwinia strain in over 70% of the patients. In addition, stomatitis was greater (69%) during induction with methotrexate - asparaginase rescue than during cytoreduction with methotrexate - folinic acid rescue (9%). High-dose methotrexate appears to delay the onset or reduce the incidence of meningeal leukemia compared to our historical experience with either no meningeal or pyrimethamine (Daraprim) prophylaxis. MOAD is superior to other regimens that the BCRP has used for adult ALL.

PUBLICATIONS

1. Esterhay RJ Jr, Wiernik PH, Grove WR, Markus SD, Wesley MN: Moderate Dose Methotrexate, Vincristine, Asparaginase and Demamethasone for Treatment of Adult Acute Lymphocytic Leukemia. Blood (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09109 03 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Alternating MOPP/SCAB vs MOPP Alone for Treatment of Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Other:	Richard Kaplan, M.D.	Senior Investigator	COB NCI
	Robert C. Young, M.D.	Chief, Medicine Branch	MB NCI

COOPERATING UNITS (if any)

Medicine Branch, DCT, NCI

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Clinical Oncology Branch

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Office of the Chief

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Forty-three patients with advanced previously untreated Hodgkin's disease have been randomized to receive either standard MOPP chemotherapy (nitrogen mustard, vincristine, procarbazine and prednisone) or standard MOPP chemotherapy alternating every other month with SCAB (streptozotocin 500 mg/m^2 IV day 1 and bleomycin 15 mg/m^2 IM days 1 and 8). No significant differences have yet emerged from the study.

MOPP chemotherapy has become the standard treatment for patients with advanced previously untreated Hodgkin's disease. The combination of streptozotocin, CCNU, adriamycin and bleomycin (SCAB) also has shown activity equal to that of alternating two effective four-drug chemotherapeutic regimens. Patients with stage III_2A, IIIB and IVA and IVB Hodgkin's disease have been randomized between standard MOPP chemotherapy and alternating every other month MOPP/SCAB.

Forty-three patients have been randomized on this study to date, 21 receiving MOPP/SCAB, 22 receiving MOPP alone. Too few ptients have completed treatment to make any assessment of the comparative effectiveness of either of these regimens.

Although MOPP chemotherapy has become standard treatment for patients with advanced, previously untreated Hodgkin's disease, there remains a sizeable patient population who will not initially respond to MOPP. SCAB also has shown activity in this same group of patients and has shown activity in previously treated patients who had received MOPP or MOPP-like regimens. Because of the activities of both of these regimens, they have been used in combination alternating them on an every-other-month basis. In order to assess whether this cyclical chemotherapy is equal to that seen with MOPP alone, a control group of patients has been treated with MOPP chemotherapy. To date, however, few patients have been randomized on either arm of the study and fewer still have completed treatment. The study is still accruing patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09110 03 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Two Chemotherapy Regimens for Previously Treated Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
Other: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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1	1	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

33 previously treated patients with advanced non-Hodgkin's lymphoma refractory to first-line therapy were treated with one of two combinations. Initially, patients who had not received Adriamycin before were treated with Adriamycin, VM-26 and Bleomycin. Patients previously exposed to Adriamycin were treated with platinum, VM-26 and bleomycin. 11 patients received the platinum regimen and only 1 of the 11 responded. In view of this poor response rate, this arm was discontinued and all patients, regardless of previous Adriamycin exposure were then put on the other regimen. Of 22 patients treated with the Adriamycin combination, there were 11 objective responses (50%) of which 6 were CR. Overall median survival for responders (CR + PR) was 11.5 months compared with 4 months for non-responders (p=.004). Five patients were treated with the Adriamycin combination despite prior Adriamycin and 3 of these 5 responded (2 CR, 1 PR). Hematologic toxicity was severe in 15/126 courses and there were 11 infectious episodes and 3 therapy-related deaths: from cardiotoxicity in 1, from sepsis in 2. We conclude that Adriamycin, VM-26 and bleomycin is an active combination in previously treated patients with NHL.

This study was initiated to study the effectiveness of 2 chemotherapeutic regimens for patients with previously treated advanced non-Hodgkin's lymphomas. Patients with biopsy proven recurrent or progressive non-Hodgkin's lymphomas of all histologies were initially allocated by prior Adriamycin treatment to 1 of 2 regimens. For those patients who had not received Adriamycin, this drug at a dose of 60 mg/m^2 IV day 1 plus VM-26 50 mg/m^2 IV day 1 and Bleomycin 10 mg/m^2 IM days 1 and 15 were administered. For those patients who had received prior Adriamycin, platinum at a dose of 50 mg/m^2 IV day 1 was substituted for the Adriamycin with the same dosages of VM-26 and Bleomycin.

In this phase of the study, 11 patients received the platinum arm. One achieved a complete response but died of treatment related toxicity (granulocytopenia and sepsis). This arm was therefore discontinued because of an apparently poor rate of activity compared to its toxicity. The Adraimycin combination arm was then opened to all patients regardless of previous exposure to Adriamycin and 22 have been treated with 6 achieving a complete remission and 5 a partial remission (overall response rate 50%). Five of these patients had previously been treated with Adriamycin and 3 of these 5 responded, nonetheless, to the Adriamcyin-containing combination (2 CR and 1 PR).

Response to Adriamycin, VM-26 and Bleomycin was found to correlate with response to previous chemotherapy. The median survival for all responders (CR + PR) was 11 1/2 months compared to 4 months for non-responders (p=0.004).

Hematologic toxicity occured in 45/126 courses and was severe (white cell nadir less than 1000, platelets less than 50,000) in 15 courses. There were 11 infectious episodes and 3 therapy-related deaths: from cardiotoxicity in 1 and from sepsis during granulocytopenia in 2.

Although the two regimens cannot be directly compared in this non-randomized trial, the VM-26, bleomycin and Adriamcyin appears to be an active and useful combination in relapsed patients regardless of exposure to Adriamycin in the past, while the platinum, VM-26, bleomycin combination, in all likelihood does not possess significant activity greater than single agent therapy.

Proposed Course: A new second-line drug combination is now underdevelopment.

Publications:

1. Kaplan RS, Bishop JF, Diggs CH, Wiernik PH: VM-26 and Bleomycin plus Adriamcyin or Cis-dichlorodiammine platinum II in patients with non-Hodgkin's lymphoma. Proc Am Assoc Cancer Res and ASCO 22: 512, 1981 (ABST).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09116 03 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Searching for the EBV antigen in Human Tumors using the PAP technique

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John C. Sutherland, M.D. Research Pathologist COB,NCI

Other: Paul H. Levine, M.D. LVC,NCI

COOPERATING UNITS (if any)
Department of Pathology
The Johns Hopkins University

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Clinical Oncology Branch

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	1	1

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Because of the ubiquity and known oncogenicity of EBV, it is appropriate to determine if EBV antigen can be identified using the PAP technique in paraffin-embedded sections of a variety of human tumors. Thus far, in working with positive controls, we have identified the VCA antigen in cultured Burkitt cells which were fixed in formalin and embedded in paraffin. Thus far we've been unable to identify the EBNA antigen in similarly processed Raji cells. We are now preparing to use 2 freshly available monoclonal antibodies to EBV utilizing both the PAP and immunofluorescent techniques on both paraffin-embedded and frozen material.

Burkitt cells will be fixed in acetone and others fixed in formalin-fixed cells will be embedded in paraffin and sectioned. Both sets of cells will be stained with monoclonal mouse anti EBV using the PAP technique. Further searching for EBV antigens in human tumors will depend on the results of these studies. If only the air-dried and acetone fixed cells are positive, then further staining of human tumors, especially lymphomas, will be restricted to fresh frozen material. If the paraffin embedded cells are positive, then the search will be enlarged to stored paraffin blocks.

PUBLICATIONS: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09118 03 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Treatment of the Resistant Phase of Chronic Myelogenous Leukemia with 5-Azacytidine and VP16-213

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles A. Schiffer, M.D. Head, Cell Component Therapy Section COB,NCI
Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch COB,NCI
Other: Helmut Kasdorf, M.D.
Roberto DeBellis, M.D.

COOPERATING UNITS (if any)

Hospital De Clinicas "Dr. Manuel Quintela", Montevideo, Uruguay

LAB/BRANCH

Clinical Oncology Branch

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A total of 27 patients with CML in blast crisis have been treated with a combination of 5-Azacytidine (150 mg/m^2, IV in 3 divided doses and VP16-213, 75 mg/m^2/day IV x 5 days). An overall respone rate of 58% (1 CR, 15 PR) was seen with a median survival of 231 days for responders and 23 days for non-responders. The overall survival was 161 days which compares favorably with other results in patients with myeloid histology. A new study has been initiated utilizing high doses of both drugs with 5-Azacytidine at 250 mg/m^2/day x 5 days by continuous IV infusion and VP16-213 at a dosage of 100 mg/m^2/day IV x 5 days.

Twenty-seven patients with chronic myelogenous leukemia in blast crisis were treated with a combination of 5-Azacytidine (150 mg/m^2/day IV in 3 divided doses x 5 days) and VP16-213 (75 mg/m^2/day x 5 days). No patient had "lymphoid" histology and terminal transferase was not present in the 12 patients tested. One complete and 15 partial responses (58% response rate) were seen with a median survival of 231 days for responders and 73 days for patients who failed to achieve a partial response (p=.008). The overall survival was 161 days (range 20-316+ days). Moderately severe nausea and vomiting, muscle aches and mucositis were the major side effects. Toxicities tended to decrease with subsequent courses and many patients could be treated on an outpatient basis.

Marrow aplasia was achieved in only four patients in the above study. In an effort to increase the response rate and the duration of response based on these initial promising results, an addendum to the protocol was prepared utilizing both drugs in high doses. In addition the 5-Azacytidine was given as an continuous infusion in order to ameilorate the gastrointestinal side effects. Eight evaluable patients have been treated to date with 5-Azacytidine, 250 mg/m^2/day IV x 5 days by continuous infusion and VP16-213, 100 mg/m^2/day IV x 5 days. Although all patients had an antileukemic response, only one PR has occurred to date. Toxicities were similar to the previous study although GI toxicity was somewhat less and the incidence and mucositis may be a little higher. In addition, one patient had moderately severe renal tubular acidosis probably second to the Azacytidine. A number of patients with poor prognostic factors including: 2 patients with atherosclerotic cardiac disease, both of whom died within five days of heart disease; two patients refractory to random donor platelets; one patient with pulmonary leukostasis and a prior pneumonectomy. Because of the influence of these other factors on response, it is too early to judge the effect of this new regimen and this study will be continued at present without modification.

PUBLICATIONS:

1. Schiffer, CA, DeBellis R, Kasdorf H, Wiernik P: Treatment of the Resistant Phase of Chronic Myelogenous Leukemia with 5-Azacytidine and VP 16-213. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09119 03COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Search for Particle-Lamellar Complexes in Human Placentas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John C. Sutherland, M.D. Research Pathologist COB,NCI

Other: William G. Banfield, M.D. CLP,NCI
Cecil W. Lee CLP,NCI

COOPERATING UNITS (if any)
Department of Obstetrics & Gynecology
University of Maryland Hospital

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NCI,NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two particle-lamellar complexes were noted in placental tissue from a normal pregnancy. These complexes were in a pericyte within the core of a villus. The purpose of this study was to determine how extensive these complexes were in placents from normal and toxic placentas. Although these complexes are seen in a wide vareity of tissues, they are most common in lymphoma and leukemia cells, especially hairy cell leukemia. What their purpose is and what function they play in cellular metabolism is unknown. A total of 10 placentas were examined, 7 from normal pregnancies and 3 from pregnancies complicated by toxemia. Besides the 1 normal placenta, these complexes were found in an additional placenta, one from a pregnancy complicated by postpartum toxemia.

PROJECT DESCRIPTION ZO1 CM 09119 03 COB

Tissues from five placentas were obtained approximately one hour after delivery and fixed in a mixture of 1% formaldehyde and 1% glutaraldehyde buffered with phosphate. These tissues were later minced, postfixed in 1% of osmium tetroxide buffered in 0.1 Ms-collidine, stained en bloc in uranyl acetate, dehydrated in graded alcohols and embedded in Epon 812. Sections cut with a diamond knife were stained with uranyl acetate and lead citrate and viewed in a Siemens Elmiskop 1A electron microscope. Six grids from 2 blocks were examined before considering a placenta negative for LPCs.

PUBLICATIONS

1. Sutherland JC, Middleton EB, Banfield WG, and Lee EH: Lamella-particle complexes in the human placenta. In Sandritter, W. (Ed. : Pathology, Research and Practice. Stuttgart, W. Germany, Gustav Fischer Verlag 169:323-329, 1980.

PROPOSED COURSE

Discontinue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09120 03 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Implement a Problem-Oriented Medical Information System (PROMIS) at the BCRP

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, M.D.	Senior Investigator	COB,NCI
Other:	John L. Foy, M.D., Ph.D.	Medical Investigator	NCHSR
	Brian K. Duvall	Computer Programmer	
	Nancy S. Brandt	Computer Operator	
	Amy M. Hament, R.N.	Nursing/Computer Applications	

COOPERATING UNITS (if any)

Intra-agency Agreement No. Y01-CM-80109
National Center for Health Services Research (NCHSR)
Office of the Assistant Secretary of Health (OASH), DHEW

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Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The BCRP, DCT has entered into an agreement with the National Center for Health Services Research (NCHSR), Office of the Assistant Secretary for Health (OASH) to develop, implement, and install a completely operational, functioning, computerized, expandable and exportable Problem-Oriented Medical Information System at the BCRP.

It is anticipated that the work should be completed in three phases, taking a total of approximately three years. Work has been completed on Phase I and Phase II has been initiated. Phase III will start upon completion of Phase II.

Phase III has not started due to unavoidable delays in the permanent computer site. It is anticipated that the contract with NCHSR will be extended beyond September 30, 1981.

The BCRP, DCT has entered into an Intra-agreement with the NCHSR, OASH, DHEW, to develop, implement, and install a completely operational, functioning, computerized expandable and exportable PROMIS system at the BCRP.

The work should be completed in three phases, taking a total of approximately three years. Phases I and III should take approximately one year each to complete. Phase II would run concurrently with Phase I for the first year and will require an additional year to complete. Phase III will start upon completion of Phase II.

The NCHSR will perform the following tasks during the phases indicated:

Phase I

A. Develop a set of system specifications for implementing the PROMIS systemn at BCRP in accordance with their current contract with the University of Vermont.

B. Schedule a critical design review of the system specifications developed in A.

C. Procure, assemble and test system hardware and software in accordance with the specifications developed.

D. Provide site preparation consultation services to BCRP for installation of the system. Actual site preparation is the responsibility of BCRP.

Phase II

A. Train BCRP personnel in the operation and maintenance of the system at BCRP, which will include:

1. Development of a training plan;
2. Training of computer personnel in hardware and software operation and maintenance;
3. Training the users of the system (i.e., physicians, nurses, pharmacists, technicians, etc.) in the operation of the system;
4. Prepare the necessary documentation for the training.

B. Demonstrate that the system is operational and that it is ready for installation at BCRP.

C. Ship and install the system at BCRP.

D. Demonstrate to the BCRP Principal Investigator that the system is fully operational and ready for use at the BCRP.

Phase III

A. Maintain and update the system software, keeping the tables and frames current.

B. Test patient population study software.

This is the third year of this three year intra-agency agreement. One Central Processing Unit (CPU) has been procured and is being used by the Baltimore Cancer Research Program (BCRP) in Baltimore. It is located in a temporary installation site. The second CPU has been ordered and will be delivered when the permanent installation site is available.

Tasks A, B, & D of Phase I under this intra-agency agreement have been completed. Task C has been partially fulfilled in that the second CPU and associated hardware have been ordered and will be assembled and tested in accordance with the specifications developed. It is now anticipated that the site will be completed by October 1981. As specified, the tasks of Phase II have been initiated. Since there has been a significant delay in the permanent site preparation, this agreement will have to be extended beyond September 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09124-02 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Radiotherapy, Chemotherapy or Combined Modality Treatment for Early Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Other:	Robert C. Young, M.D.	Chief, Medicine Branch	MB NCI
	Eli Glatstein, M.D.	Chief, Radiation Oncology Branch	ROB NCI

COOPERATING UNITS (if any)

Robert Slawson, M.D., Department of Radiotherapy, University of Maryland School of Medicine

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Clinical Oncology Branch

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NCI, NIH Baltimore, MD

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1	1	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thirty patients with stages II-IIIA$_1$ Hodgkin's disease have been randomly allocated to receive either radiotherapy, MOPP chemotherapy, or the combination of the two modalities for treatment. The study is designed to determine the best therapy for patients with early stage Hodgkin's disease. This study is an ongoing one and represents a longterm commitment to precisely define optimal treatment for this group of young individuals with a malignant disease. In additon, the study asks whether the treatment of Hodgkin's disease might be simplified from the standpoint of expense, morbidity, and comfort by minimizing treatment for subgroups of patients who require less therapy. The longterm consequences of effective therapy will be evaluated in this study over a period of years.

PHS-6040

Project Description: Z01 CM 09124-02 COB

Radiotherapy has become the standard for the treatment of early stage Hodgkin's disease. However, a number of reports suggest strongly that combined modality treatment with radiotherapy and drugs results in superior disease free survival. In some studies, superior overall survival has also been noted. More recently, subgroups of patients which are more likely to benefit from combined modality treatment have been identified. MOPP chemotherapy has become the standard treatment for patients with advanced Hodgkin's disease. Pilot studies in the literature and a pilot study performed at the BCRP suggest that early stage Hodgkin's disease may be as treatable with combination chemotherapy as is late stage disease. Indeed, early results suggest that combination chemotherapy may be as effective as the standard radiotherapy approach to this disease. This study is designed to sort out the meaning of these observations. The results will be a better understanding of the treatment requirements of patients with early stage Hodgkin's disease. This will result in long term disease free survival improvement in some groups of patients. A very important question asked by this study is whether or not the treatment of Hodgkin's disease can be simplified by using only chemotherapy alone. If the results of this study support the proposition that this may be true, expenses and morbidity causing staging procedures for Hodgkin's disease could be eliminated.

An important aspect of this study is the longterm follow-up of chronic complications of each of the therapies employed. The information gained from this follow-up will be very important in determining the best overall treatment for this disease.

Thirty patients have been randomized on this study to date. It is far too early to evaluate the relative merits of these treatments. This study is still accruing patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09135 02 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Adjuvant Combination Therapy of Resectable Adenocarcinoma of the Colon with Whole-Liver Irradiation and 5-Fluorouracil

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard S. Kaplan, M.D. Senior Investigator COB NCI

COOPERATING UNITS (if any)
Division of Radiation Therapy, University of Maryland Hospital, Baltimore,MD
All member institutions of Gastrointestinal Tumor Study Group

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A prospective randomized controlled study of liver-directed and systemic adjuvant therapy in patients with high risk colon (excluding rectal) cancers is being carried out by the Gastrointestinal Tumor Study Group. Patients are randomized to no adjuvant therapy or to 2100 R of liver irradiation combined with 3 days of IV 5-FU. Ninety patients have been entered since the study was begun in August, 1979. No conclusions regarding efficacy can be made at this early date, only 3 patients having relapsed on each arm. Myelosuppression has been more striking than expected and, in particular, thrombocytopenia has been occasionally striking. It is assumed that this toxicity reflects alteration of hepatic metabolism of 5 FU by liver irradiation. Since modification of the 5 FU dose last year, this toxicity has been reduced.

Approximately 2-3 more years of accrual will be required.

Hepatic metastases have been well established as the leading cause of failure after surgical treatment of cancer of the large bowel proximal to the rectum. Various types of single agent adjuvant systemic chemotherapy have been tested with minimal or no success. Trials of combination therapy are underway or in follow-up. The efficacy of these regimens is as yet unknown. However, the ongoing trial of liver-directed adjuvant chemotherapy (portal vein infusion of 5-FU) by Taylor and associates in England has yielded encouraging early results.

This study is being undertaken by members of the Gastrointestinal Tumor Study Group to investigate another type of liver-directed (and systemic) adjuvant therapy. Whole-liver irradiation to 2100R plus simultaneous and subsequent IV 5-FU chemotherapy is being compared in a randomized fashion to no adjuvant therapy in patients with Dukes B_2 or C colon tumors following potentially curative surgery.

Thus far 90 patients have been entered since August 1979. Only 3 patients on each arm have relapsed thus far, so no analysis of therapeutic results can be made at this early date.

However, some assessment of toxicity can be made. So far no evidence of serious hepatotoxicity has been seen, but myelosuppression from 5-FU has been apparently potentiated by the radiation. Twelve patients have had WBC $<$ 1500 and/or platelet counts $<$ 70,000. Three patients have required hospitalization, one of whom was septic. She recovered and no patient has died. The starting dose of 5-FU was reduced to minimize this problem, and myelotoxicity has indeed decreased since the reduction.

PROJECTED COURSE: This study will continue to accrue patients to a total of 200 (2-3 years).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09136 02 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Phase II Trials in Refractory Hodgkin's Disease and Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
Other: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
Vincent Lombardi Cancer Center, Georgetown

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Clinical Oncology Branch

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NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New agents with: a) unique modes of action or b) minimal bone marrow toxicity are being tested in relapsed and refractory lymphomas of Hodgkin's and non-Hodgkin's types with goals of better first-line combinations of agents and therapy which is safer to deliver to patients with involved bone marrow. The first drug being studied is Spirogermanium which has virtually no bone marrow toxicity and is unrelated to other chemotherapy agents. It is being given at 50 mg/m^2 in 150 cc D_5W over 1/2 hour thrice-weekly for 2 weeks, then weekly. Ten patients have been treated thus far and 2 have achieved complete remissions (eradication of bone marrow disease). Toxicity has been limited to mild lethargy and there has been no myelosuppression.

A phase II study of Anthracene Dicarboxaldehyde (ADC) is just beginning.

Despite the progress of the last decade in the chemotherapy of advanced lymphomas, only a small minority of the non-Hodgkin's lymphomas can be said to be cured, and when relapse occurs, complete remission becomes more difficult to achieve and death often ensues quickly. Even in Hodgkin's disease, 50% of advanced-disease patients will ultimately fail MOPP and receive progressively less successful regimens of chemotherapy. Thus there continues to be a real need for new drug combinations which will alter these situations.

Since many second-line combinations include either drugs with mechanisms of action similar to those of the first-line drugs or several agents with overlapping bone marrow toxicity, we perceive a particular need for drugs with a) unique modes of action or b) minimal bone marrow toxicity to augment the therapeutic arsenal against lymphomas, to lead ultimately to better first-line combinations and to render safer the therapy of the patient with bone marrow involvement.

The first drug being studied is Spirogermanium, being given at 50 mg/m^2 (in 150 cc of D_5W over 1/2 hour IV) 3 times a week for 2 weeks, then once per week. The drug, virtually without bone marrow toxicity, is chemically unrelated to other chemotherapy drugs and has activity against lymphomas in vitro and in vivo.

Thus far 9 patients with non-Hodgkin's lymphoma and 1 patient with refractory Hodgkin's disease have been entered. In all patients, toxicity has been notably mild and limited to mild lethargy or dizziness. No serious CNS toxicity has been observed and no myelosuppression whatsoever. Of 9 patients with NHL, 2 patients have had apparent CR's. Both of these patients consistently had bilaterally positive bone marrow biopsies prior to therapy and have gradually cleared the marrow until biopsies are now consistently normal. Four patients failed to respond and three were inevaluable for response. The fact that this non-myelosuppressive drug can be effective against NHL in the bone marrow is encouraging for its possible use in combinations.

Another agent just beginning phase II trial is ADC (Anthracene Dicarboxaldehyde) which is a new anthracene derivative with potentially less myelotoxicity and cardiotoxicity than Adriamcyin. Phase II studies in refractory lymphomas are now beginning, utilizing a dose of 260 mg/m^2 IV every 3 weeks.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 09140 01 COB

PERIOD COVERED
October 1, 1981 through September30, 1981

TITLE OF PROJECT (80 characters or less)
Phase II Trials in Primary and Metastatic Brain Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Lester Miles, M.D.	Clinical Associate	COB NCI

COOPERATING UNITS (if any)
Michael Salcman, M.D., Division of Neurologic Surgery, Univ.of Md. Hospital
Robert Slawson, M.D., Division of Radiation Therapy, Univ. of Md. Hospital

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two ongoing phase II studies investigate new types of chemotherapy in patients with primary and metastatic brain tumors refractory to surgery, radiation and standard chemotherapy.

The effect of DMSO in opening reversibly the blood-brain barrier for chemotherapeutic agents is being evaluated. Patients with indwelling Ommaya reservoirs undergo repeated CSF sampling to determine the CSF pharmacokinetics of simultaneously administered chemotherapy. Twelve patients were studied with DMSO plus cyclophosphamide. The DMSO did not alter any aspect of cyclophosphamide or alkylating activity disposition. Six patients have been treated with DMSO plus Adriamycin. Adriamycin aglycones were increased in the CSF, presumably related to either DMSO or the tumors themselves.

Another study looks at the new agent AZQ (aziridinylbenzoquinone). Thirteen patients with glioblastomas have been entered. One has had a partial response, 1 is stable, 7 have failed to respond and 4 are inevaluable. In addition, 5 patients with tumors metastatic to brain have been entered. One of these has had a response, 2 are stable, 1 failed and 1 is inevaluable.

Relatively few chemotherapeutic agents have proven useful in the treatment of primary and secondary CNS malignant tumors. Only the nitrosoureas are unequivocally active and these are able to traverse the blood-brain barrier. Even these drugs are not efficacious against metastatic tumors. We are therefore studying a number of phase II approaches to brain tumor chemotherapy in patients who have failed to respond to the standard treatments of surgery, radiation theapy and, if appropriate, nitrosourea.

In the first study, we are pursuing the demonstration by several investigators over the last two decades that some agents seem to be increased in their uptake into the brain by DMSO. We have evaluated the blood-brain barrier of mice using intravenous injections of horseradish peroxidase (HRP). Both IV DMSO and IP DMSO have dose-related effects on the blood-brain barrier in these mice and in concentrations of 15 to 20% dramatically open the barrier to 44,000 molecular weight HRP.

In appropriate patients with no other therapeutic options who happen to have Ommaya reservoirs in place, we are investigating pharmacologically whether DMSO can increase uptake of chemotherapeutic agents into the CSF of man. Twelve patients were treated with cyclophosphamide and DMSO and some of these patients also had studies utilizing cyclophosphamide alone. The DMSO did not seem to alter any aspect of cyclophosphamide pharmacokinetics in the plasma or the CSF. Many of these patients had rapid plasma half-lives which we attributed to their anticonvulsant therapy and which were not different with or without DMSO. Interestingly, the peak CSF cyclophosphamide concentration reached about half peak plasma concentrations. Thus, cyclophosphamide entered the CSF (and presumably the brain tumors) better than anticipated regardless of the administration of DMSO. This presumably relates to the presence of blood-brain barrier breakdown by the tumors themselves. One patient had a short lived clinical response to cyclophosphamide plus DMSO.

Six patients have been treated with Adriamycin plus DMSO and 2 of these were also treated without DMSO. Here again, an effect of the DMSO could not be documented. Adriamycin and its principle polar metabolite, Adrimycinol, did not reach significant concentrations in the CSF but the concentrations of total aglycones was higher than expected and certainly higher than seen in the sporadic CSF sampling previously reported by ourselves and others. Again this might relate to the presence of the brain tumors themselves or their therapy. So far, we cannot unequivocally state that we have altered the blood-brain barrier for either cyclophosphamide or Adriamycin with DMSO. However, the DMSO concentrations achieved were not measured and presumably low, and higher doses may be required to confirm any such phenomenon in man.

In a separate phase II study of brain tumor patients, we are looking at a new synthetic agent, AZQ, which is capable of crossing the blood-brain barrier and is broadly active in a number of animal tumors. AZQ is being given at a dose of 17 $^{1}/_{2}$ mg/m^{2} on days 1 and 8 of 4 week courses with dose escalation for those patients with no toxicity.

To date there have been 13 glioblastoma patients treated. One has had a documented partial remission, 1 is stable and 7 patients have had progressive disease, and 4 are inevaluable for response. With tumors metastatic to the

brain, 5 patients have been entered. One of these had a partial response which was documented but which only lasted 2 months. Another 2 patients are stable, 1 has had progressive disease and 1 is inevaluable. Toxicity from AZQ has been quite mild except that 4 patients who had all previously had nitrosoureas have developed marked cytopenia with counts as low as 6000. Although this patient with the lowest count also had sepsis (without granulocytopenia) it is clear that significant thrombocytopenia can occur with AZQ when the bone marrow has previously been exposed to nitrosourea. This had not been previously recognized.

Proposed Course: The AZQ study is continuing to accrue enough patients to make some estimate of activity in primaries and metastses. The DMSO pharmacology studies are continuing and will utilize higher doses of DMSO since no toxicity whatsoever has been seen thus far. We will also investigate the role of DMSO vis-a-vis other chemotherapy drugs. An investigation of DMSO's role in the blood-brain barrier is also continuing in animal experiments.

Publications:

1. Egorin M., Kaplan, R., Saloman, M., Miles, L., Colvin, M., Wiernik, PH and Bachur, N: Plasma and CSF pharmacokinetics of cyclophosphamide in patients treated with and without Dimethyl sulfoxide (DMSO). Proc Am Assoc Cancer Res 22: 210, 1981.

2. Kaplan, RS, Riggs, CE, Jr., Miles, L., Saloman, M., Aisner, J., Wiernik, PH, Bachur, NR: Preliminary observations on the effect of Dimethyl sulfoxide on the metabolism and distribution of Adriamycin. Proc Am Assoc Cancer Res at ASCO 22: 367, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09146 01 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of Antibody Eluted from Human Placentas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John C. Sutherland, M.D. Research Pathologist COB,NCI

COOPERATING UNITS (if any)
None

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The human placenta enjoys immunity from host rejection for unknown reasons, although there is evidence that chorionic gonadotropin may play a role in immunosuppression at the placental site. Ig, however, has been reported along the trophoblastic membrane and, in our studies, along the fetal endothelium (Fed. Proc. 40:578, 1981). The purpose of this study is to homogenize whole placentas and to characterize the Ig removed by acid elution.

Fifteen to 18 placentas will be homogenized (VirTis homogenizer) and the homogenates washed at least thrice. The washed homogenates will then be suspended in glycine buffer pH 2.8. The supernatant will be neutralized and brought to 0.15 normality with saline and then applied to a sepharose 4B-protein A column to remove IgG. The column will be washed thoroughly with PBS and Tween 80 and then cleared with 0.1N acetic acid. Maternal serum will be treated similarly. The paired serum and placental samples will then be compared for antibody titers to the TORCH and EBV antigens and tested for the presence of Ag/Ab complexes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06276 07 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Chemotherapy of Metastatic Genitourinary Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joachim Fuks, M.D.	Medical Investigator	COB,NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI
	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB,NCI

COOPERATING UNITS (if any)

Urology Division, Department of Surgery, University of Maryland Hospital

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Section of Medical Oncology

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NCI, NIH Baltimore, MD 21201

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1	1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cis-diamminedichloroplatinum (DDP) and adriamycin have been shown to have activity against some genitourinary neoplasms. DDP, 75 mg/m^2 on days 1 and 8 and adriamycin, 60 mg/m^2 on day 1, were administered intravenously to 45 patients with advanced genitourinary cancer. Courses were repeated every 21 to 28 days. Complete responses were seen in 5 of 11 patients with testicular tumor and in 3 or 10 patients with bladder carcinoma. Hematologic toxicity included neutropenia and thrombocytopenia. Non-hematologic toxicity included nausea, vomiting, nephrotoxicity, ototoxicity, and neurotoxicity. New active drug combinations have been identified for patients with advanced carcinoma of the testes, cervix or ovary but this study is being continued for patients with bladder, ureter or prostate carcinoma.

A phase II disease oriented clinical trial using cis-dichlorodiammineplatinum (DDP) and adriamycin combination has been undertaken in patients with advanced gynecological and genitourinary tumors. Forty five patients with histologically confirmed gynecological or genitourinary malignancy were entered in this study. Among these 45 patients 11 had germinal cell neoplasm, 9 ovarian cancer 10 urinary bladder carcinoma, 14 adenocarcinoma of the prostate and another patient had Wilm's tumor.

The DDP was given by 6 hours intraveous infusion and adriamycin was given intravenously over 10 minutes. The initial DDP and adriamycin dose was 75 mg/m^2 and 60 mg/m^2 respectively.

Five complete responses and 4 partial responses out of 11 evaluable patients with germinal cell tumor occurred. Partial responses have been seen in 1 patient with Wilm's tumor and in four out of 9 evaluable patients with ovarian cancer. Six of the 10 evaluable patients with bladder carcinoma achieved responses (3 CR 3 PR) 1 out of nine evaluable patients with prostate carcinoma achieved PR. Severe leukopenia (WBC < 2000/ μ l) and thrombocytopenia (plt <100,000/ μ l) occurred in 25% and 45% of evaluable courses, respectively. Non-hematologic toxicity included nausea and vomiting (45 pts), nephrotoxicity (serum creatinine > 2 mg %) (10 pts), neurotoxicity (11 pts), ototoxicity (12 pts).

Conclusions: The dose and schedule of DDP-Adriamycin used in this study is active against advanced genitourinary neoplasm but severe hematologic and non-hematologic toxicity coupled with active patients with gynecological and testicular cancer indicated that this project should be closed to patients with carcinoma of testes, cervix or ovary.

Proposed Course: Continue investigation with advanced bladder, ureter or prostate cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06919 07 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Initial Drug Therapy for Metastatic Renal Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Senior Investigator	COB,NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI
	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB,NCI
	Joachim Fuks, M.D.	Clinical Associate	COB,NCI
	Susan Markus, R.N.	Chemotherapy Nurse	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methyl-G has been given to 14 patients with renal cell carcinoma (RCC) without therapeutic effect and this study is closed. Chemotherapy failures are then given medroprogesterone and tamoxifen. To date, 0/13 patients have responded but this study is still in its preliminary stages and is being continued.

Renal cell carcinoma has notoriously been refractory to chemotherapy and radiation. Clinical studies in this area are attempting to identify new active chemotherapy agents or hormonal combinations.

PROJECT A Methyl-G

See ZO1 CM 09117 02 COB Project B.

PROJECT B Hormonal Agents

Hormonal agents, namely progestational agents, have been used in the treatment of advanced renal cell carcinoma for several years. In a recent review of the subject, the response to progestins or androgens ranged between 6 and 33 percent with an overall objective response rate of approximately 15 percent. Most responses to treatment in this tumor are, however, subjective, and this is a relatively worthless measurement of tumor response because of the diverse behavior of this tumor. In a recent clinical trial by Eastern Cooperative Oncology Group, nafoxidine (an antiestrogen) was found to have a small objective response rate in the treatment of renal cell carcinoma with two complete and one partial objective tumor responses. Another antiestrogen, tamoxifen, has shown evidence of objective response in two of four patients treated. The latter antiestrogenic compound is desirable over nafoxidine since it does not produce skin photosensitivity, but has at least a similar if not superior level of antitumor activity in human breast cancer. Several patients in a current study by the Southwest Oncology Group have had objective tumor regression.

To date, 13 patients have received the combination of medroxyprogesteron 1000 mg/wk I.M. plus tamoxifen 10 mg t.i.d. for at least eight weeks. All patients have progressed. Toxicities have included mild nausea and fluid retention (with resultant sequelai, i.e. headaches, leg swelling and mild hypertension). These effects have been easily controlled with diuretics.

Conclusion: This project is still preliminary and will require an additional 3 patients before any valid conclusions can be made.

Proposed Course: Continue investigation.

Publications:

1. Fuks, J.Z., Van Echo, D.A., Aisner, J., Mitchal, E.D., Wooley, P.V., Wiernik, P.H.: A phase II trial of Methy-G in patients with metastatic renal cell carcinoma. Cancer Clinical Trials, (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06944-06 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Treatment of Oat Cell Carcinoma of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Head,Section Medical Oncology	COB NCI
Other:	David A. Van Echo, M.D.	Senior Investigator	COB NCI
	Margaret A. Whitacre, RN	Hyperthermia Nurse	COB NCI
	Joseph Fuks, M.D.	Clinical Associate	COB NCI
	Peter H. Wiernik, M.D.	Chief,Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

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Veteran's Administration Hospital, NCI

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Clinical Oncology Branch

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INSTITUTE AND LOCATION

NIH, NCI, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Oat cell carcinoma of the lung requires aggressive combination chemotherapy. Two sequential studies at the BCRP used a combination of cyclophosphamide, doxorubicin and VP16-213 as the basic regimen. The first study showed that MER offered no benefit and the second study showed that alternating chemotherapy did not improve response or survival. The third study tests if increasing the dose and infusion time of VP16-213 can improve response and survival. The overall response rate in the two studies has been identical with 90% total and 60% complete remission (CR) rate in limited disease. Median duration of survival was 9 1/2 and 16 mos for patients with extensive and limited disease respectively. Patients with limited disease have a 20 to 30% long term disease free survival. Toxicities were managed by dose modification. With the long term survivors, complications of treatment, long term sequelae, and newer disease evolutions have become evident. Leptomeningeal carcinomatosis has been seen in 10 to 15% of patients particularly those with extensive disease and with recurrent disease. Further analyses of these factors is underway.

PROJECT A

Oat cell carcinoma of the lung can be considered a widely disseminated disease at presentation and therefore requires aggressive systemic chemotherapy. The combination of cyclophosphamide, doxorubicin and VP16-213 has been shown to be highly active in this disease. In two sequential studies the duration of survival for patients with extensive disease is greater than 9 1/2 months and greater than 15 months for patients with limited disease . Therefore the results from this combination chemotherapy alone are at least as good as any reported study today, including those with projected survivals. The two sequential studies are virtually superimposable with respect to response rate and survival. Immunotherapy as used in the first study did not add to the response rate or survival and alternating noncross-resistant combination of CCNU, vincristine, methotrexate and procarbazine also did not improve response rate or survival. The third sequential study now has been designed to test whether increasing the dose and changing to a five day infusion of VP16-213 can improve the response and survival with this combination chemotherapy. In the present study, patients are stratified according to extent of disease and then randomized to receive either the basic regimen or the regimen with high dose continuous infusion VP16. Patients who achieve complete remission receive whole brain irradiation as treatment of micrometastases. The previous study showed that patients in complete remission who received prophylactic cranial irradiation have a significantly decreased rate of intracranial metastases and a delay in the occurrence of leptomeningeal carcinomatosis.

As of April, 1981, 20 patients have been entered onto the third oat cell study which is a prospectively randomized comparison of the basic regimen and the regimen with higher dose continuous infusion VP 16. Responses are being seen with both treatments and it is too early to draw stable response or survival data. It is anticipated that the response and survival will certainly not be any worse than the previous studies. In addition to the basic study, there is a prospective trial of trimethoprim/sulfamethoxazol prophylaxis against infection as a randomized comparison with placebo. Toxicities on the current study have been similar to previous studies with primarily leukopenia. It is anticipated that there will be approximately the same degree of toxicity as in previous studies.

Proposed Course: Continue Investigation

PROJECT B

With aggressive combination chemotherapy for oat cell carcinoma of the lung there has been an increase in survival duration for all patients and occasional long term disease free survivors (approximately 20 to 30% of limited disease patients in the current studies). This improved survival has led to the recognition of previously unusual evolutions of the disease and the possibility of long term complications of therapy. The current project has thus been initiated to review patients for prognostic factors, unusual disease evolution, and late complications.

As of April, 1981, 18 patients have been recognized to have leptomeningeal carcinomatosis and in collaboration with the VAH, NCI, common features of patients with leptomeningeal carcinomatosis have been found and these include extensive disease, bone or bone marrow metastases, recurrent disease, liver involvement, partial or no response, no prophylactic cranial irradiation, and male sex. Early antimortem recognition of this complication has resulted in some patients clearing their leptomeningeal carcinomatosis with therapy. Thus, evaluation of the cerebro-spinal fluid should be performed in patients who have unusual neurological symptomatology. Also as of April, 1981, over 160 patients have been treated at the BCRP and two patients have been found to develop late aplastic anemia related to long term therapy. Two patients have had recurrent or new disease with histology other than small cell.

Proposed Course: Continue Investigation

PROJECT C

Patients who fail conventional combination chemotherapy for small cell carcinoma of the lung generally have decreased responsiveness to established agents and therefore investigation of new agents or combinations is indicated (see also report no. Z01 CM 09117 03 COB).

Proposed Course: Continue Investigation

Publications:

1. Aisner J: "Lung Cancer". In: The Science and Practice of Clinical Medicine, Vol. 6 Hematology and Oncology, Lichtman MA (ed), Grune & Stratton, Inc., New York, 1980, p 294-298.

2. Aisner J, Wiernik PH: Chemotherapy versus chemoimmunotherapy for small cell undifferentiated carcinoma of the lung. Cancer 46: 2543-2549, 1980.

3. Aisner J, Ostorw S, Govindan S, Wiernik PH: Leptomeningeal Carcinomatosis in Small Cell Carcinoma of the Lung. Medical and Pediatric Oncology 9: 47-59, 1981.

4. Aisner J: Combination chemotherapy alone for the treatment of small cell carcinoma of the lung. In: Controversies in Oncology. PH Wiernik (ed) Wiley and Sons, New York (in press).

5. Fuks JZ, Van Echo DA, Aisner J, Von Hoff D, Wiernik PH: A phase II trial of 4'-(-9-Acridinylamino)-methanesulfon-m-Aniside (AMSA) in patients with refractory small cell carcinoma of the lung. Cancer Treat Rep (in press).

6. Aisner J, Wiernik PH: Complications of Treatment and of Improved Survival in Patients with Small Cell Carcinoma of the Lung. In: Small Cell Lung Cancer, Greco FA, Bunn P, Oldham RK (eds) Grune and Stratton, New York (in press).

7. Fuks JZ, Aisner J, Carney DN, Van Echo DA, Ostrow SS, Ihde D, Wiernik PH: A phase II trial of vindesine in patients with refractory small cell carcinoma of the lung. Cancer Clin Trial (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06953 05 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Regionally Advanced or Metastatic Carcinoma of the Breast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Margaret Whitacre, RN	Hyperthermia Nurse	COB NCI
	Joseph Fuks, M.D.	Clinical Associate	COB NCI

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Section of Medical Oncology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For regionally-advanced or metastatic carcinoma (Ca) of the breast, combination chemotherapy may achieve local control and improve survival. It is used in project A to shrink primary masses and treat overt or micrometastases. Twenty-seven patients (15 with metastases and 13/27 with inflammatory Ca) were treated with combination chemotherapy. All 13 with inflammatory Ca had elimination of the inflammatory component. Twenty-four of the 27 were operable after chemotherapy and 22 had mastectomies. Five patients achieved partial remission prior to surgery and responsed after surgery to achieve complete remission. Trials in metastatic disease must stratify for prognostic variables, thus cooperative group efforts are necessary. Project B compared 3 regimens for response rate and survival duration and found CAF to be superior. MER immunotherapy was detrimental. The successor study evaluates the role of tamoxifen hormonal therapy with the CAF combination. As of April, 1981, 90 patients were entered onto study and interim analysis shows no differences. New combinations are needed for patients with refractory tumors. Project C evaluates the new combinations: mitomycin-C + vinblastine and doxorubicin + VP16. With 30 and 26 patients entered, both are highly active combinations. Project D looks at new drugs in advanced refractory disease.

Since the introduction of the 5 drug combination chemotherapy program for advanced breast cancer, there have been a large number of studies utilizing permutations of these drugs and their scheduling. In addition, the anthracycline antibiotic doxorubicin has been used in several regimens to improve the response rate duration or survival. The active combinations have also been applied to earlier breast cancer as a post operative adjuvant with demonstrated improvement in disease free interval and survival. Project A utilizes combination chemotherapy for locally advanced breast cancer with or without documented metastases (unresectable stage III and stage IV) including inflammatory carcinoma followed by surgery if the masses become operable. This study is an attempt to determine if combination chemotherapy can shrink the masses allowing for a surgical procedure and primary closure. In advanced metastatic breast cancer the attempt has been to prospectively compare combination chemotherapies which have demonstrated activity for the treatment of metastatic disease. Few previous studies prospectively compared the various treatment regimens available and stratified for the number of prognostic factors. In order to stratify for these various factors a large cooperative group effort is necessary. Project B compared 3 combination chemotherapies each of which has been shown to be an effective treatment program. In addition, this study evaluated the role of immunotherapy utilizing MER intradermal injections for its potential to increase response rate, survival and duration. The results from that study showed that the CAF combination was the superior regimen and that MER was not helpful. The successor study in the cooperative group built upon these initial observations and looked to find the additive value of tamoxifen to the CAF combination. For patients with advanced refractory breast cancer, new agents and new combinations of agents are being evaluated in order to identify secondline chemotherapy regimens which might be utilized effectively for patients failing primary combination therapy. In addition, the identification of such noncross-resistent combinations offers the potential for earlier treatment with these combinations with the planning of alternating combination chemotherapy regimens. Project C has studied the combination of doxorubicin and VP16 for patients failing primary treatment as well as mitomycin-C and vinblastine. In addition, patients who fail primary or secondary therapy are placed on phase II and phase III studies which are reported separately (see report No. Z01 CM 06973 COB).

PROJECT A: Locally Advanced Breast Carcinoma

As of April, 1981, 27 patients were treated initially with combination chemotherapy to shrink primary masses and control overt or micrometastases. Fifteen patients had clinically documented overt metastases and 13/27 had inflammatory carcinoma. After 3 to 4 courses of combination chemotherapy, 24/27 patients were considered operable and 22/24 underwent surgical procedures. Four had simple mastectomy, 10 had modified radical mastectomy and 8 had radical mastectomies. All but 3 patients were able to obtain primary closure without skin grafts. All 13 patients with inflammatory carcinoma had elimination of the inflammatory component of the disease. Three patients failed only at the local site and the other patients either failed distally (7) or simultaneously at local and distal sites (5). Two patients died without evidence of disease from other causes and 5 patients remain alive without evidence of recurrent disease.

Proposed Course: Continue Investigation

PROJECT B

Primary chemotherapy for metastatic disease. As of April, 1981, 388/431 patients were evaluable for response on the first study which compared: CMF, CAF and CAFVP each with and without MER. With complete plus partial response rates for the combinations of 34, 54 and 57% respectively. The CAF regimen was superior to CMF in response and survival durations and MER produced no benefit. Thus, it was concluded that the CAF regimen was superior overall. The successor study looks to determine the additive role of tamoxifen to CAF combination chemotherapy. Patients are stratified according to estrogen receptor data, menopausal status and dominant site of disease and then randomized to receive either CAF or CAF + tamoxifen. As of April, 1981, 90 evaluable patients have been entered onto study and interim evaluation shows no statistically significant differences in response frequency, duration or survival. The successor protocol is accruing at an appreciable rate and proposals for new studies are under discussion.

Proposed Course: Continue Investigation

PROJECT C:

In the treatment of Advanced Refractory Breast Carcinoma as of April, 1980, 29 patients previously treated and refractory to combination chemotherapy without doxorubicin were treated with a combination of doxorubicin + VP16-213. There were 3 complete and 8 partial responses. Doxorubicin + VP16 is therefore an active combination in advanced breast cancer which is noncross-resistant with conventional chemotherapy. It is, however, myelotoxic in this previously treated group.

As of April, 1981, 31 patients with refractory previously treated advanced disease were treated with mitomycin-C and vinblastine. Among 30 evaluable patients, 1 had a complete, 11 had partial responses, and 4 had stabilization. Median duration of response was 127 days. Toxicity included moderate to severe myelosuppression. The combination of mitymycin-C and vinblastine appears to be an active combination as secondline treatment for metastatic breast cancer and appears to be noncross-resistant with primary therapy.

Proposed Course: Continue Investigation

PROJECT D:

Patients failing primary and secondary therapy with combination chemotherapy are treated with phase II or phase I agents which are filed separately (see report no. Z01 CM 06973 COB of Dr. David Van Echo.)

Proposed Course: Continue Investigation

Publications:

1. Ostrow S, Egorin M, Aisner J, Bachur N, Wiernik PH: High dose Cis-diamminedichloroplatinum therapy in patients with advanced breast cancer: Pharmakokinetics, toxicity, and therapeutic efficacy. Cancer Clinical Trials 3: 23-27, 1980.

2. Aisner J: "Breast Cancer". In: The Science and Practice of Clinical Medicine. Vol. 6, Hematology and Oncology, Lichtman MA (ed), Grune and Stratton, Inc. New York, 1980, pp 290-298.

3. Konits PH, Aisner J, Van Echo DA, Lichtenfeld K, Wiernik PH: Mitomycin C and vinblastine chemotherapy for advanced breast cancer. Cancer (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06960 05 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

New Drug Therapy of Previously Treated Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB,NCI
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COOPERATING UNITS (if any)

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Clinical Oncology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical Studies have been undertaken to identify new agents or combinations of agents that may have activity in patients with relapsed acute leukemia. The following studies have been completed AMSA (20% CR rate in ANLL) and AZQ (dose finding only). The following projects are in progress: Ara-C + TdR, DHAD, nitrous oxide, whole body hyperthermia and AMSA vs. Poly I G-L-Lysine (the later study for maintenance therapy in recurrant leukemia only).

Relapse from complete remission occurs in almost all adult patients with leukemia and patients become refractory to primary induction therapy. New agents are necessary to produce response and survival. Patients who relapse post initial therapy are eligible. Most relapsed patients are then treated with newer agents, however, responses are invariably infrequent and of short duration. Relapsed leukemic patients who are refractory to their initial therapies or whose initial response is very brief (or non existant) are eligible for these studies.

PROJECT A AMSA

AMSA an acridine derivative has been given to 33 patients with relapsed leukemia. The dose used was 60 $mg/m^2/d$ daily until aplasia (8-12 days). Twenty two patients with ANLL, 5 with ALL and 6 with CML in blast phase were entered on study. Five patients with ANLL achieved CR (20%) and one patient with ALL a PR. Duration of response was approximately 3 months. Toxicities included prolonged myelosuppression, mucositis and transient liver function abnormalities.

Conclusions: AMSA as a single agent has approximately a 20% CR rate in relapsed ANLL. The schedule used in this study is not superior to that reported by other institutions and may be more toxic.

Proposed Course: Close project.

PROJECT B Ara-C Plus TdR

Ara-C plus TdR was given to 53 patients with relapsed leukemia 90% of whom had received extensive prior therapy with and were resistant to Ara-C. Both drugs were given as simultaneous 24 hour continuous infusions until bone marrow aplasia (7-12 days). Initially the TdR dose was fixed at 8 $gm/m^2/d$ and the Ara-C was escalated from 100 to 250 $mg/m^2/d$ in cohorts of 3-21 patients. At the highest dose level 7/15 ANLL patients achieved a CR or PR (47%), 0/5 ALL responded and 1/1 CML-B patient achieved a CR. Duration of response is in excess of a median of 120 days. Toxicities included myelosuppression, nausea and vomiting, diarrhea and enteritis.

Subsequently 19 additional patients were treated with Ara-C fixed at 250 $mg/m^2/d$ and TdR escalated to 20, 30 and 40 $gm/m^2/d$. In this group of patients there are 3 CR, 1 PR in 13 evaluable ANLL patients and no responses in ALL or CML-B patients. Toxicities are similar in this schedule to the lower TdR dose schedule except for the appearance of a severe skin rash in 13/19 patients.

Conclusion: The combination of Ara-C and TdR is a highly active combination for relapsed ANLL patients. Response data for other forms of leukemia are still incomplete.

Proposed Course: Continue investigation.

PROJECT C

AZQ is a benzoquinone derivative with antitumor activity designed to penetrate the CNS. Twenty two patients with relapsed leukemia were entered on a dose finding study of AZQ given daily x 7 days. The dose ranged from 8-28 $mg/m^2/d$. Toxicities were mild and included nausea, alopecia and mucositis. Severe bone marrow aplasia occurred at the highest dose level. No responses were observed.

Plasma and CSF pharmacokinetic studies were done in several patients and it was seen that AZQ achieves significant levels in the CSF.

Conclusions: AZQ is an attractive compound to explore in acute leukemia because of its bone marrow effects and penetrance of the CNS. Phase II studies of AZQ at 24 $mg/m^2/d$ x 7 will produce a minimal of toxicity and consistant bone marrow hypoplasia.

Proposed Course: A phase II trial in CALG-B will be activated this year.

PROJECT D Dyhydroxanthracenedione (DHAD)

DHAD as an anthracenedione derivative with preclinical antitumor activity approximately twice adriamycin.

To date 8 patients with relapsed leukemia have been entered on to dose finding study of DHAD given as a single dose daily x 7. The dose employed has been 8-9 $mg/m^2/d$. Toxicities have included nausea, mucositis and bone marrow hypoplasia.

Conclusions: This study is too preliminary for comment.

Proposed Course: Continue investigation.

PROJECT E Nitrous Oxide

Followed as a separate project (see Z01 CM 06994 04 COB)

PROJECT F Whole Body Hyperthermia

Follow as a separate project (see Z01 CM 06994 04 COB)

PROJECT G AMSA vs. Poly IC-L-Lysine for Maintenance Therapy

AMSA has known activity as an induction agent for relapsed leukemia but little is known as to its value for maintenance. Poly IC-L-lysine is a synthetic RNA which induced interferon production in humans. Patients with relapsed leukemia who achieve a complete response are randomized to receive either AMSA 60 $mg/m^2/d$ x 7 over 8-10 weeks or Poly IC-L-lysine 12 mg/m^2 every other day for 5 doses every 8-10 weeks. To date only two patients have been entered on protocol.

Conclusions: This study is too preliminary for comment.

Proposed Course Continue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06970 04 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cis-dichlorodiammine Platinum II Neurotoxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joachim Fuks, M.D.	Clinical Associate	COB,NCI
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	Merrill Egorin, M.D.	Scientific Expert	LCB,NCI
	Andre LeRoy, Ph.D.	Biomedical Engineer	R BEI

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to elucidate the mechanism of cis-dichlorodiammine platinum neurotoxicity serial nerve conduction studies, histobiochemical and electron microscopy studies of sural nerve biopsies were done in patients receiving DDP. Three of the 10 patients treated with DDP developed sensory polyneuropathy. Histochemical and electron microscopy studies on sural nerve biopsy performed on two patients with clinical polyneuropathy have showed axonal degeneration.

Cis-diamminedichloroplatinum (DDP) has been found to have antitumor activity in head-neck, ovarian, bladder, and testicular cancer. There have been occasional case reports implicating DDP as a causal agent for peripheral nerve damage in patients treated with DDP at standard doses and schedules (50 to 100 mg/m^2 every three to four weeks).

This study was designed to evaluate nerve function of patients who received DDP using serial neuropathological, biochemical and electrophysiologic studies. Serial nerve conduction studies, prior to and during DDP therapy have been performed on 10 patients. Threee of the 10 evaluable patients developed sensory polyneuropathy and sural nerve biopsies performed on two of these 3 patients showed axonal degneration by histochemical and electron microscopy studies.

Conclusion: The temporal relationship between the administration of DDP, patients symptoms, serial nerve conduction studies histochemical and electron microscopy studies on sural nerves in these patients clearly implicates DDP as a causal factor in peripheral neuropathy.

Proposed course: Discontinue investigation.

Publications:

1. Reinstein, L., Ostrow, S., Wiernik, P.H.: Serial nerve conduction studies in cis-platinum (II) DDP peripheral neuropathy. Arch. Phys. Med. Rehabil. 59:558, 1978.

2. Ostrow, S., Hahn, D., Wiernik, P.H., Richards, R.D.: Ophthalmologic toxicity after cis-dichlorodiammine platinum (II) therapy. Cancer Treat. Rep. 62:1591-1594, 1978.

3. Reinstein, L., Ostrow, S., Wiernik, P.H.: Peripheral neuropathy after cis-platinum II (DDP) therapy. Arch. Phys. Med. Rehabil. 61:280-282,1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06973 04 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Phase I Studies of New Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phase I studies are pharmacology and toxicity trials in humans of new anticancer agents that have completed preclinical toxicity, pharmacology and anti-tumor activity. Such studies are completed when dose-limiting toxicity is encountered. A study has been completed for of Dihydroxyanthracenedione (NSC 301739) and for Aclacinomycin-A (NSC 208734). A current study is in progress investigating VP16-213 given as a 24 hour continuous infusion for five days.

Phase I studies are studies in humans of the pharmacology and toxicity of new anti-cancer agents that have already had preclinical toxicity, pharmacology and anti-tumor activity testing completed. These studies usually require 20-30 patients with refractory malignant disease. Initial dosages are based on toxicity data from laboratory animals. Dosages are escalated according to standard schemes. Three patients are treated at each non-toxic dose level and six patients at each level with reversable acceptable toxicity. When dose limiting toxicity is encountered the study is closed.

PROJECT A

Dihydroxyanthracenedione (DHAD) is an anthraquinone compound which has been investigated because of its structural relationships to the anthracyclines. It has DNA intercalating properties and preclinical antitumor activity equal to or superior to Adriamycin. In addition, since DHAD lacks a sugar moiety, it is theorized that it will have less clinical cardiac toxicity which is the major limitation to the use of the anthracyclines. Sixteen patients have been treated with DHAD using a single dose intravenous bolus every day x3 schedule. Courses are repeated every three weeks. Dose limiting toxicity is reversible related leukopenia. A minor tumor response has been seen in a patient with soft tissue sarcoma.

Proposed course: Study closed. A phase II study in soft tissue sarcoma has been started.

PROJECT B

Aclacinomycin-A (ACM-A) is an anthracycline analogue currently in clinical trial in Japan. It appears to have similar preclinical and clinical spectrum of antitumor activity as Adriamycin. It is reported to have less nonhematologic side-effects (hair loss, nausea-vomiting) than Adriamycin and is felt to have less cardiac toxicity. Eighteen patients have received ACM-A on a single dose intravenous bolus every three weeks at doses of 60-120 mg/m^2. Dose limiting toxicity is myelosuppression. Other toxicities seen are nausea, vomiting, transient liver function abnormalities and cardiac arrythmias. Significant alopecia did not occur. Detailed plasma and urine pharmacokinetics of ACM-A and its metabolites were studied and previously undescribed metabolites have been identified.

Conclusions: ACM-A has hematologic and nonhematologic toxicities similar to other anthracyclines except for alopecia.

Proposed course: Study closed. A phase II study in breast carcinoma will be initiated in CALGB.

PROJECT C VP16-213

VP16-213 is an epipodophytoxin derivative with known antitumor activity in oat cell carcinoma of the lung and acute nonlymphocytic leukemia. There is some evidence that scheduling is important in enhancing the antitumor activity of VP16-213. Little information exists concerning continuous infusion administration of VP16-213.

Seven patients with refractory malignancies have received VP16-213 as a 5 day continuous infusion at doses of 75-150 $mg/m^2/d$. The only toxicity has been myelosuppression.

Conclusions: A much larger total dose per course of VP16-213 can be administered by continuous infusion. Dose limiting toxicity will be myelosuppression probably at 125 or 150 $mg/m^2/d$ + 5.

Proposed Course: Continue study.

Publications:

1. Van Echo, D.A., Whitacre, M.Y., Aisner, J., Wiernik, P.H.: A phase I trial of Dihydroxyanthracenedione. Cancer Treatment Reports, (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06974 04 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Whole Body Hyperthermia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI
	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB,NCI
	Nicholas Bachur,M.D.,Ph.D.	Chief, Lab. of Clinical Biochemistry	LCB,NCI
	Philip Konits, M.D.	Clinical Associate	COB,NCI
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	Merrill Egorin, M.D.	Expert, Lab. Clin. Biochemistry	LCB,NCI
	Dean Brenner, M.D.	Medical Investigator	COB,NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed utilizing equipment provided by the National Aeronautics and Space Administration to induce whole body hyperthermia (WBH) to 42.°C with minor morbidity to patients. Studies are currently in progress to assess the pharmacokinetics of cyclophosphamide (CTX) plus WBH; CTX plus Adriamycin plus WBH and the therapeutic effectiveness of these combinations in adenocarcinoma of the lung (CTX & WBH) and soft-tissue sarcomas (CTX-ADM-WBH). These studies are still in their preliminary stages and no conclusions are possible at this time. Other studies are underway to access the clinical effect of WBH in acute leukemia and oat cell carcinoma.

Clinical whole body hyperthermia (WBH) has been examined to assess the feasibility of combining cyclophosphamide (CTX) with WBH 42°C for four hours in patients with adenocarcinoma of the lung. In addition, seven patients with soft-tissue sarcoma have received the combination of Adriamycin (ADR) + CTX and WBH 41.8°C for two hours. Other aspects of the project include pharmacokinetic studies of the chemotherapeutic agents during WBH, and studies of endocrine function and physiologic alterations during WBH.

PROJECT A CTX + WBH

Seven patients with metastatic adenocarcinoma of the lung have been given CTX 1.5 mg/m^2 as a two hour infusion during WBH 41.8°C for four hours 2/5 patients evaluable for response have objective tumor regressions lasting 2-7 months. Toxicities have included myelosuppression secondary to CTX in addition to the usual symptoms from WBH. Pharmacokinetics are being done on CTX. Patients serve as their own controls and receive at least one course of CTX without WBH in order to compare the results with CTX-WBH courses.

PROJECT B ADR-CTX + WBH

Nine patients with soft-tissue sarcomas have received the combination of ADR 45 mg/m^2 during WBH 41.8°C for two hours followed by CTX 1.0 gm/m^2 as a one hour infusion four hours following WBH. 2/5 evaluable patients have achieved a pathologically documented complete response. Four patients have progressed. Two patients are too early to evaluate for response. Preliminary pharmacokinetic studies on CTX indicate that unchanged (inactive) parent compound CTX may be excreted at an accelerated rate through the urine suggesting that WBH inhibits hepatic microsomal activation of CTX. Studies of ADR pharmacokinetics are too preliminary for comment.

CONCLUSION:

WBH can safely be combined with a variety of chemotherapeutic agents in an effort to augment antitumor activity. It is essential that pharmacokinetic studies be integrated as part of any WBH program since WBH may adversely affect drug metabolism.

These projects are open for further investigation and in the future will investigate the pharmacology of other anti-cancer agents when combined with WBH.

Proposed course: Continue investigation.

Clinical whole body hyperthermia (WBH) has been examined to assess the feasibility of combining cyclophosphamide (CTX) with WBH 42°C for four hours in patients with adenocarcinoma of the lung. In addition, seven patients with soft-tissue sarcoma have received the combination of Adriamycin (ADR) + CTX and WBH 41.8°C for two hours. Other aspects of the project include pharmacokinetic studies of the chemotherapeutic agents during WBH, and studies of endocrine function and physiologic alterations during WBH.

PROJECT A CTX + WBH

Seven patients with metastatic adenocarcinoma of the lung have been given CTX 1.5 mg/m^2 as a two hour infusion during WBH 41.8°C for four hours 2/5 patients evaluable for response have objective tumor regressions lasting 2-7 months. Toxicities have included myelosuppression secondary to CTX in addition to the usual symptoms from WBH. Pharmacokinetics are being done on CTX. Patients serve as their own controls and receive at least one course of CTX without WBH in order to compare the results with CTX-WBH courses.

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CONCLUSION:

WBH can safely be combined with a variety of chemotherapeutic agents in an effort to augment antitumor activity. It is essential that pharmacokinetic studies be integrated as part of any WBH program since WBH may adversely affect drug metabolism.

These projects is open for further investigation and in the future will investigate the pharmacology of other anti-cancer agents when combined with WBH.

Proposed course: Continue investigation.

PROJECT C WBH In Relapsed Acute Leukemia

WBC 42-42.5°C for 2 hours q weekly was administered to 1 patient with ANLL and 1 patient with ALL. Neither patient received any clinical benefit.

Conclusion: This study is too early to evaluate.

Proposed Course: Continue investigation.

PROJECT D Endocrine Effects of WBH

Thyroid hormones consisting of Thyroxine (T_4), Trilodothyronine (T_3), reverse Trilodothyronine (rT_3) and Thyrotrophin Stimulating Hormone (TSH) were sequentially drawn over a 48 hour period in 5 patients with cancer undergoing whole body hyperthermia. Results indicate dramatic reciprocal changes of T_3 and rT_3 in which rT_3 levels had risen over 100% in 24 hr period. T_4 levels had risen slightly and TSH levels did not change significantly. At the 48 hour both rT_3 and T_3 levels began to return to baseline. This data suggest that acute heat stress provides stimulus for changes of T_4 peripheral metabolism causing a lowering of T_3 levels, a calorgenic metabolite.

Publications:

1. Ostrow, S.S., Van Echo, D.A., Whitacre, M., Aisner, J., Simon, R., Wiernik, P.H.: Physiologic alterations in patients undergoing whole body hyperthermia for the treatment of cancer. Cancer Treatment Reports, (in press), 1980.

2. Ostrow, S., Van Echo, D.A., Egorin, M., Whitacre, M., Growcho, L., Aisner, J., Colvin, M., Bachur, N., Wiernik, P.H.: Alteration of cyclophosphamide pharmacokinetics by whole body hyperthermia. J. National Cancer Institute (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06975 04 COB

PERIOD COVERED
October 1, 1980 though September 30, 1981

TITLE OF PROJECT (80 characters or less)
Treatment of Non-Oat Cell Carcinoma of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	David A. Van Echo, M.D.	Senior Investigator	COB NCI
	Margaret Whitacre, RN	Hyperthermia Nurse	COB NCI

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Medical Oncology

INSTITUTE AND LOCATION
NIH, NCI Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Most patients with non-small cell lung cancer (NSCLC) present with unresectable disease and a large proportion have disease outside radiotherapy ports. Many drugs have been evaluated for NSCLC but they have been disappointing. Combination chemotherapy has not shown any convincing evidence of survival benefit independent of prognostic factors. Platinum however, may play a role in combination chemotherapy. The current study thus utilizes cyclophosphamide, doxorubicin, and VP 16 (active in oat cell carcinoma) + platinum. Sixty patients were entered on study; 33 received four drugs and 27 received three drugs. There are 4 complete responses (CR) and 5 partial responses (PR) for the the four drug arm and four PR's in the three drug combination. It is too early to stratify for histologic subtypes or performance status. Toxicities include myelosuppression, mucositis and pharyngitis. Patients who fail primary chemotherapy receive phase II agents or secondline combinations. To date 20 patients have been studied with vindesine + platinum. There have been 0/12 responses for vindesine and 2/8 responses for the two drug combination. It is too early to develop stable response or survival information.

PROJECT A

Advanced non-oat cell carcinoma of the lung with disease outside the realm of surgery and radiotherapy presents a significant problem and thus the development of new treatments or combination chemotherapy programs for this disease is warranted. Single agent chemotherapy for these diseases tends to produce disappointing responses and several reports have suggested that combinations including platinum may have increased activity. Cyclophosphamide and doxorubicin are single agents with very low order of activity but there is animal data to suggest that they may be synergistic. Since there is pathological and biochemical evidence to suggest that the lung cancers are related, this study builds upon the observation in patients with oat cell or mixed oat cell and non-oat cell lung cancers may have response to the three drug combination of cyclophosphamide, doxorubicin and VP16. The study randomizes between the three drug combination with or without added platinum. The three drug combination of cyclophosphamide, doxorubicin and platinum has been proposed as a very active combination in several reports although it is not clear that the response is independent of prognostic factors such as performance status. The current study thus seeks to determine the value of platinum when added to the three drug combination of cyclophosphamide, doxorubicin and VP 16.

As of April, 1981, 60 patients have been entered onto study. Thirty-three patients have received the 4 drug combination and 27 patients have received the 3 drug combination. Among patients with the 4 drug combination, there have been 4 complete responses and 5 partial responses and among the patients who received the 3 drug combination there have been 4 partial responses. The majority of the responses occur in the patients with adenocarcinoma although the numbers remain relatively small when subcatagorized according to histology and performance status. Toxicity has been principally myelosuppression and primarily leukopenia and has been managed by dosage modification. There have been 6 instances of bacteremia during periods of neutropenia and 1 episode of Klebsiella pneumonia.

Proposed Course: Continue Investigation

PROJECT B:

Patients who fail primary therapy for non-small cell carcinoma of the lung tend to have a poor prognosis and tend to be refractory to conventional agents. Thus, the appropriate management of such patients would be to test new agents or new combinations. (see also report no. Z01 CM 06973 04 COB). As secondline treatment patients with non-small cell lung cancer were assigned to receive vindesine alone or vindesine + platinum according to whether or not they had prior exposure to platinum. Twenty patients have been entered onto study, 12 patients onto vindesin alone and 8 with the 2 drug combination. There have been no responses among the 12 patients who received vindesine and 2/8 patients who received the 2 drug combination have had partial responses. The data is too early to develop meaningful response or survival data. Toxicity has been mild.

Proposed Course: Continue Investigation

Publications:

1. Aisner J: "Lung Cancer". In: The Science and Practice of Clinical Medicine, Vol. 6 Hematology and Oncology, Lichtman MA (ed), Harper and Row, Hagerstown, Maryland, 1980, pp 357-368.

2. Hankins JR, Satterfield JR, Aisner J, Wiernik PH, McLaughlin JS: Pericardial window for malignant pericardial effusion. Ann Thorac Surg 30: 465-471, 1980.

3. Aisner J, Wiernik PH: Chemotherapy in the treatment of malignant mesothelioma. Seminars in Oncology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06976 04 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Augmentation of Chemotherapeutic Agents with DMSO

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Joseph Fuks, M.D.	Clinical Associate	COB NCI
	Merrill Egorin, M.D.	Scientific Expert	LCB NCI
	Nicholas Bachur, M.D.,Ph.D	Chief, Lab Clinical Biochemistry	LCB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Medical Oncology

INSTITUTE AND LOCATION
NIH, NCI Baltimore, MD. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many advanced cancers such as renal cell carcinoma and squamous cell carcinoma of the lung have proven particularly refractory to chemotherapy. It is therefore warranted to test new agents or new experimental modalities in such tumors. Animal studies suggest that chemotherapeutic activity of certain chemotherapeutic agents can be augmented by the use of dimethylsulfoxide (DMSO). Patients with metastatic squamous cell carcinoma of the lung were therefore treated with DMSO and cyclophosphamide. Fourteen patients were treated with 5 or 6% DMSO over 3 days and 1500 mg/m^2 of cyclophosphamide. Serial blood and urine samples were collected to assess pharmacokinetics of cyclophosphamide. No antitumor responses were seen although 9/14 patients had stabilization. Toxicity was mainly hematologic similar to cyclophosphamide alone. The plasma pharmacokinetics of cyclophosphamide in this study are similar to previously reported results for cyclophosphamide alone but the 24 hour urinary excretions of cyclophosphamide is lower than previously reported. The low order of clinical responsiveness in this tumor suggest that other antineoplastic agents or more sensative tumors should be tested.

PHS-6040

There have been many advances in the drug treatment of many human malignancies. Several cancers such as metastatic squamous cell carcinoma of the lung have proven relatively refractory to conventional drug treatment. Therefore, new agents or new experimental modalities for the treatment of these diseases is warranted. It has recently been demonstrated that the antitumor activity of antineoplastic agents, especially alkylating agents, can be enhanced by the administration of dimethylsulfoxide. Patients with advanced squamous cell carcinoma of the lung were given dimethylsulfoxide and then treated with intravenous cyclophosphamide to test whether this treatment might increase the permeability of the drug and augment the response that might be seen from cyclophosphamide. Pharmacokinetics were performed to follow drug disposition. Fourteen patients received 5 liters of 5 or 6% DMSO solution over 3 days and then 1500 mg/m^2 of cyclophosphamide intravenously on the third day. Serial blood and urine samples were collected to assess the pharmacokinetics of cyclophosphamide. No antitumor response were observed and this likely precludes a 20% level of activity. Toxicity was mainly hematologic and similar to cyclophosphamide alone. Unmetabolized cyclophosphamide and alkylating activity concentrations were determined in plasma, CSF and urine. Plasma pharmacokinetics of cyclophosphamide in this study are similar to previously reported results for cyclophosphamide alone but the 24 hour urinary excretion of cyclophosphamide was much lower than previously reported. Further studies in tumors more responsive to cyclophosphamide or the use of additional agents seems warranted.

Proposed Course: Continue Investigation

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09113 03 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Treatment of Advanced Gynecological Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dean E. Brenner, M.D.	Scientific Expert	COB,NCI
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	Arlene A. Forastiere, M.D.	Senior Investigator	COB,NCI
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Clinical Oncology Branch

SECTION
Section of Medical Oncology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Second line salvage chemotherapy for ovarian carcinoma, endometrial carcinoma, or cervical carcinoma rarely exceeds 10% to 20% response rates. Thus new drugs are needed. Thirty-two patients with advanced gynecologic malignancies, 11 ovarian, 17 cervical, 4 endometrial carcinoma received 120 mg/m^2 of mAMSA. Among 30 evaluable patients, there were 3 partial responses: cervical-2, endometrial-1. This suggests minimal activity for this drug in carcinoma of the cervix and less in ovarian carcinoma. For spirogermanium in advanced gynecologic malignancies, 11 patients have been treated, 6 with ovarian and 5 with squamous cell cervical carcinoma. The data are too early to evaluate.

For newly diagnosed advanced ovarian carcinoma, doxorubicin, cis-platinum, VP-16 and cyclophosphamide is being tested. Five patients have been entered but 2 are still unevaluable. There has been one complete response and 2 failures. The data are thus too early for evaluation.

PHS-6040

Tumors of the female genital tract constitute 20% of the overall cancer incidence in the females. Salvage chemotherapy remains poor. Patients with carcinoma of the ovary, carcinoma of the cervix, or carcinoma of the endometrium, following initial treatment with surgery or radiation therapy rarely respond to chemotherapy. Therefore, the introduction of new cytotoxic techniques in the testing of this group of patients is important to current chemotherapeutic armamentarium. mAMSA is one of a series of acridines synthesized in an effort to design agents which would bind to DNA and would have antitumor activity. Current phase II studies in gynecologic tumors have been performed at our institution. We find little response rate in carcinoma of the cervix and carcinoma of the ovary. Because of the few patients with endometrial carcinoma that are treated with chemotherapy, there is not enough data in this tumor to determine mAMSA's activity.

Spirogermanium is a new azapiran with anti-tumor activity whose activity appears to be through the inhibition of protein synthesis. DNA and RNA synthesis have not initially been altered. In vitro activity has been found against HELA, L1210, and human malignant lymphoma cells. Initial studies in Europe have suggested activity in advanced ovarian carcinomas.

PROJECT A: Advanced Refractory Carcinoma of the Ovary.

Eleven patients with carcinoma of the ovary were entered onto the mAMSA protocol. All these patients had late-stage disease and were previously treated with chemotherapy. No responses in 9 evaluable patients were observed. There was severe myelotoxicity in 4 of the patients and mild gastrointestinal toxicity in 2. We believe that there is little activity of this drug in ovarian carcinoma.

Six patients with advanced ovarian carcinoma have been treated with spirogermanium. After 3 weeks of QOD treatment, there is clinical evidence of progression in 2. Toxicity for this drug has been mild with lethargy and fatigue, one episode of nausea and vomiting and one episode of intravenous site burning. Further patients are needed on this protocol to define the activity of the drug.

PROPOSED COURSE: Continue investigation with spirogermanium and other new agents.

PROJECT B: Carcinoma of the Cervix.

Seventeen patients with advanced carcinoma of the cervix were entered on m-AMSA. There were 2 partial responses of short duration. Fifteen of 17 patients were treated had prior radiation or chemotherapy. There was myelotoxicity in approximately 1/3 of patients and mild gastrointestinal toxicity. We believe that this drug has minimal activity for this disease.

Spirogermanium has been administered to 5 patients with advanced carcinoma of the cervix. One patient had progressive disease after 6 weeks. A second patient has had a clear objective/subjective response. Other patients are not evaluable for response as it is too early.

PROPOSED COURSE: Continue investigation with spirogermanium and other new drugs.

PROJECT C: Carcinoma of the endometrium.

Four patients with advanced carcinoma of the endometrium have been entered onto m-AMSA. One patient responded and there was one patient with a long duration of stabilization. No toxicity was observed. There are too few patients for a meaningful evaluation of activity.

PROPOSED COURSE: Continue investigation.

PROJECT D

For newly diagnosed patients with ovarian carcinoma the 4 drug combination of cyclophosphamide, doxrubicin, cis-platinum, and VP16-213 is tested to assess response and duration. As of April 1981 5 patients have been entered onto study and 3 are evaluable for response with 1 partial response and 2 progressive disease. The data are thus too early to draw meaningful conclusions regarding the value of this combination.

PROPOSED COURSE: Continue investigation.

PUBLICATIONS:

1. Whitley N., Brenner D., Francis A., Villa Santa U., Aisner J., Wiernik P.: Use of the computed tomographic whole body scanner for staging and follow-up of patients with advanced ovarian carcinoma. Invest. Radiology, accepted for publication.

2. Brenner D.: Chemotherapy of carcinoma of the cervix. In : Scott R.M., Prempree T. (eds). Carcinoma of the Cervix. Thomas C.A., Springfield, Illinois, (1981) (in press).

3. Brenner D.: Carcinoma of the Cervix - a review. Am. J. Med. Sci. accepted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09117 03 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Phase II Studies with New Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Merrill Egorin, M.D.	Expert, Lab. Clinical Biochemistry	COB,NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phase II studies are therapeutic investigations of new agents that have finished Phase I testing (i.e. clinical toxicology and pharmacology studies). Completed negative Phase II trials have been done with 6-thioguanine in colorectal carcinoma and Methy-G in renal cell carcinoma. Trials are currently underway with Methyl-G in GU tumors and esophageal carcinoma, DHAD in soft tissue sarcomas, AMSA in malignant melanoma, AZQ in oat cell carcinoma of the lung and head and neck malignancies; and neocarzinostatin plus VP16-213 in hepatocellular carcinoma.

Phase II studies are therapeutic trials of new anticancer agents that have finished clinical pharmacokinetic and toxicity testing (Phase I trial). A minimum of 14 patients with refractory metastatic malignant disease are entered in each disease category to exclude a possible 15-20% response rate with 95% confidence limits. Patients are expected to have measurable disease and an expected survival of two months. If responses are seen, additional patients are entered to adequately define a response rate.

PROJECT A Colorectal Carcinoma

Intravenous 6-thioguanine in colorectal carcinoma. Nineteen patients have been entered on study at a dose of 800-1000 mg/m^2 IV bolus every three weeks. Toxicities are emesis and leukopenia. No responses were seen. A method has been devised using a high pressure liquid chromatography technique to measure parent compound and pharmacokinetics.

Conclusion: Closed. Intravenous 6-thioguanine is ineffective in colorectal carcinoma.

PROJECT B Renal Cell Carcinoma

Methyl-G in Renal Cell Carcinoma: Fourteen patients were treated with Methyl-G at 500 mg/m^2 as a one hour infusion every three weeks. No antitumor responses were seen. Toxicities were myalgias, weakness and diarrhea.

Conclusions: Study closed. Methyl-G is ineffective in renal cell carcinoma.

PROJECT C Oat Cell Carcinoma of the Lung

AZQ in Oat Cell Carcinoma (OCC): Four patients with relapsed OCC of the lung received AZQ at 20 mg/m^2 as a 1/2 hour infusion d1 and d8 every three weeks. No antitumor responses have been seen.

Conclusions: Study too early to evaluate.

PROJECT D Malignant Melanoma

AMSA in Malignant Melanoma: Thirteen patients have been entered on study and treated at a starting dose of 150 mg/m^2 every three weeks. The study is too early to evaluate for response. No significant toxicities have been seen suggesting that malignant melanoma patients have more bone marrow tolarence for treatment with myelotoxic agents.

Conclusions: Too early to evaluate

PROJECT E GU Malignancies

Methyl-G in GU Malignancies: Four patients with bladder carcinoma have been entered on study at 5-600 mg/m^2/week. One patient with transitional cell carcinoma of the renal pelvis has achieved a partial response (PR). Toxicities have been diarrhea and neuromuscular.

Conclusions: Too early to evaluate for response. Continue study.

PROJECT F Esophageal Carcinoma

Methyl-G in Esophageal Carcinoma: Study recently activated and three more patients have been entered. To be done in collaboration with the Latin American-USA Exchange. Too early to evaluate for response.

Conclusions: Continue study.

PROJECT G Hepatoma

NCS + VP 16-213 in Hepatoma: Twelve patients have been entered on-study. Survival on this study is directly related to pretreatment performance status. Although no significant tumor regressions have been seen, stabilization has occurred in several patients for as long as 12 months.

Conclusions: Too early to evaluate for response. Study open.

Proposed Course: Continue investigation.

PROJECT H Head and Neck Malignancies

AZQ in head and neck malignancies. Three patients have been entered on study at 20 mg/m^2 d1 and day 8. Toxicities have been myelosuppression.

Conclusions: Too early to evaluate for response. Study open.

PROJECT I DHAD

DHAD in soft tissue sarcomas. Two patients have been entered on study at 4 mg/m^2/d x 3.

Conclusions: Too early to evaluate. Study open.

Publications:

1. Fuks, J.Z., Van Echo, D.A., Aisner, J., Mitchel, E.P., Wooley, P.V., Wiernik, P.H.: A phase II trial of Methyl-G in patients with metastatic renal cell carcinoma. Cancer Clinical Trials (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09123 02 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Evaluation of Biomarkers in Patients with Advanced Malignant Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB NCI
Other:	Margaret A. Whitacre, RN	Hyperthermia Nurse	COB NCI
	David A. Van Echo, M.D.	Senior Investigator	COB NCI
	Joseph Fuks, M.D.	Clinical Associate	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
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Frederick Cancer Research Center

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Clinical Oncology Branch

SECTION
Section of Medical Oncology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biomarkers, when available, allow for certain malignancies to be either diagnosed early, recurrences to be diagnosed early, or allow for sequential analysis to determine the course of treatment. These parameters are usually more sensitive indicators of disease than conventional diagnostic techniques. In an attempt to identify biomarkers from patients with advanced malignancies, particularly those with lung cancer, blood samples were drawn at the time of admission and sequentially during treatment. Sixty-three patients have been entered onto study and 62 patients have had multiple blood samples drawn for CEA, calcitonin, vasopressin, ACTH, and C3dp-C, and enolases. Laboratory data has been entered onto the DCRT computer and corresponding clinical information from each time of blood drawing has been accrued and is currently being entered onto the same computers for correlative analysis. Approximately 35 patients have been analyzed by both modalities and at the present time the data is too preliminary to draw any firm conclusions about the value of any of the biomarkers.

Diagnostic techniques and technology allow for the evaluation of tumor masses only when they have accumulated a sufficient numbers of cells to be either palpable or demonstrable radiographically. Such accumulations require a large number of cells and generally signify advanced disease. Biomarkers when available allow for the detection of tumor cells in much smaller deposits. Therefore, if biomarkers were available one might diagnose tumors earlier, or one might follow the results of treatment sequentially to determine if the patients tumor burden had become sufficiently low. One might also use biomarkers to determine when and if a patient relapsed from response.

In order to identify biomarkers, patients with advanced cancer, particularly those with lung cancer were followed sequentially with serial blood samples and the results of the biomarkers assayed. The sequential analysis was entered onto DCRT computers and graphed. The correlative clincal data for each blood drawing period was logued and analyzed for each of the patients including treatment courses, performance status, measurable disease, and progress of treatment.

As of April, 1981, 63 patients have been entered onto study and 62 patients have been sequentially tested for biomarkers. There were 40 patients with small cell lung cancer, 2 patients with mixed small cell and squamous cell carcinoma, 3 with squamous cell carcinoma, 14 with adenocarcinoma of the lung, 1 large cell carcinoma of the lung, 2 patients with acute myelogenous leukemia and 1 patient with acute lymphoblastic leukemia. Blood samples were assayed for CEA, calcitonin, vasopressin, ACTH and a compliment fragment C3dp-C. In addition some selected samples were assayed for enolases. There has been a wide variation of results from tests for example CEA's have varied from 0 to 450 units. Sequential analysis of the biomarkers has been entered onto DCRT computers and have been graphed for clinical correlation of disease status, treatment, performance status, weight loss and other clincal features. These clinical features will be analyzed for each of the times at which a blood sample was drawn and the results correlated to determine if any of the biomarkers fluctuate according to disease status or treatment. To date 35 patients have been analyzed but the information is too preliminary to draw any conclusions.

Proposed Course: Continue Investigation

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09137 02 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Evaluation of Δ9-THC as an Antiemetic in Patients with Malignancies Undergoing Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Nicholas Bachur,M.D.,Ph.D.	Chief, Lab. Clinical Biochemistry	LCB,NCI
	Merrill J. Egorin, M.D.	Scientific Expert	LCB,NCI
	Dean Brenner, M.D.	Clinical Associate	COB,NCI
	Charles Riggs, M.D.	Clinical Associate	LCB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Medical Oncology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Δ9 Tetrahydrocannabinol (THC) has been shown in previous clinical observations to ameliorate nausea and vomiting associated with chemotherapy. This current double blind, placebo controlled study is designed to assess the antiemetic efficacy of THC in cancer patients undergoing intensive chemotherapy. In addition, since THC can alter hepatic-microsomal drug metabolism of certain drugs as evidenced by induced tolerance to THC this study examines the effects of THC on the plasma pharmacokinetics of antineoplastic agents.

Nausea and vomiting, an almost universal side effect of chemotherapy, remains a major problem to patients, not infrequently causing them to withdraw from treatment programs. Currently employed drugs for achieving anti-emesis, including phenothiazines, often fail to ameliorate chemotherapy-related nausea and vomiting. Δ^9-THC, the active ingrediant in marihuana has been identified as possessing antiemetic effect. THC can be administered orally or by inhalation. Several reports have suggested that THC:

1. is a more effective antiemetic agent than Compazine
2. can induce tolerance, thus subsequent THC dose may need to be increased
3. may stimulate induction of enzymes
4. may have drug interactions with chemotherapeutic agents

Patients, ages 21 to 65 who receive chemotherapy with cis-platinum; adriamycin and cyclophosphamide alone or in drug combinations are allowed to participate. Patients are randomized in double blind fashion to receive Δ^9-THC, Compazine or placebo. Patients give information on nausea, vomiting, sedation, incoordination, "high". During this study blood samples are drawn periodically to study plasma level of Δ^9-THC and pharmacokinetics of adriamycin, cyclophosphamide and/or cis-platinum.

As of April 1980 five patients have been evaluated. Some anti-emetic effect has been noted but because of a small patient group the code has not been broken yet and the assessment of exact anti-emetic efficacy of THC as well its plasma levels and interactions with chemotherapeutic agents is not yet evaluable.

Proposed Course: Continue investigation

Publications:

1. Riggs, C.E., Egorin, M.J., Fuks, J.Z., Schnaper, N., Duffey, P., Colvin, O.M., Aisner, J., Wiernik, P.H., Bachur, N.R.: Initial observation on the effects of Δ^9-tetrahydrocannabinol on the plasma pharmacokinetics of cyclophosphamide and doxorubicin. J. Clin. Pharmacol. 21:131-137, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09139 02 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Use of Computed Tomographic Scanning in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To prospectively study the capabilities computed tomography (CT) in oncology, 20 patients with advanced carcinoma of the cervix have been evaluated pre-operatively by the CT to correlate extent of local disease and presence of retroperitoneal adenopathy. Sensitivity of finding this adenopathy is 67% and specificity 92%. Accuracy predicting final staging is 65% compared to post-operative results. Prospective studies are underway in ovarian carcinoma and 17 patients have clinical-pathological correlation for 67 CT scans. For 83% of the patients and 88% of CT scans, CT was found to contribute management information and provided decision making information. Clinical decisions were made on the basis of CT alone in 43% of scans, where other clinical evaluations were not helpful. A new CT voluming technique has been developed which allows for more accurate CT tumor measurements. Current studies on patients with ovarian carcinoma, sarcomas, and small cell carcinoma of the lung are underway.

PHS-6040 (Rev. 2-81)

Computed tomagraphy (CT) is a new diagnostic tool that allows for accurate assessment of masses or fluids in closed body spaces. There is little information on the application of this tool as a routine method for medical oncology. Project A seeks to use CT as a means of guiding biopsies into difficult body areas. Patients with advanced carcinoma of the cervix commonly relapse distally due to retroperitoneal node involvement. Retroperitoneal node sampling is used to determine the location of radiotherapy portals and the prognostic indications as well as for the acceptability of surgery for these patients. Project B looks at the role of the CT scan in the preoperative staging and follow-up of patients with advanced carcinoma of the cervix. Patients undergo CT scans, then have correlation with non invasive testing and laparotomy. Patients with ovarian carcinomas being treated with chemotherapy are difficult to assess because of its intraabdominal spread. The CT provides visualization of abdominal and retroperitoneal structures which is unavailable. Project C looks at the role of CT scan to evaluate the clinical course of patients undergoing chemotherapy particularly those with advanced ovarian carcinoma. Measurement of tumor response to therapy by standard roentgenographic or nuclide scan techniques is fraught with measurement errors. The CT scan allows the determination of sequential cross-sectional areas, allowing the computation of the volume of a given organ or mass. Project D is an attempt to correlate tumor volume or volume of in vivo organs or ex-vivo organs with results of direct measurements in order to assess the ability of this tool to quantify response.

PROJECT A: Biopsy under CT Scan guidance.

Biopsies of livers in 13 patients under CT scan guidance have been performed without morbidity. Adequate tissue and pathology was obtained in 12 out of 13 patients. Pathologic diagnosis in these biopsy patients has been obtained in 12 out of 13 patients.

PROPOSED COURSE: Continue investigation.

PROJECT B: Pre-operative assessment, staging, and node status in patients with advanced carcinoma of the cervix.

Twenty patients with advanced carcinoma of the cervix have had pre-operative abdomino-pelvic CT scans performed prior to surgery. There were 13 true negative, 4 true positive, 1 false positive and 2 false negative studies for a sensitivity of 67% and a specificity of 92%. CT staging agreed with pathologic staging in 13 out of 20 patients (65%). CT had difficulty detecting pelvic sidewall involvement, and microscopic invasion into adjacent visceral pelvic structures (e.g. bladder and rectum).

PROPOSED COURSE: Continue investigation.

PROJECT C: Response to therapy as documented by CT scan in patients with ovarian adenocarcinomas.

Seventeen patients with epithelial ovarian carcinoma had 22 whole body scans performed prior to or following laparotomy. In this group of patients, a high pathologic correlation was found when the CT was positive at liver, ascitic peritoneal, mesenteric, and omental sites. When the CT was negative, high pathologic correlation was found only at the ascitic and mesenteric sites. Repetitive CT scans were performed on 18 patients followed prospectively during treatment. When CT results were compared to physical examination, other radiological studies, and clinical status, 83% of patients and 88% of CT scans performed contributed useful management information. In 43% of scans performed, decisions were made on the basis of CT only. Such data suggests that the CT scanner is very useful in the staging and follow-up of patients with ovarian carcinoma, but the CT failed to detect small size metastases within the abdominal or pelvic cavities.

PROPOSED COURSE: Continue investigation.

PROJECT D: Estimation of tumor volumes by CT scanner.

Accuracy studies performed with inanimate objects, cadaver organs, and balloons inserted into the stomach and vagina and enlarged with a known volume of contrast material have documented the accuracy of this technique. Tumor volume estimations are now underway in patients with ovarian carcinoma, sarcomas, and small cell carcinomas of the lung. Correlations with response and progressive tumor in these patients have been accurate. CT voluming is able to define in an exact manner ($\pm$10%) the degree of tumor increase or decrease in size.

PROPOSED COURSE: Continue investigation.

PUBLICATIONS:

1. Whitley N., Brenner D., Francis A., Villa Santa U., Aisner J., Wiernik P.: Use of the computed tomographic whole body scanner for staging and follow-up of patients with advanced ovarian carcinoma. Invest. Radiology, accepted for publication.

2. Brenner D.E., Whitley N., Goldstein W.Z., Aisner J.: Computed tomographic demonstration of peritoneal mesothelioma. Lancet (in press) 1981.

3. Whitley N., Brenner D., Aisner J., Wiernik P.: Use of computed tomography (CT) in the preoperative evaluation of cervical carcinoma. Proc. XV Int. Congress of Radiology, Brussels, 24 June-1 July 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09150 01 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Squamous Cell Carcinoma of the Head and Neck, and Esophagus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Results of standard treatment for squamous cell carcinoma (SCC) of the head and neck or esophagus are poor. New approaches employing combined modality treatment, new combination chemotherapy regimens and new agents are needed. In Project A, newly diagnosed pts with esophageal SCC receive 2 cycles of cis-platinum, bleomycin and VP 16-213 prior to local treatment (surgery or radiotherapy). Those with recurrent disease receive the 3 drug combination until disease progression. To date, among 6 pts there are 2 partial responses (PR),1 minor response,1 early death,and 2 are too early to evaluate. In Project B, a combined modality approach is employed for previously untreated, pts with unresectable head and neck SCC. Two cycles of cyclophosphamide and cis-platinum are given prior to radiotherapy and then continued during radiotherapy. One pt has been entered. In Project C, advanced, recurrent head and neck cancer is treated with the combination of cis-platinum and cyclophosphamide. To date, responses in 12 evaluable pts are: 2 CR and 6 PR (66%). Project D evaluates new agents (see also report Z01 CM 09117 03 COB). A phase II trial for recurrent head and neck cancer with aziridinylbenzoquinone (AZQ) has accrued 4 pts.

The prognosis for patients with advanced squamous cell carcinoma of the head and neck or esophagus is very poor with conventional approaches to treatment (surgery and radiotherapy). Five year survival for stage III and IV head and neck carcinomas ranges from 10-25% depending on primary site. Overall survival for esophageal cancer patients is less than 5% at 5-year due to the presence of occult metastases in most patients at diagnosis. There are few antineoplastic agents with significant activity against these tumor types. When chemotherapy is used to treat disease which has not been controlled by surgery and radiotherapy neither single nor combination chemotherapy produces a significant effect on survival. Response rates for head and neck cancer vary from 20-50% depending on the regimen and are in the range of 15-20% for esophageal cancer. The majority of responses are partial and the duration of response is brief. In contrast, results of trials employing cis-platinum containing combination chemotherapy prior to local treatment (surgery or radiotherapy) have demonstrated an improvement in overall response rate and in the number of patients achieving complete regression of disease. The theoretical advantange of this combined modality approach is that more drug may reach the tumor bed if given prior to disruption of the vascular supply by surgery or radiotherapy.

To further investigate this approach to treatment of advanced epidermoid carcinoma of esophagus (Project A) and head and neck (Project B), we are evaluating the therapeutic effacacy of two cis-platinum containing chemotherapy regimens. This chemotherapy will be given as initial treatment, prior to surgery or radiotherapy to reduce tumor bulk so that unresectable disease may become surgically approachable or to reduce the number of hypoxic cells so that radiotherapy will be more effective in sterilizing tumor bed.

For previously treated patients, there are two trials evaluating chemotherapy for epidermoid carcinoma of the head and neck. One trial, Project C, evaluates a combination of conventional agents - cis-platinum and cyclophosphamide. This combination is of interest because of possible synergism between these two drugs. This regimen may represent treatment with low toxicity and substantial activity. The drugs are easily administered in a low dose to out-patients with minimal toxicity. Project D evaluates new agents for previously treated patients with head and neck cancer. Currently a phase II agent, Aziridinylbenzoquinone (AZQ), is being tested in those patients refractory to conventional chemotherapy (see also report Z01 CM 09117 03 COB).

PROJECT A: Cis-platinum, Bleomycin and VP 16-213 for the Treatment of Squamous Cell Carcinoma of the Esophagus.

As of April 1, 1981, six patients have been entered into this trial. Two patients with recurrent disease (liver, lung, soft tissue metastases) are partial responders at 3+ and 6+ months. There is one non-responder, one early death one week into treatment and two patients are too early to evaluate.

Proposed Course: Continue investigation.

PROJECT B: Cis-platinum, Cyclophosphamide and Radiotherapy Combined Modality Treatment of Advanced Head and Neck Cancer.

This trial has just been initiated. As of April 1, 1981 one patient has been entered.

Proposed Course: Continue investigation.

PROJECT C: Cis-platinum and Cyclophosphamide for Advanced, Recurrent Epidermoid Carcinoma of the Head and Neck

As of April 1, 1981, 15 patients have been entered into this trial. There are 2 CR, 6 PR, 1 minor response, 3 progressions and 3 are too early to evaluate. Results are too preliminary to comment on response duration. Toxicity consists mainly of mild nausea and vomiting. Two patients have required in hospital treatment for prolonged nausea and vomiting. There has been no nephrotoxicity. Myelosuppression consists of depression of all three blood elements, leukopenia being most prominant. There have been no treatment related infections or bleeding complications.

Proposed Course: Continue Investigation.

PROJECT D: Aziridinylbenzoquinone (AZQ) for Epidermoid Carcinoma of the Head and Neck Refractory to Conventional Chemotherapy.

This study is being conducted using a day one and eight schedule every 28 days. Four patients have been entered into the study. No antitumor activity has been observed. Toxicity has consisted of myelosuppression only, primarily leukopenia (see also report Z01 CM 09117 03 COB).

Proposed Course: Continue Investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06217-12 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Prevention of Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C.A. deJongh	Guest Worker	COB,NCI
	M.R. Moody	Research Microbiologist	COB,NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB,NCI
	C.L. Fortner	Head, Clinical Research Pharmacy	COB,NCI

COOPERATING UNITS (if any)

None

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Clinical Oncology Branch

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	6	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The risk of infection increases as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. At the Baltimore Cancer Research Program an intensive infection surveillance program assesses the ongoing infectious disease status of the patients and the Program as a whole. From this data base have grown appropriate basic infection control policies and specific research approaches including evaluation of laminar air flow rooms, simpler means of air filtration and reverse isolation, oral nonabsorbable antibiotics and techniques to prevent axillary and oral infections. Trimethoprim/sulfamethoxazole and nalidixic acid, both absorbable antibiotics, are being evaluated as a means of suppressing the aerobic gram-negative flora while preserving colonization resistance. These programs are enforced by the infection control nurse. Ketoconazole, an imidazole derivative with good activity against Candida sp., will be included in the oral antibiotic regimens and compared to the antifungal currently in use: nystatin.

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice which potentially damage host defense mechanisms and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection.

Specific Projects:

A. Surveillance - The cornerstone of an infection control program is an accurate understanding of the infections presently occurring including the sites and etiologic agents, apparent predisposing factors, routes of acquisition of hospital organisms, and changing patterns of morbidity and mortality. At the Baltimore Cancer Research Program, this is accomplished by the daily rounds of an infection control nurse, twice weekly infectious disease rounds, daily evaluation of all infected or potentially infected patients, and an extensive program of both patient and environmental microbial culturing. The data derived is summarized regularly and utilized to adjust, on a continuing basis, the programs in infection control.

B. Basic Infection Control Procedures - The Infection Control Policy Manual has been continually updated with enforcement pursued by the infection control nurse. The following procedures are standard: 1) urinary, intravenous, and hyperalimentation catheters are avoided except under extenuating circumstances, 2) all intravenous solutions are prepared in laminar air flow hoods, all bottles and tubing are replaced daily, and butterfly needles are changed every other day, 3) personal hygiene is emphasized including handwashing and the use of special germicidal hand lotions, 4) all in patients are seen regularly by the infection control nurse for discussion of preventive techniques, 5) results of patient and environmental surveillance cultures are utilized to direct specific control techniques. These techniques have substantially reduced nosocomial infection.

C. In an attempt to further 1) evaluate the preservation of "colonization resistance", 2) improve patient tolerance, and 3) redue cost, relapsed acute leukemia patients were randomly allocated to receive either gentamicin plus nystatin (GN) or trimethoprim/sulfamethoxazole (TMP/SMZ) plus nystatin. Also, 40 patients with oat cell carcinoma of the lung who were to receive intensive myelosuppressive chemotherapy were randomly allocated to receive trimethoprim/sulfamethoxazole or a placebo. Extensive microbiologic studies of the patients with oat cell carcinoma indicated that TMP/SMZ does adequately suppress the aerobic gram-negative microflora of the alimentary canal without suppressing the anaerobic flora, presumably thereby preserving "colonization resistance". More patients will need to be studied to determine the full prophylactic effect but the trend is toward a reduction in gram-negative infections in the TMP/SMZ treated group. Also as of this date, 44 patients with acute leukemia have been evaluated. Those receiving TMP/SMZ have had rates of infection comparable to those receiving GN, indicating that TMP/SMZ+N is effective for infection prophylaxis in this patient population. Acquisition and infection with

gentamicin-resistant gram-negative rods have been minimal. Patient tolerance and compliance are markedly improved. The cost of TMP/SMZ+N is much less than GN. These results indicate that this new approach to infection prevention may prove to be equally efficacious, yet less toxic, more tolerable and less expensive than total microbial suppression with oral nonabsorbable antibiotics.

D. Based on the results above that TMP/SMZ+N is an effective form of infection prophylaxis and based on reports from elsewhere that nalidixic acid will, similarly to TMP/SMZ, suppress alimentary tract aerobes but not anaerobes, a new trial was initiated. Newly diagnosed, previously untreated patients with ANLL who are not infected at the time of admission and relapsed acute leukemic patients are randomly allocated to TMP/SMZ+N or nalidixic acid plus nystatin (NA+N). Eighty-three patients have been entered to date, of whom 52 are evaluable, with initial results suggesting advantage for the TMP/SMZ+N regimen with regard to infection prevention. However, allergic reactions do occur with sulfa compounds whereas nalidixic acid has no apparent significant side effects. As many as 10 more patients are in the process of being evaluated and final results should be available in the immediate future.

E. Laminar air flow room isolators have been recently installed at the BCRP.

A major problem is that newly admitted patients with ANLL who are infected at the time of admission have a reduced complete remission rate and consequently a reduced overall survival time. Remission rates are low (< 30% compared to 80% for noninfected patients) because many of these patients die of infection before receiving an adequate trial of cytotoxic therapy. Death is usually due to the rapid occurrence of new infection, often from an acquired fungus or from a recently acquired antibiotic-resistant gram-negative bacillus.

Most of the careful evaluations of LAF isolation microbial suppression techniques have excluded from evaluation patients who presented with infection. This was done to ensure comparability between groups and to reduce confusion during evaluation. As a result, there is no clear understanding at the present time as to the degree of value of LAF isolation-microbial suppression for the patient admitted with pre-existing infection. It can be assumed however that this approach will substantially reduce the incidence of new infection and, as a result, it can be hypothesized that with reduced infection and reduced infectious death, the remission rate should increase and approach that of the patient admitted without infection. This protocol seeks to resolve this question by randomly allocating patients to the current standard approach at the BCRP (Control group) or to that same approach with the addition of complete reverse isolation within the laminar air flow room (LAF group). In both groups, the remission induction chemotherapy will be the same and supportive care will be equivalent with the only difference in the management between the two groups being the utilization of total barrier reverse isolation nursing in a laminar air flow unit or not. Eight patients have been included in this study and their data is being analyzed, but a significantly higher number of patients will be necessary to complete the evaluation.

Additional evaluations in the LAF environment will include: comparison of remission induction potential for patients with ANLL over the age of 60 years. These patients have a poorer response rate than younger patients because death often occurs because of supervening infection. It is possible that a substantial reduction in infection among these patients will result in an increased remission rate. A third group of patients are those in complete remission of ANLL who will be receiving intensive cytoreductive maintenance therapy. These patients require the maximum infection prevention available due to the high risk involved with this form of maintenance.

F. The Effect of Chemotherapy and Oral Antibiotics on Intestinal Absorption - Since patients who are receiving cytotoxic therapy for acute leukemia or who are receiving prophylactic oral antibiotics for infection prevention frequently experience anorexia, nausea, vomiting, diarrhea and mucositis, it is possible that intestinal absorption may be impaired. Patients with acute leukemia about to receive cytotoxic therapy and prophylactic oral antibiotics are being studied prospectively to determine whether the chemotherapy, the oral antibiotics, both or neither have a substantial effect on intestinal absorption function. Fourteen ANLL patients have been studied before therapy, following cytotoxic therapy and following the use of oral prophylactic antibiotics. Evaluation has included serum vitamin A concentration, karotine, folate, and serum and urinary d-xylose. The results of the patients studied indicate that there is no major effect on intestinal absorption with the exception of a moderate reduction in d-xylose absorption following the institution of oral nonabsorbable antibiotics.

G. Ketoconazole, an imidazole derivative with low order of toxicity, has significant in vitro efficacy against Candida and Torulopsis and borderline efficacy against Aspergillus sp. Ketoconazole will be added to the oral antibiotics trimethoprim/sulfamethoxazole (TMP/SMZ) or nalidixic acid (NA) and compared to the current prophylactic regimens of TMP/SMZ or NA plus nystatin to determine its efficacy in preventing candidiasis and aspergillosis.

H. Many of the infections that occur in cancer patients are caused by microorganizms that are acquired by the patient from the hospital environment. The purpose of this continuing project is to monitor the patients' physical environment, the products used in their care, and the personnel involved in their care, in order to detect and eliminate, insofar as possible, the sources of such organisms. Regularly scheduled cultures of laminar air flow rooms are performed once or twice weekly to monitor protective procedures; general personnel studies are carried out twice per year and persons in contact with patients in laminar air flow rooms are cultured on a weekly basis. Isolation rooms are monitored for the presence of antibiotic resistant microorganisms following cleaning procedures when the isolated individual leaves the room, and weekly cultures of food, air, water, room surfaces and, less frequently, pharmaceuticals for the LAF rooms, are done.

Cultures of food during the past year were not consistently free from bacteria. Penicillium and Cladosporium species were grown from baked potato, Rhodotorula and Cladosporium sp from mashed potato, Enterobacter

agglomerans from custard, Group D streptococci from a casserole of ground beef and macaroni, and Aspergillus niger from veal parmigiana. Of the non-cooked food, several species of non-fermenting gram negative bacilli and Escherichia coli were recovered from a culture of toasted white bread; Coca cola grew the yeast Rhodotorula and Candida parapsilosis, and cultures of puffed rice grew Aspergillus flavus. Such results allowed recommendations to be made for elimination of some foods and for care in handling other food items that were undoubtedly contaminated after having been cooked. Extensive studies were performed to evaluate supplies, procedures and personnel for new LAF rooms. Surfaces were relatively bacteria free except for occasional cultures of splash areas. Linen supplies were sterile with rare exceptions. Of the pharmaceuticals employed, only nystatin suspension was contaminated (Bacillus sp.). The patients' gut flora was effectively suppressed, but gingival and nose cultures continued to harbor microorganisms. On the basis of the appearance of several microorganisms in the nose cultures that had not been recovered from earlier samples, the decision was reached to use an inhibitory nose cream in an attempt to suppress such flora.

Preliminary work in a collaborative study initiated by Dr. DePaola of the University of Maryland dental school revealed that denture cups and unclean dentures are often heavily contaminated by a variety of yeasts and gram negative bacilli. Microbiologic support will be provided for his effort to eliminate this source of oral contamination.

Publications:

1. Schimpff, S.C.: Infection prevention during granulocytopenia. In Remington, J.S. and Swartz, M.N. (Eds.): Current Clinical Topics in Infectious Diseases 1980. New York, McGraw-Hill Book Company, 1980, pp. 85-106.

2. Wade, J.C. and Schimpff, S.C.: Infections in patients with suppressed cellular immunity. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. II. New York, Raven Press, (in press) 1981.

3. Schimpff, S.C. and Young, V.M.: Epidemiology and prevention of infection in the immunocompromised host. In Young, L.S. and Rubin, R.H. (Eds.): Clinical Approach to Infection in the Immunocompromised Host. New York, Plenum Press (in press) 1981.

4. Newman, K.A. and Schimpff, S.C.: Protective isolation: What policies and procedures are necessary? Asepsis 1:2-3, 1980.

5. Schimpff, S.C.: The laminar air flow room--an effective means for preventing infection. In Wiernik, P.H. (Ed.): Controversies in Oncology. New York, John Wiley and Sons, Inc., Publ., (in press) 1981.

6. Newman, K.A., Schimpff, S.C. and Wade, J.C.: Antibiotic prophylaxis of infection for patients with granulocytopenia. In Verhoef, J., Peterson, P.K. and Quie, P.G. (Eds.): Infections in the Immunocompromised Host-Pathogenesis, Prevention and Therapy. Amsterdam, Elsevier/North-Holland Biomedical Press, 1980, pp. 187-204.

7. Schimpff, S.C.: Infection prevention during profound granulocytopenia: new approaches to alimentary canal microbial suppression. Ann. Intern. Med. 93:358-361, 1980.

8. Wade, J.C., Schimpff, S.C., Hargadon, M.T., Fortner, C.L., Young, V.M. and Wiernik, P.H.: A comparison of trimethoprim-sulfamethoxazole plus nystatin with gentamicin plus nystatin in the prevention of infections in acute leukemia. N. Engl. J. Med. 304:1057-1062, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06219-12 COB

PERIOD COVERED
October 1, 1980 through September 1, 1981

TITLE OF PROJECT (80 characters or less)
Studies on Cancer Patient Microbial Flora: Acquisition, Colonization and Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection and Microbiological Research	COB,NCI
Other:	K.A. Newman	Infection Control Nurse	COB,NCI
	M.R. Moody	Research Microbiologist	COB,NCI

COOPERATING UNITS (if any)
None

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.50	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to monitor the patients' microbial flora which may give rise to infection, to determine sources of hospital acquired flora when possible, and to study characteristics of unusual organisms of clinical significance. Such organisms may be subspeciated by phage typing, serotyping or biotyping to allow determination of spread. A review of cultures, particularly blood cultures, revealed that during the last year gram-positive cocci outnumbered gram-negative bacilli as a cause of bacteremias. Staphylococcus epidermidis and streptococci both increased significantly in number. Antibiotic resistant coryneform bacteria are still encountered in the patients and further studies have been carried out to evaluate their characteristics. Nasal surveys for filamentous fungi confirmed the presence of the organisms in the air as has been shown in air samples. Staphylococcus aureus surveys of personnel showed the carrier rate to be sharply decreased.

Opportunistic microorganisms that are considered "normal" microflora in a normal human host, are the chief cause of infection in cancer patients whose host defenses are impaired. This project is designed to monitor the cancer patients' flora, to determine sources of such flora, and to study unusual microorganisms that are recovered.

Baseline surveillance cultures of 135 consecutive patients with acute nonlymphocytic leukemia admitted to the BCRP for their first induction chemotherapy shortly after initial diagnosis and who had received no recent antibiotic therapy, indicated that there was a higher than expected incidence of colonization with gram-negative bacilli in the nose, gingiva and axillas along with the expected colonization by gram-negative bacilli in the rectum. Among these colonizing gram-negative bacilli, P. aeruginosa and, to a lesser extent, Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis were most likely to be associated with a subsequent bacteremia during periods of mucosal damage and granulocytopenia. Surveillance cultures also indicated those patients at highest risk of having yeast infections such as those caused by Torulopsis glabrata or Candida sp. Results of nasal surveillance cultures detected a subpopulation of patients at greatest risk for colonization and subsequent infection with Aspergillus flavus. Surveillance cultures have been utilized for the design of systemic therapeutic antibiotic protocols and for the monitoring of oral nonabsorbable antimicrobial regimens for alimentary canal microbial suppression.

The coryneform bacterium that is susceptible to vancomycin and occasionally to rifampin, but to none of the other antibiotics tested, is still occasionally encountered in the patient population. It was recovered from samples of approximately 21 patients last year. All of the strains thus far recovered have been run through a series of 50 biochemical reactions by means of 50L API strips and the results are under comparison with those obtained with conventional techniques.

The addition of surveillance nose cultures for filamentous fungi reported last year have been continued, as infections caused by these organisms remain a problem in the cancer patient. These cultures reveal that considerable variations in the number of positive cultures occur over time. For example, in January 1980, 8.3% of the 157 fungal nose cultures grew filamentous fungi, all from different patients, whereas in February 1980, 17.7% of the total 141 nose cultures for fungi yielded these organisms, including 9 Aspergillus flavus from 8 patients.

Filamentous fungi had been recovered from "street" marijuana in an earlier study. Patients at the BCRP are receiving marijuana or a placebo cigarette to ameliorate the nausea that can accompany the administration of some chemotherapeutic agents. These cigarettes as well as cigarettes and cigars from various brands of standard tobacco, pipe tobacco, and "street" marijuana were tested for the presence of bacteria and/or filamentous fungi. Bacillus spp. were isolated from all tobacco products; no bacteria or fungi were isolated from the placebo cigarette or the one made with federally controlled marijuana. Aspergillus flavus, A. niger, and Cladosporium spp. were recovered from the two samples of "street" marijuana.

The last survey of hospital personnel to determine Staphylococcus aureus carriers revealed that the carriage rate had dropped to a low of approximately 6%.

Publications:

1. Schimpff, S.C. and Young, V.M. Epidemiology and prevention of infection in the compromised host, In Rubin, R.H. and Young, L.S. (Eds.): Clinical Approach to Infection in the Immunocompromised Host. New York, New York, Plenum Publishing Corp., (in press) 1981.

2. Young, V.M., Moody, M.R. and Morris, M.J. Phase variation in the genus Serratia. J. Med. Microbiol. 13:341-343, 1980.

3. Young, V.M., Moody, M.R. and Morris M.J. Distribution of Serratia marcescens serotypes in cancer patients. J. Med. Microbiol. 13:333-339, 1980.

4. Newman, K.A., Schimpff, S.C., Young, V.M. and Wiernik, P.H. Lessons learned from surveillance cultures from patients with acute nonlymphocytic leukemia: usefulness for epidemiologic, preventive and therapeutic research. Am. J. Med. 70:423-431, 1981.

5. Young, V.M.: Surveillance cultures of immunosuppressed patients. Clin. Microb. News Let. 3:19-18, 1981.

6. Young, V.M., Meyers, W.F., Moody, M.R. and Schimpff, S.C.: The emergence of coryneform bacteria as a cause of nosocomial infections in compromised hosts. Am. J. Med. 70:646-650, 1981.

7. Young, V.M.: Hemophilus influenzae. In Peterson, J.W., Davis, C.P. and Alperin, L. (Eds.): Textbook for Medical Microbiology. Addison Wesley Publ. Co., (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06223-12 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on Pseudomonas aeruginosa in a Cancer Research Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. Tillman	Laboratory Scientist II	COB,NCI
	S. C. Schimpff	Head, Section of Infection and Microbiological Research	COB,NCI

COOPERATING UNITS (if any)

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Clinical Oncology Branch

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.66	.50	.16

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pseudomonas aeruginosa is one of the chief causative agents of infection in severely granulocytopenic patients with acute leukemia and other malignancies. The prime objectives of this study has been to monitor its epidemiology in cancer patients and its natural history in the hospital environs. Recovery rates from blood cultures are higher than last year. P. caryophylic from food was probably the original source of its incidence in the upper respiratory tract of five patients and in several denture cups. P. cepacia was isolated from denture cups in three patients at a later time. A strain of P. stutzeri with an unusual cross-resistance to penicillins was isolated from Micro-Bact, the toilets and sink sprays in the LAF units.

Pseudomonas aeruginosa remains a frequent cause of infection in cancer patients, and the prevention of infections by this ubiquitous organism continues to be an important goal. This project monitors the incidence, epidemiology, and natural history of P. aeruginosa. The role of other nonfermentative gram-negative bacilli in these patients and their environs is also evaluated on a continuous basis.

Serotyping of all strains of P. aeruginosa recovered is still performed on a routine basis. Over the last two years, the incidence of P. aeruginosa has increased. Recovery of this bacterium from blood was twice as high this year when compared to the previous year. P. aeruginosa continues to be infrequently isolated from the hospital environment.

Recovery of a nonfermentative gram-negative bacilli from patients as well as their environment has increased. The recovery of bacilli less frequently encountered in these patients such as Moraxella, Bordetella, Acinetobacter and nonsaccharolytic pseudomonads has risen while recovery of fluorescent pseudomonads other than P. aeruginosa has decreased.

Preliminary work in a collaborative study initiated by Dr. DePaola of the University of Maryland School of Dentistry revealed that denture cups and unclean dentures are often heavily contaminated by a variety of yeasts, gram-negative and gram-positive bacilli. One species that had been infrequently isolated at the BCRP, Pseudomonas caryophylii, was recovered from several denture cups during a one-month period in which the bacterium was also recovered from cultures of the upper respiratory tract of five patients. However, the earliest isolation of this organism was from cultures of toasted white bread. Cultures of several dental cups during a later period contained P. cepacia; this species was isolated from lesions and gum cultures of three patients. Although this organism was found to be able to assimilate chlorhexidine, a compound used for handwashing at the BCRP, no likely source of transmission, such as hands, food, etc., was found.

A nonfermentative gram-negative bacillus was isolated from the water supply in the toilets and sink sprays of the LAF units. It was identified as Pseudomonas stutzeri. This species is usually susceptible to many different antibiotics, but this strain is unusual with cross-resistance to the ureidopenicillins and the semisynthetic penicillins. Further studies showed that this strain was able to survive in low numbers in undiluted Micro-Bact, a phenolic compound used as a disinfectant in the water supply. However, the actual dilution of Micro-Bact used as a disinfectant was neither inhibitory nor bactericidal. Various other disinfectants are being studied to determine their effectiveness against this bacterium.

Publications:

1. Moody, M.R., Young, V.M. and Glor, D.E.: Type associated differences in alternate pathway activation by Pseudomonas aeruginosa. J. Infect. Dis. (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06264-12 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection and Microbiological Research	COB,NCI
Other:	K.A. Newman	Infection Control Nurse	COB,NCI
	C.A. deJongh	Guest Worker	COB,NCI
	M.R. Moody	Research Microbiologist	COB,NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB,NCI

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SECTION

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INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies such as Hodgkin's disease and oat cell carcinoma of the lung. The aims of this project are to catalogue and identify the infections, identify the predisposing factors and evaluate new techniques for more rapid and/or accurate diagnosis of infection. This program has been in progress for over nine years and has allowed meaningful evaluation of the types of infection occurring in different types of tumor patients. Specific problems have been identified such as the nature of hospital acquisition of potential pathogens including bacteria and fungi. This epidemiologic data has allowed appropriate and logical development of infection prevention techniques and application of newer methods of infection diagnosis such that there has been a reduction of infection morbidity and mortality.

Objectives:

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifested both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Formal infectious disease rounds are held twice weekly to review each recognized infection, discuss management and evaluate how each infection might have been prevented. New evaluations completed, or in progress, this year are included below.

A. Evaluation of Periodontal Hygiene and Infection

Infection remains the leading cause of mortality in patients undergoing myelosuppressive therapy for ANLL. Most adults have periodontitis, a chronic, usually asymptomatic oral infection that has been shown to often produce acute exacerbations and systemic complications during myelosuppressive therapy. Nineteen patients receiving first remission induction chemotherapy were studied. At admission, detailed indices of the periodontal status were developed by the dental staff utilizing standardized objective criteria (sulcular depth measurements, plaque index, diagnostic radiographs). The patients were randomly assigned to receive either limited or intensive oral hygiene care prior to and during chemotherapy. All acute infections in these patients were then documented by the medical and dental investigators. Compliance with oral nonabsorbable antibiotics was good and equivalent between all groups; days of granulocytopenia were comparable. All patients had moderate to severe periodontitis at admission; 4 patients presented with oral complaints and resultant fever. The 19 patients experienced 45 acute infections during therapy, of which 7 (16%) were clinically documented as periodontal in origin and 7 (16%) were other oral infections. All periodontal exacerbations occurred in patients receiving limited oral hygiene care; no such complications occurred in patients receiving intensive oral hygiene care. Incidence of mucositis or pulmonary/pharyngeal infection was not associated with degree of periodontal disease. Oral symptomatology and clinical incidence of infection were both correlated with granulocytopenia (greater than 100/ul). Intensive oral hygiene prior to and during chemotherapy resulted in elimination of acute periodontal exacerbations. Thorough oral examination, prophylaxis, and rigorous oral hygiene prior to and during chemotherapy is therefore recommended in these patients.

B. Hepatitis - Forty-two consecutive patients with ANLL who achieved a complete remission following induction therapy with daunorubicin and cytosine arabinoside were evaluated for the development of hepatitis. Hepatitis was defined as a transaminase elevation of 2.5 x normal on two consecutive

determinations five days apart but occurring within 160 days of the first blood product transfusion. Hepatitis secondary to drugs or primary disease were excluded and serologic determinations for differentiation or viral hepatitis A or B, cytomegalovirus, herpes or Toxoplasma were performed. As part of leukemia therapy, patients were randomly allocated to receive or not receive levamisole as immunotherapy. Of the 42 patients with a complete remission, 31 developed nonA/nonB hepatitis, an additional two patients developed Hb_sAg positive hepatitis and the remaining nine patients had no hepatitis. There was no difference in the number of transfused blood product units given to the groups with or without hepatitis (72 versus 73 units per patient). The median survival for patients who develop nonA/nonB hepatitis was significantly longer (549 days) than was the survival of those patients who did not develop hepatitis (322 days). Patients who received levamisole and who developed nonA/nonB hepatitis had the longest median survival (767 days). Three patients presently remain in complete remission (1053+, 1190+, 1558+ days); each of these three had nonA/nonB hepatitis. Median peak SGOT levels correlated with the complete remission duration and total survival. Thus, hepatitis of the nonA/nonB type was found to be associated with an increase in the complete remission duration and the total survival of patients with acute nonlymphocytic leukemia.

C. Staphylococcus epidermidis - At the BCRP, Staphylococcus epidermidis has been found to be an increasingly significant pathogen among granulocytopenic patients with acute leukemia. S. epidermidis is usually thought of as a contaminant when it is present in blood cultures, but in the past three years there has been an increasing frequency of proven septicemia and other significant infections caused by S. epidermidis. No bacteremias were noted prior to 1977, but an incidence of 2.0/1000 hospital days for patients with acute leukemia was noted in 1977 followed by 5.8 in 1978 and 7.6 through mid-1979. Prior to 1977, all S. epidermidis infections were skin related; since that time 15% were skin, 14% were sino-pulmonary, 35% alimentary tract and 35% bacteremias of unknown origin. In attempting to determine the cause for the increased frequency of this infection, bacteriophage typing indicated that a common environmental source could not be implicated. There was no association with the use of intravenous catheters or the use of steel needles. No association could be found with changes in antileukemia cytotoxic therapy or the use of systemic antimicrobials. All bacteremias occurred in the setting of a granulocyte count less than 100/ul in patients who had some degree of mucosal damage along the alimentary canal. The colonization index (mean quantitation of S. epidermidis from surveillance cultures) was found to be substantially higher for patients given gentamicin-nystatin prophylaxis (2.3) compared to those given gentamicin-vancomycin-nystatin (0.7). With the reinstitution of vancomycin, the bacteremia rate returned during the last six months of 1979 to 2.0/1000 days. Although most isolates of S. epidermidis have been cephalosporin susceptible, blood cultures have tended to remain continuously positive despite the use of full dosage of cephalothin or cefazolin. The substitution of vancomycin has produced sterile blood cultures, but infection resolution is frequently slow until granulocyte recovery occurs.

D. Venous Access - Access to the venous system is essential for the management of patients with acute leukemia, however, the effects of cytotoxic agents, antibiotics such as amphotericin and cephalosporins and other agents all have sclerosing effects on peripheral veins often resulting in extreme difficulty with blood drawing or further therapeutic administrations. Two systems for improved venous access have been evaluated at the Baltimore Cancer Research Program. Arteriovenous fistula were placed in 28 patients requiring an average of 78 minutes for surgery. Of the 28 fistula created, only 8 were considered to be of benefit to the patient. In another approach to venous access, 104 large bore indwelling catheters (Hickman catheters) were placed via the subclavian vein in 98 patients. These catheters exit via subcutaneous tunnel in the midchest region reducing the opportunity for local infection. Most patients were markedly thrombocytopenic and granulocytopenic at the time of placement, yet insertion was completed in an average of 39 minutes with minimal complications. The median catheter functional duration has been 100+ days. Only nine Hickman catheters required removal; four for mechanical failures, three for proven infection and two for suspected infection. Catheter-related bacteremias have occurred in two patients of which one was cleared with systemic antibiotics. Multiple catheter exit site infections have also occurred but have cleared with increased site care and/or systemic antibiotics without the necessity of catheter removal. The catheters remained patent without clotting and have been useful for blood drawing, chemotherapy and other therapy administration, hyperalimentation plus central venous pressure monitoring and platelet pheresis. The use of Hickman catheters has proved to be a practical, safe, effective means for improved venous access.

E. Overall Review of Aspergillus Cases: More than 100 cases of invasive aspergillosis have occurred at the BCRP since 1969. These cases are being reviewed with regard to underlying diagnosis, granulocytopenia, chemotherapy, systemic antibacterial antibiotics and other predisposing factors. Site of infection, organism causing the infection, diagnostic techniques and difficulties with microbiologic identification, treatment and x-ray patterns will also be evaluated. Special emphasis will be put on Aspergillus sinusitis, an entity reported rarely in the immunocompromised patient, yet a common site of infection at the BCRP.

F. Amikacin and Gentamicin Resistance Patterns - In January 1979 the BCRP began using amikacin exclusively as the aminoglycoside in combinations for empiric antibiotic therapy. This was initiated on the basis of a very high incidence of gentamicin- and tobramycin-resistant gram-negative bacilli. The question to be answered now is whether or not there has been the development of an increasing frequency of amikacin resistance and whether or not there has been a decreasing frequency of gentamicin/tobramycin resistance. The initial evaluation, now almost completed, consists of a retrospective evaluation of all MIC data generated on all diagnostic and surveillance cultures for gram-negative rods obtained during the last 18 months, since it is only from September 1979 on that minimum inhibitory concentrations (MICs) for amikacin and gentamicin have been obtained routinely for all gram-negative bacilli isolated from BCRP patients. No shift in resistance patterns has been detected in the preliminary evaluation of the available data.

Publications:

1. Schimpff, S.C.: Infection in patients with cancer. In Lichtman, M.A. and Wiernik, P.H. (Eds.): The Science and Practice of Clinical Medicine. New York, Grune and Stratton, (in press) 1981.

2. Wade, J.C. and Schimpff, S.C.: Infections in patients with suppressed cellular immunity. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. I. New York, Raven Press, (in press) 1981.

3. Schimpff, S.C.: Pseudomonas bacteremia in granulocytopenic cancer patients. J. Inf. Dis. (in press) 1981.

4. Schimpff, S.C.: Infections in patients with acute leukemia. In Gunz F. and Henderson, E.S. (Eds.): Leukemia. New York, New York, Academic Press, (in press) 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06266-12 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Therapy of Infection in Granulocytopenic Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection and Microbiological Research	COB,NCI
Other:	C.A. deJongh	Guest Worker	COB,NCI
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	P.H. Wiernik	Chief, Clinical Oncology Branch	COB,NCI
	G.M. Thomas	Diagnostic Microbiologist	COB,NCI
	R.S. Finley	Pharmacist	COB,NCI
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of consecutive antibiotic trials for suspected sepsis in granulocytopenic cancer patients began in 1969 and continues to the present. Initial evaluations proved the usefulness of carbenicillin and gentamicin and later trials, including a large multiinstitutional trial, indicated that carbenicillin (ticarcillin) plus gentamicin had the best efficacy-to-toxicity ratio of the 3 possible two-drug combinations of carbenicillin (ticarcillin), gentamicin and cephalothin. With the addition of early granulocyte transfusions for poor risk patients, a response rate of over 90% was achieved. A new penicillin derivative, mezlocillin, with very broad activity was utilized alone in 50 consecutive patients but proved less than satisfactory in the absence of concommitant aminoglycoside therapy. Therefore, ticarcillin plus amikacin became standard therapy to which piperacillin (a new broad spectrum penicillin) plus amikacin and moxalactam (a new broad spectrum cephalosporin) plus amikacin have been compared. Moxalactam plus amikacin proved to be probably more effective overall because of improved results with Klebsiella sp and S. aureus.

Project Description: Z01 CM 06266-12 COB

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRP Section of Infection Research has continuing programs designed to improve the therapeutic approach to these infections.

A. Bacterial infections

1. Piperacillin is a new broad penicillin with a much broader spectrum than ticarcillin against gram-negative bacilli. It has the safety of penicillin plus their rapid bactericidal mode of action. Therefore, piperacillin plus amikacin was compared to ticarcillin plus amikacin in a randomized, prospective double blind trial including 128 febrile episodes in neutropenic patients. The response rates were equivalent despite the broader spectrum of piperacillin. Surprisingly, hypokalemia occurred as frequently among the piperacillin as the ticarcillin treated patients despite a piperacillin sodium content which is only 20% that of ticarcillin. Nevertheless, the piperacillin plus amikacin regimen must be regarded as highly effective and minimally toxic.

2. Moxalactam is a cephalosporin-like compound which, unlike currently marketed cephalosporins, has activity against Pseudomonas aeruginosa. A combination of moxalactam with amikacin has not only a broad spectrum but includes synergistic activity against P. aeruginosa, K. pneumoniae, E. coli and Staphylococcus aureus. As a result, moxalactam plus amikacin was compared to ticarcillin plus amikacin in a prospective, randomized double blind trial for febrile neutropenic cancer patients. One-hundred ninety-one febrile episodes were evaluated. The overall response rates were equivalent, despite the large number of patients studied there were insufficient organisms in individual categories to make efficacy comparisons. Hypokalemia and nephrotoxicity rates were also equivalent. Moxalactam plus amikacin must be considered as highly effective and minimally toxic.

3. The next empiric antibiotic evaluation consisted of a prospective, randomized comparison of three two-drug combinations: 1) ticarcillin plus amikacin (control), 2) moxalactam plus amikacin, and 3) azlocillin plus amikacin. This protocol seeks to determine if the penicillin, azlocillin (which unlike ticarcillin has activity against Klebsiella pneumoniae and increased activity against P. aeruginosa), or the cephalosporin, moxalactam (which unlike ticarcillin has activity against Klebsiella pneumoniae and increased activity against S. aureus) will be superior to ticarcillin (plus amikacin) and concurrently produce less toxicity (especially hypokalemia). This study also examines the issues of 1) length of antibiotic therapy required and 2) development of fungal superinfection. One hundred patients have been included in this study, and analysis of the data is in progress. Up to this moment both efficacy and toxicity seem to be equivalent.

4. A new antibiotic study will be initiated soon and will consist of the comparison of the combination of beta-lactam plus amikacin, now considered as standard regimen, to a combination of Moxalactam plus piperacillin. Both, moxalactam and piperacillin, are new β-lactam antibio-

tics with activity against the four most common pathogens in the granulocytopenic cancer patient. Furthermore in vitro evaluations have shown that these antibiotics also have synergistic activity against most gram negative bacilli. This combination will be devoid of nephro and ototoxicity inherent with aminoglycoside administration suggesting the potential for an entirely new approach to empiric antibiotic therapy.

5. Therapy of infectious episodes among granulocytopenic cancer patients consists of a combination of drugs which usually includes an aminoglycoside such as amikacin and it is associated with ototoxicity and nephrotoxicity. Audiometric measurements have been performed for the past three years on all patients receiving aminoglycosides. Emphasis is being placed on determining the time course and total dosage required to cause clinically significant auditory dysfunction. Although evaluation of the data obtained continues, the incidence of ototoxicity is in the range of that reported in other studies despite the high doses of amikacin in use at the BCRP over the last 12 months.

6. Amikacin dosing project: The objective of this study, now completed, was to establish the predictability of a pharmacokinetic dosing method to establish amikacin dosages based on lean body weight, estimated creatinine clearance, a standard amikacin elimination rate constant and desired peak (approximately 26 ug/ml) and trough (approximately 8 ug/ml) concentrations. Steady state serum peak and trough concentrations were measured by a radioimmunoassay technique and the levels used to calculate the patient specific elimination rate constant and volume of distribution.

B. Fungal infections

1. Ketoconazole is a new imidazole derivative with excellent activity against Candida sp. Furthermore the drug is devoid of significant toxicity, is readily absorbed when taken orally and its relatively long half life allows administration every 24 hours. For these reasons a study of ketoconazole as therapy of local and disseminated Candida infections will be initiated promptly. Pharmacokinetics and in vitro microbiological data will also be obtained in this study.

C. Viral infections

1. Acyclovir is a new antiviral agent with minimal toxicity which is one-hundred fold more active against the herpes viruses than adenine arabinoside. In a prospective, randomized, double blind study, acyclovir will be compared to placebo for patients with early stages of herpes zoster to determine if it will prevent progression of the localized disease and dissemination. Acyclovir will also be compared to placebo in a study which will try to answer questions concerning efficacy and toxicity of the drug in the therapy of immunocompromised patients with infections due to herpes simplex, varicella-zoster virus or cytomegalovirus. Patients with life threatening infections due to herpes simplex or varicella-zoster virus will be eligible for an open study of therapy with acyclovir.

D. In vitro evaluation of new antibacterial antibiotics.

1. Three new semisynthetic penicillins, piperacillin, mezlocillin and azlocillin, and three new cephalosporins, cefotaxime, moxalactam, and cefoperazone have become available for investigational use. Combinations of these antibiotics should prove useful for empiric therapy of the febrile, neutropenic patient provided they do not show antagonistic effects such as seen with cefoxitin, a second generation cephalosporin, and piperacillin. The various combinations of the new penicillins as well as ticarcillin with the third generation cephalosporins plus the combination of each antibiotic with amikacin are being used to test their effectiveness against a battery of commonly infecting bacteria.

2. In collaboration with Dr. Harold Standiford at the University of Maryland, five volunteers who received mezlocillin, ticarcillin, and gentamicin alone or in combination are being tested for beta-lactam and gentamicin concentrations and the serum bactericidal activity on a battery of stock microorganisms.

3. The new penicillins, mezlocillin and piperacillin, as well as ticarcillin will be combined with gentamicin or amikacin, and the results compared to determine the in vitro efficacy of these combinations on a battery of stock strains.

4. The effect of Bacterial Strain Mucoidy on Synergy Assays--The combination of moxalactam plus piperacillin has been shown to have very broad antibacterial activity, is not associated with antagonism and frequently demonstrates synergy against a broad range of gram-negative bacilli including P. aeruginosa and Staphylococcus aureus. Their use in combination would be unlikely to be associated with serious adverse sequelae. However, this combination appears to be antagonistic when mucoid strains of P. aeruginosa from patients with cystic fibrosis are tested. Mucoid variants from non-mucoid strains of P. aeruginosa recovered from patients at the BCRP and non-mucoid revertants from mucoid strains recovered from patients with cystic fibrosis from the Matsen study will be tested with the moxalactam-piperacillin combination.

5. Cerebrospinal Fluid Levels of Ticarcillin--Gram-negative meningitis is a relatively common occurrence at the Shock-Trauma Unit at the University of Maryland Hospital. In a collaborative study, patients who received ticarcillin who had a lumbar puncture performed had a sample of cerebrospinal fluid collected and studied for the concentration of the antibiotic in the cerebrospinal fluid. Analysis of data is in progress.

6. Rate of Bactericidal Action of Antibiotic Combinations as a Function of Time--The rate at which bacteria are killed by penicillin in vitro was found to vary strikingly within realtively narrow zones of concentration, and with many species there was a sharply defined concentration of penicillin at which it was optimally effective and in excess of which the rate at which the organisms were killed were paradoxically reduced rather than increased. Various antibiotic combinations of some

of the newer penicillins and a cephalosporin were inhibitory and bactericidal at low concentrations whereas certain high concentrations were neither inhibitory nor bactericidal. The therapeutic implications of this paradox is that the treatment of infections caused by a bacterium that has a narrow antibiotic effective range, small doses of the antibiotics at appropriate intervals may be more effective than extremely large doses which maintain tissue or serum concentrations at excessively high levels. Therefore, the rate of bactericidal action of these antibiotics as a function of the concentrations on the bacterial strains exhibiting this reduced activity at high concentrations will be studied.

7. Anthracycline and Aminoglycoside Interaction--Daunorubicin can increase amikacin and gentamicin MICs for Klebsiella pneumoniae and Pseudomonas aeruginosa but not on Escherichia coli. The effect of four anthracyclines, daunorubicin, adriamycin, 7-O-methyl nogarol, and aclacinomycin A, on the MICs of gentamicin, sisomicin and amikacin for K. pneumoniae and P. aeruginosa will be determined. E. coli will be the negative control bacterium and ticarcillin will be the negative control antibiotic. Aminoglycoside uptake by the test strains will be assessed alone and in the presence of the anthracyclines.

8. Characterization of Antibiotic Resistance in Gram-Negative Bacilli Recovered from Patients at the BCRP--R plasmids are responsible for most of the drug resistance expressed by clinical isolates of gram-negative bacilli. The appearance of antibiotic resistance within bacterial species previously considered to be "universally susceptible" to certain antimicrobial agents indicates that these organisms now harbor one or more R factors. The monitoring of the persistance and dissemination of R plasmids in specialized hospital environments such as intensive care units and burn wards is of the utmost importance. The epidemiological evidence for in vivo R factor transfer must always be accompanied by a characterization of the plasmid concerned. Many drug resistance genes reside upon DNA sequences which have the capacity for excising themselves from one chromosome and inserting themselves into another; such genes are called transposons. Antibiotic resistance mediated by transposons are likely in gentamicin and tobramycin resistance. In Enterobacter cloacae that was resistant to gentamicin and tobramycin was isolated from the rectal cultures of a patient with AML at the BCRP. Earlier isolates of this species were not resistant. However, a P. aeruginosa strain that was resistant to these aminoglycosides was part of the rectal flora. A retrospective study of these bacterial strains recovered from this patient during a four-month period has been undertaken to demonstrate and characterize any R plasmid(s) that may be harbored by these resistant clinical isolates. Multiresistant gram-negative bacilli recovered frm other patients are also under investigation.

Publications:

1. Wade, J.C. and Schimpff, S.C.: Antibiotic therapy for febrile granulocytopenic patients. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. I, New York, Raven Press, (in press) 1981.

2. Love, L.J., Schimpff, S.C., Schiffer, C.A. and Wiernik, P.H.: Improved prognosis for granulocytopenic patients with gram-negative bacteremia. Am. J. Med. 68:643-648, 1980.

3. Wade, J.C., Schimpff, S.C., Newman, K.A., Fortner, C.L., Moody, M.R., Young, V.M. and Wiernik, P.H.: Potential of mezlocillin for empiric single agent therapy in febrile granulocytopenic cancer patients. Antimicrob. Ag. Chemother. 18:299-306, 1980.

4. Wade, J.C., Schimpff, S.C. and Wiernik, P.H.: Antibiotic combination-associated nephrotoxicity in granulocytopenic patients with cancer. Arch. Intern. Med. (in press) 1981.

5. Schimpff, S.C.: Infection in the abnormal host. In DuPont, H.L. and Pickering, L. (Eds.): Textbook of Infectious Diseases. Menlow Park, California, Wesley Publ. Co., (in press) 1981.

6. Wade, J.C. and Schimpff, S.C.: Approaches to therapy of bacterial infections in the granulocytopenic patient. In Klastersky, J. (Ed.): EORTC Monograph--Proceedings of the EORTC-International Antimicrobial Therapy Project Group Symposium. New York, New York, Raven Press, (in press) 1981.

7. Schimpff, S.C.: Intravenous trimethoprim/sulfamethoxazole plus ticarcillin as empiric antibiotic therapy for granulocytopenic patients. Arch. Intern. Med. (in press) 1981.

8. Moody, M.R., Young, V.M., Morris, M.J. and Schimpff, S.C.: In vitro activity of miconazole, miconazole nitrate, and ketaconazole alone and combined with rifampin against Candida spp. and Torulopsis glabrata recovered from cancer patients. Antimicrob. Ag. Chemother. 17:871-875, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06951-05 COB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Infection and Microbiological Research in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection and Microbiological Research	COB,NCI
Other:	C.A. deJongh	Guest Worker	COB,NCI
	K.A. Newman	Infection Control Nurse	COB,NCI
	M.R. Moody	Research Microbiologist	COB,NCI
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	G.M. Thomas	Diagnostic Microbiologist	COB,NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Infection and Microbiological Research

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aim of this program is an integration of research, education, and patient care with regard to the infections arising in patients with cancer. The research areas include infection epidemiology, diagnosis, prevention and treatment. Education includes the teaching of BCRP clinical associates the fundamental problems and techniques for infection management, teaching the nursing staff basic prevention and treatment modalities, and teaching the University of Maryland Infectious Disease fellows and students concepts of infection control in the tumor patient. A program of continuing education through seminars, symposia and publications serves the medical community at large. Patient care includes patient education in infection prevention in addition to infection diagnosis, prevention and management.

Project Description: Z01 CM 06951-05 COB

The frequency and severity of infection in patients with advanced cancer necessitates an ongoing program of 1) infection research, 2) education and 3) patient care. The aims of this program are to integrate these three areas into the ongoing clinical and laboratory programs at the BCRP.

Research areas include infection epidemiology, diagnosis, prevention and therapy (see #6264, #6219, #6223, #6266, and #6217).

Education includes the training of BCRP clinical associates, University of Maryland Infectious Disease fellows, and Department of Medicine rotating residents in the management approaches necessary for optimum care of the infected or potentially infected cancer patient. This is done through daily work rounds plus informal discussions. Nursing education, done largely by the infection control nurse, is accomplished by twice monthly teaching conferences plus informal patient management discussions.

An integrated team approach has been developed, based around twice weekly formal infection rounds, and includes active participation by physicians and microbiologists from the Infection and Microbiological Research Section and the Cell Component Therapy Section, the infection control nurse, pharmacist, and attending physicians. This approach ensures thorough discussion of the various management approaches for each patient while reviewing the requirements of protocol studies.

A televised symposium, originating from New York, was directed live to over 9000 physicians in 20 cities in February 1981, addressing issues on severe gram negative infections including a filmed section elaborating the techniques of infection prevention at the BCRP.

Publications:

1. Schimpff, S.C. and Wiernik, P.H.: Infection in cancer patients (predisposing factors, sites, organisms and general approach to diagnosis). In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., (in press) 1981.

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3. Schimpff, S.C. and Wiernik, P.H.: Infections in the patient with cellular immune dysfunction. In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., (in press) 1981.

4. Schimpff, S.C. and Wiernik, P.H.: Infection prevention in patients with cancer. In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06988-04 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Radiation-Related Thyroid Disorders in Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. Salvatore	Secretary, Section of Infection and Microbiological Research	COB,NCI

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.125	.062	.062

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Late complications in the treatment of Hodgkin's disease with radiation therapy are increasingly being recognized. Thyroid dysfunction following irradiation is one such complication which has not been widely recognized, probably because of the subtle clinical nature of minor thyroid hormone imbalance. Two hundred thirty-two patients with Hodgkin's disease followed at BCRP have been evaluated for post-therapy thyroid dysfunction. Forty-one percent of those with mantle radiation therapy had an elevated TSH, but normal T_4, but an additional 25% had an elevated TSH plus a depressed T_4. Thus, 66% of 169 patients had evidence of thyroid dysfunction following mantle radiation therapy.

One patient has been detected with irradiation-associated thyroid carcinoma. He had received initial irradiation in 1962.

Project Description: Z01 CM 06988-04 COB

Two hundred fourteen patients with Hodgkin's disease returning to the Baltimore Cancer Research Program for routine clinic visits had a serum sample obtained for thyroid function tests. Some patients had multiple serial serum samples taken over many months. Serum thyroxine (T_4), T_3 resin uptake by thyroxine binding globulin, the effective thyroxine ratio and the serum thyroid stimulating hormone (TSH) were measured. Patients with stages I-IIIA receiving radiation therapy or radiation plus adjuvant chemotherapy and patients with stages IIIB-IV received chemotherapy alone. Radiation therapy was delivered from a cobalt 60 source with midplane dose of approximately 4000 rads given as 200 rads per day, five days per week for four weeks weighted to give two-thirds of the dose from the anterior port and one-third through the posterior port.

Major Findings:

Of 169 patients who received mantle radiation therapy, 69 (or 41%) were found to have an elevated TSH but normal thyroid hormone level. An additional 43 patients (25%) had an elevated TSH plus an abnormally low T_4. Combined, 112 of 169 patients (66%) had evidence of thyroid dysfunction following mantle radiation therapy. Among the 45 patients who had not received mantle radiation therapy, 3 (7%) had an elevated TSH but none had depressed thyroid hormone levels.

With an incidence of 25% of patients developing chemical hypothyroidism within a few years after radiation therapy, it is clear that this is a major complication of irradiation for Hodgkin's disease. All such patients should therefore have TSH determinations done every six months for the first five years following irradiation with determination of thyroxine levels should the TSH be elevated. Replacement therapy with sodium levothyroxine will usually be sufficient. For those patients with elevations of TSH alone, it is appropriate to begin replacement therapy for this state of compensated hypothyroidism. Patients may benefit symptomatically, the lessened production of TSH will prevent the rare occurrence of sella turcica damage, and, since elevated TSH in the presence of radiation damaged thyroid tissue is known to be carcinogenic, TSH suppression may lessen the potential for later development of thyroid carcinoma.

One patient has now developed thyroid carcinoma, nearly 20 years after initial irradiation. His TSH levels had been elevated but never suppressed with levothyroxine. Carcinoma was detected during surgery for a nodule which was benign; the carcinoma was multicentric in both lobes.

Publications:

1. Schimpff, S.C.: Carcinoma of the thyroid. In Aisner, J. and Chang, P. (Eds.): Advances in Cancer Treatment Research. The Netherlands, Martinus Nijhoff, Publ., 1980, pp. 88-102.

2. Schimpff, S.C.: Hormonally active tumors. In Lichtman, M.A. and Wiernik, P.H. (Eds.): The Science and Practice of Clinical Medicine. New York, Grune and Stratton, 1980 pp. 302-305.

3. Schimpff, S.C., Diggs, C.H., Wiswell, J.G., Salvatore, P.C. and Wiernik, P.H.: Radiation-related thyroid dysfunction: implications for the treatment of Hodgkin's disease. Ann. Intern. Med. 92:91-98, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06275 12 COB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cell Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Barbara Kotelba-Witkowka	Visiting Scientist	
	Janice P. Dutcher, M.D.	Medical Investigator	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI
	Gerald Johnson, M.D.		

COOPERATING UNITS (if any)
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Dr. Paul Terasaki, University of California, Los Angeles; University of Md. Department of Nuclear Medicine; Baltimore Rh Typing Lab

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.0	4.5	4.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year there has been an expansion of the HLA matched donor pool and increased use of autologous frozen platelets. One can predict the long-term platelet alloimmunization status of patients with acute nonlymphocytic leukemia by the end of induction therapy. With few exceptions patients who do not develop lymphocytotoxic antibodies within six to eight weeks of platelet transfusion never subsequently developed antibodies despite subsequent transfusions. A randomized study comparing the rate of alloimmunization in patients receiving lymphocyte depleted and standard platelet concentrates is in progress. Platelet cryopreservation using glycerol-glycose produces inferior results to platelets frozen with DMSO in vitro and in vivo. Platelets stored in the presence of Amberlite resin which serves to preserve higher ambient pH's can maintain morphologic integrity and ATP levels compared to platelets stored for long periods of time at ambient temperature without the resin. Nonmatched ^{111}Indium labeled granulocytes do not migrate to sites of infection in alloimmunized patients and in vitro techniques which may be suitable as a crossmatch are being studied.

The Cell Component Therapy Section provides platelet and granulocyte transfusions for the prophylaxis and treatment of hemorrhage and infection in patients with leukemia and other types of cancer receiving intensive therapy. In addition an active laboratory interested in various aspects of granulocyte and platelet function is in operation.

I. PLATELET TRANSFUSION

Approximately 16,500 units of platelet concentrate were transfused in 1979/80. Approximately 5,000 units were collected and processed by the Cell Component Therapy Section and consisted of HLA matched platelets, platelets collected with granulocytes, autologous and HLA matched platelets collected for freezing. The majority of the platelets transfused were random donor platelets prepared by the Community Blood & Plasma Center. In addition, the Community Blood & Plasma Service provides HLA matched platelets for the BCRP and recruited approximately 800 more donors to the BCRP HLA donor file. Recently this donor file was transferred to the PROMIS computer system at the BCRP. The computer file searches for donors based on a system capitalizing on the cross-reactivity amongst the HLA antigens.

Alloimmunization remains the major transfusion problem faced in the management of patients with acute leukemia. It has been demonstrated by our group that there was an absence of a dose response relationship between the number of platelet transfusions that patients with leukemia received during induction and the subsequent development of alloimmunization. Long-term follow-up of this group of patients was then undertaken to measure the clinical course of alloimmunized patients as well to possibly develop a means of prospectively identifying patients more likely to become alloimmunized. Patients were studied who survived more than six months and received large numbers of random donor products throughout their life. A total of 114 patients with ANLL were studied. Of these approximately 45% were either alloimmunized on admission or developed lymphocytotoxic antibody within six to eight weeks of beginning induction therapy. Except for a few patients who lost their antibody at times they were profoundly ill, this group of patients was dependent upon histocompatible platelet transfusions throughout their lives. Conversely, 66 patients did not make lymphocytotoxic antibody during induction therapy despite multiple transfusions of random donor blood products. Only five of these patients subsequently made lymphocytotoxic antibody. It thus appears that one can identify with greater than 90% confidence at the end of induction therapy the long-term antibody behavior of patients with ANLL. One need not be concerned therefore about withholding prophylactic transfusion in the latter group because these patients are unlikely to ever become alloimmunized. Furthermore, we now focus our freezing program almost entirely on patients identified as being alloimmunized after induction. Unfortunately, analysis of a large number of medical factors including: sex, age, timing of chemotherapy, HLA types, infection status, remission rate and development of other antibodies could not distinguish between the two groups. It is therefore impossible at this time to prospectively identify patients more or less likely to become alloimmunized.

Platelet cryopreservation remains one of the major interests of the unit and last year approximately 1300 units of autologous frozen platelets were

administered to patients with leukemia. Most of these patients were alloimmunized and many had no other donors available. Throughout the years, the results using platelets frozen with 5% DMSO have been quite consistent with count increments approximately 60 to 70% of that noted with fresh platelets. Experiments were also performed using a newer freezing methodology with glycerol-glucose as cryoprotective agents which had been reported to produce excellent results in research in other laboratories. Experiments in our laboratory however revealed that significant morphologic and functional damage occurred using this method with marked decreases in ATP levels and poor responses to aggregating agents. Nine experiments were done in which patients had platelets frozen either using DMSO or glycerol. The results with DMSO were similar to our larger experience but the recoveries using glycerol were only half of the DMSO recoveries. In addition the glycerol method was more cumbersome and involved considerably more technologist time. It was therefore concluded that unless significant improvements in this technique occur that the DMSO methodology remains the procedure of choice at this time.

In an effort to prevent alloimmunization, a study has been in progress for approximately two years which compares the rate of alloimmunization in patients receiving induction chemotherapy who receive either standard platelet preparations or platelets which have been rendered partially leukocyte poor by an extra centrifugation. All patients in this study also received frozen red cells to limit the antigenic exposure to the platelets. Approximately 60 patients have been randomized on the study to date which should close within the next year. Should the study be "positive" it could markedly influence blood banking practice in that some sort of leukocyte poor blood would be necessary for patients with leukemia. In addition to this expensive proposition, approximately 20 to 30% of the platelets are lost during the extra centrifugation and this would obviously depleat platelet supplies.

An analysis was undertaken of the incidence of refractoriness to platelet specific antigens in patients with acute leukemia. It has been known that a certain percentage of alloimmunized patients will not respond to perfectly HLA matched platelets presumably because of antibody to platelet specific antigen. Of approximately 200 patients with ANLL analyzed, only 7 (3.5%) had apparent refractoriness due to platelet specific antigen (as defined by refractoriness in the absence of lymphocytotoxic antibody and/or refractoriness to perfectly HLA matched platelets). This would therefore appear to be a somewhat uncommon problem in patients with acute leukemia. An Elisa assay has been developed in the laboratory which it is hoped will be able to serve as a means of donor selection for both HLA antigens and for platelet specific antigens. The test is done in microtiter plates and appears to give reproducible results with known positive and negative sera. Studies are underway to determine the best means of preserving platelets in the microtiter plates so that the plates can be made up in advance and stored for future use. Preliminary results indicate that platelets can be frozen and utilized in this test although the background results are somewhat higher using this technique. We are presently studying whether cell preparations prepared in the usual fashion and then dessicated while on the plate can serve as a means of preservation. Platelets from two of the patients who are apparently refractory to platelets specific antigens were

tested using the Elisa assay and were found to have the PLA 1 antigen present. Therefore their refractoriness was not related to antibody against this most well characterized of the platelet antigens. Two sources of PLA 1 antisera have been found and concentrated eluates of the antibody have been made and are frozen away for reference and known positives. A search is presently underway for normal donors who are PLA 1 negative to serve as controls for future tests.

Presently platelets can be stored at ambient temperature for only 72 hours. During this storage time the pH of the suspending media gradually falls and the platelets are rendered nonviable. In an effort to increase the buffering capacity of the suspending media to control this fall in pH, platelets were stored in plasma to which amberlite resin beads had been added which had been "preloaded" with phosphate ion. Platelets were stored in twice the normal concentration with either one or two grams of the resin and then compared to control platelets. As expected, the pH, morphology, ATP levels and response to aggregating agents deteriorated rapidly in the control platelets with essentially no viable platelets remaining after twenty-four hours. In contrast, the pH of the resin platelets was maintained greater than 6.5 for up to 5 days with good maintenance of ATP levels for up to 72 hours and preservation of discoid morphology for up to 72 hours. Electron microscopy confirmed the preservation of microtubules and discoid platelets with the resin for up to 5 days. It is felt that this improved platelet preservation was related to the maintenance of the pH as mediated by the resin beads which both bind CO_2 (documented directly by study of plasma gases) and buffered by the phosphate ions. In addition, it is possible that the exogenous supply of extra phosphate might have been of metabolic benefit to the platelet. Although not of immediate practical importance, this would appear to be an excellent system to study the effects of long-term storage on platelets as well as the effect of the pH of the suspending media on platelets during storage.

An analysis of large numbers of HLA matched transfusions is in progress. Particular attention is being paid to transfusions which were mismatched for antigens felt not to be expressed on platelets (i.e. HLA-B12, 44, and 8). The possible predictive effects of a lymphocytotoxic crossmatch will also be studied in these patients.

Non-A, Non-B hepatitis continues to be a significant problem in patients receiving large numbers of transfusions. The BCRP donor file now consists entirely of donors with normal SGPT values which are being repeated periodically on these donors. This provides a unique opportunity to determine whether this maneuver (which is likely to become standard practice in the country within the next two years) will decrease the rate of hepatitis in our patient population.

II. GRANULOCYTE TRANSFUSION THERAPY

Therapeutic granulocyte transfusions continue to be administered as early in the course of seriously infected patients as possible. Although the number of neutropenic patients has increased throughout the years we have noted that our use of granulocyte transfusion has leveled off, attesting to improved results seen with antibiotics and the short periods of aplasia noted with more

effective antileukemic therapy. Because of the considerably high yields of granulocytes obtained with the IBM 2997 machine we attempt to utilize this machine whenever possible in preference to the Haemonetics Model 30. The yield of granulocytes with the IBM is approximately 25 x 10^9. With this dose we frequently note circulation of transfused granulocytes the day after transfusion, a phenomenon we rarely ever saw using lower doses of granulocytes. In addition to attempts to increase the dose of granulocytes administered, we also utilized granulocytes from patients with chronic myelogenous leukemia whenever such patients were available. We have noted the frequent occurence of temporary engraftment of CML cells with circulation of granulocytes for days to weeks after the transfusion. This has invariably resulted in salutary effects on the patients' infections and no episode suggestive of graft versus host have ever been seen in patients with leukemia. Engraftment has been demonstrated by the presence of the Philadelphia chromosome and the graft has invariably been rejected when the patients entered remission.

The major problem which remains in granulocyte transfusion is the study of the effect of alloimmunization on the results of transfusion. A previous study demonstrated that patients receiving prophylactic granulocyte transfusions became rapidly alloimmunized with the development of lymphocytotoxic and leukoagglutinating antibody and a much increased incidence of often severe transfusion reactions. It has become our policy not to administer granulocytes to patients who are known to be alloimmunized unless a closely matched donor is available. The definition of "matching" is problematic however. In an effort to study this question further, we are utilizing ^{111} Indium-labeled granulocyte in alloimmunized patients to determine whether the presence of antibody will block the migration of labeled cells to known sites of infection. In preliminary experiments it was demonstrated in 20 non-alloimmunized patients that Indium-labeled granulocytes will migrate to sites of infection within 30 minutes of infusion producing good quality scans even in patients with rather small areas of infection. The technique requires only 10^8 cells and does not require the administration of a granulocyte transfusion. In contrast to the results in non-alloimmunized patients, granulocytes do not seem to migrate to sites of infection in alloimmunized patients (defined as patients refractory to platelet tranfusion with lymphocytotoxic antibody present). A total of 12 patients have been studied to date and in 9 of these patients there was no migration of granulocytes to infected sites as determined by post infusion scanning. Two of the 3 patients in who migration did occur received granulocytes which were fortuitously matched for HLA antigens and in whom the lymphocytotoxic antibodies crossmatch was negative. These studies indicate that nonmatched granulocytes are unlikely to be of benefit for alloimmunized patients and confirm our clinical approach to this problem. Further studies are necessary however to determine whether it is the HLA antigen system or granulocyte specific antigens which are of importance in this regard. Our leukemia patients tend to have both antibodies present when they are alloimmunized. In the next phase of the study therefore we will attempt to utilize donors who are closely HLA matched with the alloimmunized patients which should allow us to determine the best means of prospective crossmatching. In this regard we are studing a slide technique using staphlococcus protein A which directly measures the binding of staph to granulocytes which have been

previously fixed on a slide. The potential advantage of this technique is that granulocytes can be fixed on the slide which can then be stored away for future use for crossmatching. We hopefully will have access to sera containing granulocyte specific antibody from other laboratories for the use in this study.

III. Other Laboratory Functions

A. Lymphocyte and blast collection for other DCT laboratories in Baltimore and Bethesda.

B. Cryopreservation of blasts from patients with leukemia for immunotherapy studies. Blasts are cryopreserved using 10% DMSO with good post recoveries which have shown to be quite adequate for neuraminadase treatment for use in the BCRP ANLL protocol.

C. Therapeutic Plasmapheresis continues to be performed on selected patients including patients with Goillain-Barre syndrome, myasthenia gravis, ITP, and recently an interesting patient with post transfusion purpura. The latter patient had a dramatic rise in platelet count which correlated precisely with removal of anti-PLA 1 antibody and improvment in hemostasis.

D. Stem cells from the circulating blood of selected patients with CML have been frozen using 10% DMSO. Recent results in other laboratories using this autologous rescue approach have not been very successful, however, and it is unlikely that we will pursue this program except perhaps to use the cells as a transfusion rescue should serious infection occur in these patients.

PUBLICATIONS:

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2. Schiffer CA, Aisner J: Platelet and granulocyte transfusion therapy for patients with cancer in Clinical Practice of Blood Transfusion, Petz LD and Swisher SN, Eds., Churchill-Livingstone, New York (in press).

3. Aisner J, Schiffer CA, Wiernik PH: Cell support. in: Fundamentals of Clinical Hematology, Spivak JL (Ed), Harper and Row, Hagerstown, MD, pp 357-68, 1980.

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10. Schiffer CA, Aisner J, Dutcher JP, Daly PA, Wiernik PH: A Clinical Program of Platelet Cryopreservation. in Progress in Pheresis, WR Vogler, Ed., (In press).

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13. Dutcher J, Schiffer CA, Aisner J, Wiernik PH: Alloimmunization following Platelet Transfusion: The Absence of a Dose Response Relationship. Blood 57:395-398, 1980.

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15. Kotelba-Witkowska B, Schiffer CA: Cryopreservation of Platelet Concentrates Using Glycerol-Glucose. Transfusion (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06043 10 COB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Automated Monitoring of Drug Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	William R. Grove, B.S.	Asst. Dir., Clin. Res. Pharmacy Sect.	COB,NCI
	Larry Yamamoto, M.D.	Acting Chief, Health Services Research, USPHS Hospital	HSR,NIH
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)

Health Services Research, USPHS Hospital, Baltimore, Maryland

LAB/BRANCH

Clinical Oncology Branch

SECTION

Clinical Research Pharmacy Section

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.25	1.0

CHECK APPROPRIATE BOX(ES)

☒(a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide an efficient and rapid method of automated collection of data concerning the clinical trials of experimental drugs.

The system of computer processing of drug administration information provides accurate periodic summaries of all BCRP patients' drug therapy. Each drug card is typed containing the name of the drug, dose, route of administration, frequency, date, and patient's name and medical record number. Each card also contains the direct administration record as charted by the nurses at the time of administration. These cards are processed by optical character recognition and produce a weekly summary of all drugs ordered.

This system fulfills all the requirements of drug ordering, administration and recording in addition to providing machine-readable data for computer input. This system eliminates the transcription operations usually associated with preparation of input for computers and provides a data pool for clinical studies.

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs. This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing three copies: the pharmacy copy (for entry to a patient's drug profile), a copy for medical records and a copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration or omission of each drug. A monthly drug utilization report is also obtained as a byproduct.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patient's health, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration, and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists, and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. The system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

This study is complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06237 10 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of a Clinical Research Pharmacy Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Section	COB,NCI
Other:	William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB,NCI
	Rebecca Finley, Pharm.D.	Clinical Pharmacist	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒(a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the role of the pharmacist in the patient care/research areas, and as a member of the health care team actively contributing to the patient's care and research efforts.

The pharmacist, utilizing new methods, has become a valuable member of the research health care team contributing directly to patient care and research. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improved drug prescribing practices by the promotion of rational and safe drug use, the prevention of potential drug-drug interactions, the detection and prevention of adverse drug reactions, the detection of drug-induced laboratory test abnormalities, the detection of possible drug-induced disease, the provision for easily accessible drug information, the continuation of education for various members of the health care team, counseling of the patient on proper use of drugs, and the detection and prevention of intravenous admixture incompatibilities.

The objective of this study is to determine the role of the pharmacist in research patient care area.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the care of the patient. The pharmacist attends morning and evening ward rounds and infectious disease rounds, reviews charts, interviews patients and is familiar with the patient's condition. Pharmacy rounds are convened immediately after the morning medical rounds. All pharmacists attend, as all new problems are discussed. This provides the pharmacist with all of the patient's current problems, therefore, he can intelligently discuss therapy with the patient's physicians during the working day, as the need occurs. With this information, he can also provide the staff with unsolicited information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile and investigational drug record. The pharmacist receives a direct copy of the physican's drug order and transcribes it to the patient's drug profile and chemotherapy record. The drug record includes the patient's name, primary and secondary problems, physician's name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages and then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems, and possible adverse drug reactions. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system includes evaluating the necessity of continued medication orders, pharmacokinetics, proper drugs, and correct dosage.

The pharmacist, utilizing the above methods, has become a valuable member of the research health care team and contributes directly to the care of the patient. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improved drug prescribing practices by promoting the safe and rational use of drugs, the prevention of potential drug-drug interactions, the detection and prevention of adverse drug reactions, the detection of drug-induced laboratory test abnormalities, the detection of possible drug-induced diseases, the provision of easily accessible drug information, counseling of the patient of proper use of drugs, and the detection and prevention of intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs, and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better research/patient care and has enhanced the quality of the research data.

Drug information is becoming a major by-product of locating the pharmacist in the patient care area. This information is being requested by all members of the health care team. Continuing education is also involved in this process of providing drug information. The impact of this service will continue to be studied by the audit process.

The pharmacist is making rounds with a nurse to improve compliance of patients taking non-absorbable oral antibiotics and other medications. A pharmacist is doing audiograms of patients receiving potentially ototoxic agents.

Present studies will be continued, and additional methods to improve patient care will be further researched.

PUBLICATIONS:

1. Burks, L., Aisner, J., Fortner, C.L. and Wiernik, P.H., Meperidine hydrochloride for the treatment of shaking chills and fever, Arch. Intern. Med. 140:483-484, 1980.

2. Wade, J.C., Schimpff, S.C., Newman, K.A., Fortner, C.L., Moody, M.R., Young, V.M. and Wiernik, P.H., Potential of Mezlocillin as empiric single-agent therapy in febrile granulocytopenic cancer patients, Antimicrob. Agents Chemother, 18:299-306, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06912 07 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Grove, B.S.	Asst. Dir., Clin. Res. Pharmacy Sect.	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	Judy Gundel, B.S.	Clinical Pharmacist	COB,NCI
	Robert Esterhay, M.D.	Senior Investigator	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
PROMIS Laboratory, Dept. of Medicine, University of Vermont

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal. A cathode ray screen terminal and a printer have now been installed in the pharmacy.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. The standarized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Section of the BCRP or the PROMIS Laboratory, University of Vermont.

The antineoplastic drug dislays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

The objectives of this project are to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal. A terminal and a printer have now been installed in the pharmacy.

These displays are to be complete, concise, and accurate summaries of currently available literature. Additional antineoplastic drugs are added to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. This information is then organized to a specific format to conform with the over 20,000 displays currently included in the PROMIS system. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Section of the BCRP or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS Laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Previously entered data may be updated as necessary by providing the new information and references to the PROMIS laboratory for incorporation.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06943 06 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Audit of the Outpatient Drug Profile

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, B.S. Asst. Dir., Clin. Res. Pharmacy Sect. COB,NCI

Other: Clarence L. Fortner, M.S. Head, Clinical Res. Pharmacy Sect. COB,NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to promote compliance and knowledge of state pharmacy laws through an audit of the outpatient prescription file. The process audited is the pharmacist's completion of the required information on the prescription.

The prescriptions from one day's outpatient prescription file were audited for complete drug information (drug name, dose, quantity, directions), patient name, physician signature, date, complete filing information (manufacturer, lot number, expiration date, pharmacist's initials). In addition, a lot number verification of the source was conducted. For controlled substance prescriptions, the audit also included the patient's address, physician, DEA/SS number, sequential prescription numbers, and the red "C".

Forty-five prescriptions were audited. The following results were obtained: Drug name, 45/45; Dose, 45/45; Quantity, 41/45; Date, 41/45; Manufacturer, 30/45; Lot number, 43/45 and Expiration Date, 38/45. Items which were not being completely filled out were brought to the attention of the staff.

The purpose of this study is to promote compliance and knowledge of state pharmacy laws through an audit of the outpatient prescription file. The process audited is the pharmacist's completion of the required information on the prescription.

The prescriptions from one day's outpatient prescription file were audited for complete drug information (drug name, dose, quantity, directions), patient name, physician signature, date, complete filing information (manufacturer, lot number, expiration date, pharmacist's initials). In addition, a lot number verification of the source was conducted. For controlled substance prescriptions, the audit also included the patient's address, physician, DEA/SS number, sequential prescription numbers, and the red "C".

Forty-five prescriptions were audited. The following results were obtained: Drug name, 45/45; Dose, 43/45; Quantity, 41/45; Directions, 42/45; Date, 41/45; Manufacturer, 30/45; Lot number, 43/45 and Expiration date, 38/45. Items which were not being completely filled out were brought to the attention of the staff.

This study is complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06961 05 COB

PERIOD COVERED

Otober 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Establishment of a Pharmacy Clinical Clerkship in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	William R. Grove, B.S.	Asst Dir, Clin. Res. Pharmacy Sect.	COB,NCI
	Robert Kerr, Pharm. D.	Dir., Clinical Pharmacy, UM	
	Rebecca Finley, Pharm. D.	Clinical Pharmacist	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)

University of Maryland, School of Pharmacy

LAB/BRANCH

Clinical Oncology Branch

SECTION

Clinical Research Pharmacy Section

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Clinical Research Pharmacy Section of the BCRP has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation.

The objective is to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols; i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities, and the management of non-neoplastic concurrent medical/social problems.

The pharmacy student should gain experience in both the inpatient and ambulatory areas.

The Clinical Research Pharmacy Service has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation.

The objectives are to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols; i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems. They will learn the effective and reliable reference sources for the most current information on cancer chemotherapy.

These students will be directed through the Clinical Research Pharmacy Service with support from the Clinical Oncology Branch.

This program had six rotations during the past year and will continue for further evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06990 04 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Role of the Pharmacist in Monitoring Patients Receiving Ototoxic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Freda Danhauer, M.S.	Clinical Pharmacist	COB,NCI
Other:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
	Stephen C. Schimpff, M.D.	Head, Infection & Micro. Res. Sect.	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to evaluate the feasibility of pharmacist performed audiometric monitoring of patients who are receiving potentially ototoxic therapy.

Patients receiving potentially ototoxic drugs have baseline pure tone audiograms performed by the pharmacist utilizing a portable audiometer. A follow-up audiogram is obtained at the completion of therapy. All abnormal follow-up audiograms are noted and brought to the attention of the physician. Additional follow-up audiograms are scheduled in those patients with abnormal results to determine the reversibility of the toxicity. This function will allow the pharmacist significant patient contact and a greatly increased awareness of the need for a multidisciplinary approach to patient care. This function will also provide a significant opportunity for the patient to utilize the pharmacist as a source person concerning their drug therapy.

This study will investigate how the pharmacist can contribute significantly to the care of the patient by performing clinically relevant audiometric studies of patients at high risk of developing ototoxicity.

This study was designed to evaluate the feasibility of having a pharmacist conduct pure-tone audiometric studies on patients receiving potentially ototoxic drugs. All audiograms are conducted in the patient's semi-private room utilizing a portable Beltone Audiometer (Model 10D).

Training of a pharmacist was accomplished in conjunction with the audiology department of the University of Maryland Hospital. This pharmacist, in turn, educated the other pharmacists in the proper technique of obtaining a pure-tone audiogram.

The pharmacist is responsible for obtaining baseline and follow-up audiograms on all patients randomly allocated to receive empiric antibiotic therapy which includes an aminoglycoside or other potential ototoxic drugs; i.e., cis-platinum. Abnormal findings are relayed to the patient's physicians.

The pharmacist has been able to demonstrate a 5% overall occurence of ototoxity associated with aminoglycoside antibiotics.

This function has given the pharmacist a significant and productive amount of patient contact time and interaction with research protocols. Not only has this increased the pharmacist's awareness of the need for a multidisciplinary approach to patient care and research data collection, but it has increased the patient's utilization of the pharmacist as a drug information source person.

This study will investigate the ability of the pharmacist to contribute to the care of the patient and to the compilation of research data by conducting accurate audiometric studies of patients receiving ototoxic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06997 03 COB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comparative Auditory Toxicity of Aminoglycoside Antibiotics in Leukopenic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Freda Danhauer, B.S.	Clinical Pharmacist	COB,NCI
Other:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
	Stephen C. Schimpff, M.D.	Head, Infection & Micro. Res. Sect.	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Clinical Research Pharmacy Section

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to assess the relative auditory toxicity among three different aminoglycosides. Audiograms are to be obtained by a pharmacist utilizing a portable audiometer. It is our intention to study the monitoring function as performed by a pharmacist as well as to present the results of the auditory toxicity portion of the study.

Thirty-two patients received ticarcillin (T) and gentamicin (G), 29 received ticarcillin and netilmicin (N), and 29 received ticarcillin and amikacin (A). Two patients receiving T & G (6.2%), one patient receiving T & N (3.4%), and one patient receiving T & A (3.4%) developed auditory toxicity with bilateral decreases of at least 20 db at one or more frequencies. Pharmacists were well received by the patients and successfully obtained and interpreted pre- and post-aminoglycoside audiograms.

The objective of this study is to assess the relative incidence of auditory toxicity among three different aminoglycosides (gentamicin, netilmicin, and amikacin), each administered in combination with ticarcillin. It is our intention to study the monitoring function as performed by a pharmacist as well as to present the results of the auditory toxicity.

Ototoxicity, both auditory and vestibular, secondary to aminoglycoside antibiotics, has previously been reported with gentamicin and amikacin. Ninety patients received one of these combinations of empiric antibiotic therapy. Baseline and serial audiograms were obtained on these patients. There was no difference among the three groups in the average duration of aminoglycoside exposure, age, for underlying disease. Two patients receiving ticarcillin and gentamicin (6.2%), one patient receiving ticarcillin and netilmicin (3.4%), and one patient receiving ticarcillin and amikacin (3.4%) developed auditory toxicity with bilateral decreases of at least 20 db at one or more frequencies. None of these five patients were receiving other drugs which have been associated with ototoxicity during antibiotic therapy. Serum creatinines were monitored at least every other day with dosage changes made at the first sign of rising creatinine. None of the patients with ototoxicity demonstrated nephrotoxicity.

The present study is complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09112 02 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
An Evaluation of the Effect of Chemotherapy and Oral Antibiotics on Gastrointestinal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Grove, B.S.	Asst. Dir., Clin. Res. Pharmacy Sect.	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	Stephen C. Schimpff, M.D.	Head, Infection & Micro. Res. Sect.	COB,NCI
	Kathryn A. Newman, R.N.	Infections Control Nurse, Infection & Microbiology Res. Sect.	COB,NCI
	Peter P. Lamy, Ph.D.	Professor, School of Pharmacy University of Maryland	
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
University of Maryland School of Pharmacy, Baltimore, MD

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to evaluate the effect of chemotherapy and oral antibiotics on gastrointestinal function in a population of patients with acute non-lymphocytic leukemia in remission.

Eligible patients are those patients receiving the chemotherapy combination of thioguanine and cytosine arabinoside for maintenance chemotherapy and receiving a specific group of oral antibiotics.

The following laboratory tests are performed as a baseline, repeated at the end of the chemotherapy course and again while on oral antibiotics: serum carotene, serum Vitamin A, serum folic acid, serum D-xylose (2 hr) and urine D-xylose (5 hr). Twenty patients have been entered on study. Eleven patients have completed both sets of follow-up tests. Eight patients failed to complete both sets of follow-up tests, mostly due to the need for hospitalization to receive systems antibiotics. One patient remains on study. Because of the high drop-out rate in the second phase of the study, the study will focus on the effect of chemotherapy on gastrointestinal function. All 20 patients had the tests repeated at the end of their chemotherapy course.

The objective of this study is to evaluate the effect of chemotherapy and oral antibiotics on gastrointestinal function in a population of patients with acute nonlymphocytic leukemia in remission.

Eligible patients are those patients receiving the chemotherapy combination of thioguanine and cytosine arabinoside for maintenance chemotherapy and receiving any of the following combinations of oral antibiotics for infection prevention: gentamicin + nystatin; gentamicin + vancomycin + nystatin; nalidixic acid + nystatin; trimethoprim-sulfamethoxazole + nystatin.

The following laboratory tests are performed as a baseline, repeated at the end of the chemotherapy course and again while on oral antibiotics: serum carotene, serum Vitamin A, serum folic acid, serum D-xylose (2 hr) and urine D-xylose (5 hr).

Surveillance cultures of nose, gums and rectum are obtained prior to the use of oral antibiotics and repeated in one week, to verify compliance to the oral regimen. This microbiological information will be correlated to the results of the absorption studies to determine if there is a difference due to the various oral antibiotic regimens.

Twenty patients have been entered on study. Due to the severity of granulocytopenia induced by the chemotherapy, eight patients required hospitalization for systemic antibiotics during the second phase of the study, and were considered inevaluable. The study will focus on the effect of chemotherapy on gastrointestinal absorption during the first phase of the project.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09121 02 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Prevention of Shaking Chills and Fever with Intravenous Meperidine or Hydrocortisone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rebecca Finley, Pharm. D.	Clinical Pharmacist	COB,NCI
Other:	Joseph Aisner, M.D.	Head, Medical Oncology Section	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Section	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to evaluate and compare the efficacies of intravenous Meperidine and Hydrocortisone in preventing shaking chills associated with Amphotericin therapy. Patients are randomized to receive either Meperidine or Hydrocortisone by IV push prior to infusion, or as an addition to the infusion. The occurrence of shaking chills and the time interval between the beginning of the amphotericin infusion and the chill are being evaluated.

This is a double-blind randomized trial designed to evaluate and compare the efficacies of intravenous Meperidine and Hydrocortisone in the prevention of shaking chills in patients receiving Amphotericin therapy. In addition, the route of administration (IV push vs addition to infusion bottle) is also being evaluated. Patients are eligible for the study if they have previously experienced shaking chills with Amphotericin therapy.

Patients receiving Amphotericin therapy are randomized to receive one of the following randomizations: 1) 50 mg of Meperidine, IV push, 5 minutes before the Ampotericin infusion; 2) 25 mg of Hydrocortisone, IV push, 5 minutes before the Amphotericin; 3) 50 mg of Meperidine added to the Amphotericin infusion; or 4) 25 mg. of Hydrocortisone added to the Amphotericin infusion.

Criteria which is being evaluated includes, the occurrence of shaking chills, the time interval between the beginning of the infusion to the onset of the shaking chills and adverse reactions related to any of the randomizations. Forty-four randomizations have been completed with a total of 42 evaluable trials. The data is summarized below.

Results:

	Chill	No Chill
Infusion-hydrocortisone	6	2
Infusion-meperidine	7	2
IV-push-hydrocortisone	5	9
IV-push-meperidine	7	4

At this point there is no apparent difference in the efficacy of the 4 regimens in preventing shaking chills associated with Amphotericin B therapy.

The only adverse reaction which has been reported was one episode of transient hypotension which was believed to be associated with meperidine in the Amphotericin B infusion.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09122 02 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Amikacin Dosing Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rebecca Finley, Pharm. D.	Clinical Pharmacist	COB,NCI
Other:	Stephen C. Schimpff, M.D.	Head, Infection & Microbiology Research Section	COB,NCI
	Carlos DeJongh	Clinical Assoc., Infection & Microbiology Research Section	COB,NCI
	Kathryn Newman, R.N	Infection Control Nurse, Infection & Microbiology Res. Sect.	COB,NCI
	Clarence L. Forner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
	Ellis S. Caplan, M.D.	Clinical Assoc. Professor, Infectious Disease Division, UMH	
	James C. Wade, M.D.		

COOPERATING UNITS (if any)

University of Maryland Infectious Disease Division

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

X ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study was to establish the predictability of a pharmacokinetic dosing method (adapted to accomodate a TI 59 Programmable Calculator) to establish amikacin dosages based on lean body weight, estimated creatinine clearance, a standard amikacin elimination rate constant and desired peak (25 mcg/ml) and trough (5-8 mcg/ml) concentrations. Steady state serum peak and trough concentrations were measured by a radioimmune assay technique. The measured levels were then used to calculate the patient specific elimination rate constant and volume of distribution. If necessary the maintenance dose was adjusted using these values. One hundred sixty-three consecutive granulocytopenic patients participating in an empiric antibiotic trial had amikacin dosages calculated in this manner. One hundred twelve evaluable trials were completed using this initial dosing method. The median peak concentration was 20.4 mcg/ml and the median trough concentration was 6.25 mcg/ml using this method. One hundred fifteen dosage adjustments were made using the second method. The median peak concentration was 25.3 mcg/ml and the median trough concentration was 8.1 mcg/ml using the adjustment method described above.

The objective of this study was to establish the predictability of a pharmacokinetic dosing method (adapted to accomodate at TI 59 Programmable Calculator) to establish amikacin dosages based on lean body weight, estimated creatinine clearance, a standard amikacin elimination rate constant and desired peak (25 mcg/ml) and trough (5-8 mcg/ml) concentrations. Steady state serum peak and trough concentrations were measured by a radioimmune assay technique. The measured levels were then used to calculate the patient specific elimination rate constant and volume of distribution. If necessary the maintenance dose was adjusted using these values.

One hundred sixty-three consecutive granulocytopenic patients participating in an empiric antibiotic trial had amikacin dosages calculated in this manner. One hundred twelve evaluable trials were completed using this initial dosing method. The median peak concentration was 20.4 mcg/ml and the median trough concentration was 6.25 mcg/ml using this method.

One hundred fifteen dosage adjustments were made using the second method. The median peak concentration was 25.3 mcg/ml and the median trough concentration was 8.1 mcg/ml using the adjustment method described above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09141 01 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Use of the NBI 3000 Word Processor for Generating Patient Drug Administration Records

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Grove, B.S.	Asst. Dir., Clin. Res. Pharmacy Sect.	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI,NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1		1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ✓

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The medication ordering and administration system for the branch has been converted from an individual drug card system, which required processing by a computer, to a system designed around the capabilities of a NBI 3000 word processor.

The new system provides for complete control of the drug records processing requirements. Use of outside computer facilities is no longer necessary.

The operator of the NBI 3000 word processor enters the information from all drug records during the week including inpatient orders with nursing administration records, outpatient clinic drug orders, and outpatient prescriptions. The NBI 3000 is then directed to produce a printout for each patient's weekly drug record, in triplicate. The three copies provide the information for the patient's chart, for the pharmacy profile and for the billing document.

All forms involved in the ordering, charting, and recording of patient drug administration have been changed during the past year. This change was made necessary by the termination of available computer facilities which had processed the drug forms in the past. A NBI 3000 word processor has replaced the computer.

An operator enters drug administration information from the ordering sheets onto a weekly document within the NBI 3000. This includes outpatient prescriptions, inpatient drug orders and outpatient clinic drug orders. The operator also duplicates the charting record for nursing administration for the following weeks.

At the conclusion of each week's entries, the operator directs the word processor to sort the file alphabetically by patient name and by drug name, and print a separate weekly drug summary of all medications received by each patient. This report is printed in triplicate, with copies provided for the patient's medical record, the pharmacy profile, and the billing document.

The complete and accurate records necessary for clinical research purposes are thus maintained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09142 01 COB

PERIOD COVERED
October 1, 1980 to September 1, 1981

TITLE OF PROJECT (80 characters or less)
Use of the NBI-3000 Word Processor for Maintaining Investigational Drug Records

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Grove, B.S.	Asst. Dir., Clin. Res. Pharmary Sect.	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
Other:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2		.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The necessity of maintaining complete and accurate drug records of investigational drugs is essential. In the past this has required the tedious and time-consuming function of manually recording the daily chemotherapy entries into a notebook organized by each investigational drug.

This project is designed to investigate the feasibility of replacing the manual system with the use of a NBI 3000 word processor.

Simultaneous audits of the NBI 3000 generated record with the manual record have shown the NBI 3000 to be accurate and complete. As soon as all details have been resolved, the record will be maintained exclusively on the NBI 3000.

All drugs ordered and administered to B.C.R.P. patients are entered onto a NBI 3000 word processor to produce the weekly drug reports. By coding each entry for investigational drugs, protocol drugs, or other study drugs, the operator is able to later extract this subset of information from the main document. Through the capabilities of the word processor, this information is organized by drug and by date to produce a record similar to the one which has previously been maintained manually.

The procedures are written so that the secretary to the Section can produce the record, saving 5-10 hours per weeks of pharmacist time.

Preliminary audits comparing the NBI generated record with the manual record have indicated no loss of accuracy or completeness. Certain details remain to be resolved before this investigational drug record becomes used exclusively.

Methods of using the NBI 3000 word processor for other functions will be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09143 01 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Comparison of Two Amikacin Dosing Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rebecca Finley, Pharm. D.	Clinical Pharmacist	COB,NCI
Other:	Stephen C. Schimpff, M.D.	Head, Infection & Micro. Res. Sect.	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharm. Sect.	COB,NCI
	Kathryn Newman, R.N.	Infection Control Nurse, Infect. & Microbiology Res. Sect.	COB,NCI
	Carlos DeJongh, M.D.	Clinical Associate, Infect. & Microbiology Res. Sect.	COB,NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

X☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this study are to evaluate and compare two amikacin dosing methods in regards to toxicities, efficacy and serum concentrations achieved by each of the methods. The first dosing method is 600 mg/m^2/day (or approximately 15 mg/kg) divided into 4 doses. The second dosing method establishes optimal serum concentrations based on pharmacokinetic parameters. All granulocytopenic patients participating in an empiric antibiotic trial are randomized to receive amikacin using one of the methods described above plus either ticarcillin, azlocillin or moxalactam. Amikacin serum concentrations are measured for all patients. Those patients who have been randomized to receive pharmacokinetic dosing have adjustments made as necessary based on serum concentrations.

All patients are being monitored for evidence of renal and auditory toxicity.

PHS-6040

The objectives of this study are to evaluate and compare two amikacin dosing methods in regards to toxicities, efficacy and serum concentrations achieved by each of the methods. The first dosing method is 600 mg/m^2/day (or approximately 15 mg/kg) divided into 4 does. The second dosing method establishes optimal serum concentrations based on pharmacokinetic parameters. All granulocytopenic patients participating in an empiric antibiotic trial are randomized to receive amikacin using one of the methods described above plus either ticarcillin, azlocillin or moxalactam. Amikacin serum concentrations are measured for all patients. Those patients who have been randomized to receive pharmacokinetic dosing have adjustments made as necessary based on serum concentrations.

All patients are being monitored for evidence of renal and auditory toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09144 01 COB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Ticarcillin and Piperacillin on Amikacin Serum Concentrations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rebecca Finley, Pharm. D.	Clinical Pharmacist	COB,NCI
Other:	Kathryn Newman, R.N.	Infection Control Nurse, Infection & Microbiology Res. Sect.	COB,NCI
	Stephen C. Schimpff, M.D.	Head, Infection & Microbiology Research Section	COB,NCI
	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
	Marcia Moody, Ph.D.	Infection & Microbiology Res. Sect.	COB,NCI

COOPERATING UNITS (if any)

University of Maryland Infectious Disease Division

LAB/BRANCH

Clinical Oncology Branch

SECTION

Clinical Research Pharmacy Section

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been reported that semi-synthetic penicillins interact with some aminoglycoside antibiotics to form a conjugate that inactivates both agents. The objective of this project is to determine: 1) if either ticarcillin or piperacillin alter concentrations of amikacin in normal human serum over specified periods of time under various storage conditions, and 2) if the effects of these two penicillins on amikacin concentrations are different. Twenty aliquots of normal serum will have amikacin added to yield a concentration of 25 mcg/ml. Ten aliquots will have piperacillin added to yield a concentration of 125 mcg/ml and the remaining 10 aliquots will have ticarcillin added to yield the same concentration. Five aliquots of each group will be stored at 37°C and the remaining 5 aliquots of each group will be stored at 25°C. Samples from each group will be frozen at - 70°C at specified time intervals over a 24 hour period and amikacin concentrations will be determined at the end of the 24 hour period.

It has been reported that semi-synthetic penicillins interact with some aminoglycoside antibiotics to form a conjugate that inactivates both agents. The objective of this project is to determine: 1) if either ticarcillin or piperacillin alter concentrations of amikacin in normal human serum over specified periods of time under various storage conditions and 2) if the effects of these two penicillins on amikacin concentrations are different. Twenty aliquots of normal serum will have amikacin added to yield a concentration of 25 mcg/ml. Ten aliquots will have piperacillin added to yield a concentration of 125 mcg/ml and the remaining 10 aliquots will have ticarcillin added to yield the same concentration. Five aliquots of each group will be stored at 37°C and the remaining 5 aliquots of each group will be stored at 25°C. Samples from each group will be frozen at -70°C at specified time intervals over a 24 hour period and amikacin concentrations will be determined at the end of the 24 hour period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09145 01 COB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Ototoxicity with Pharmacokinetically Dosed Amikacin in Granulocytopenic Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Freda Danhauer, B.S.	Clinical Pharmacist	COB,NCI
Other:	Clarence L. Fortner, M.S.	Head, Clinical Res. Pharmacy Sect.	COB,NCI
	Stephen C. Schimpff, M.D.	Head, Infection & Micro. Res. Sect.	COB,NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB,NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to assess the incidence of auditory toxicity for pharmacokinetically dosed amikacin. Audiograms are to be obtained utilizing a portable audiometer.

Initial audiograms were obtained for 91 (55%) of the empiric antibiotic treatment courses. Sixty-one (37%) of the patients with a baseline audiogram had a follow-up test upon completion of therapy. Of the 61 evaluable courses, 13 developed ototoxicity with 10 (17%) not attributed to any other drug or disease process. Ototoxicty was defined as a change of 20 db or more at a specific frequency in one or both ears.

The objective of this study was to assess the relative ototoxicity of pharmacokinetically dosed amikacin. The aminoglycoside was given in conjunction with either ticarcillin or moxalactam for an empiric antibiotic protocol.

To obtain and maintain effective amikacin serum concentrations larger daily doses (mg/kg/day) than that recommended by the manufacturer were required. In one previous study, a 24% incidence of ototoxicity occurred with the administration of larger daily doses. Of the 61 evaluable, 13 developed ototoxicity with 10 (17%) not attributable to any other drug or disease process. There was no statistical difference in age, weight, total dose, peak concentration or trough concentration between the 2 groups. A difference between 12.5 days and 8 days of antibiotic therapy for ototoxic and non-ototoxic treatment courses, respectively, was statistically significant (Wilcoxan test $p=.026$). Other risk factors implicated in the development of ototoxicity were previous aminoglycoside exposure and an abnormal baseline audiogram.

SUMMARY
Laboratory of Clinical Biochemistry
October 1, 1980 to September 30, 1981

After two years of interactive work at the University of Maryland Health Sciences Campus, the Laboratory of Clinical Biochemistry is established in the scientific community at both an academic level and a collaborative research level with neighboring medical and research groups. The continued success of collaborative research in clinical pharmacology between the Clinical Oncology Branch and the Laboratory of Clinical Biochemistry has lead to new investigation of Phase I and Phase II studies and to areas of drug-drug interaction, and the effects of response modifiers such as hyperthermia on pharmacokinetics.

Our research in the biochemical pharmacology of antineoplastic agents concentrates on the resolution and understanding of free radical formation from drugs. We have shown that biochemically produced free radical intermediates exist with a number of important and highly effective anticancer agents such as the anthracycline antibiotics, actinomycin D, mitomycin C, and streptonigrin. We have previously shown conclusively the biochemical mechanisms by which these free radicals are generated intracellularly. Now our research efforts are aimed at investigating the physical-chemical characteristics of the drug free radicals in an effort to clarify the structure activity relationships of these drugs and to allow molecular modification for altered free radical formation. In our recent research we have shown that free radical formation from anthracyclines as catalyzed by NADPH cytochrome P450 reductase or xanthine oxidase yields a symmetrical free radical without a lag period under anaerobic conditions. This radical converts to a secondary asymmetric free radical signal indicating a change in physical state. The second signal is generated from the aglycone metabolite of the drug. With manipulation of the solvent system, we demonstrated hyperfine structure generated from both the anthracycline parent and from aglycone product indicating that both moieties produce free radicals. The hyperfine structure shows localization of the free electron in the A- and B-rings. Correlated with the free radical anion concentration, we equated production of the aglycone metabolite; and through the use of competitive substrates and inhibitors of free radical formation, vitamin K3, vitamin E, and DMPO we could modify the reaction sequence of electron transfer.

The production of actinomycin D free radical has progressed through a chemical study of the reduction of actinomycin D and of two analogs prepared in our Laboratory. This was done to determine the physical-chemical characteristics of the actinomycin D free radical.

In our project study of the carbonyl reductases that are ubiquitous in the human and other mammalian tissues, we have shown that these enzymes are very heterogeneous, that the profile of enzymes obtained from rabbit livers are similar to those seen in human liver, and that these enzymes exhibit stereospecificity both for the alcohol product that is generated as well as for the NADPH cofactor utilized.

Continuing studies of the pharmacodynamics of cancer chemotherapeutic agents such as the study of hyperthermic effects on cyclophosphamide metabolism in vitro compared to a previous in vivo study that we did last year in our clinical pharmacology study shows that rat liver metabolism of cyclophosphamide is depressed at elevated temperatures, i.e., temperatures above 40.5°. Thus cyclophosphamide

may not be a suitable drug for combination with systemic hyperthermia.

In research on animal pharmacology of new anticancer agents, we compared the cellular pharmacology of a new series of active anticancer agents, N,N-dimethyl adriamycin, N,N-dimethyl daunorubicin, and adriamycin octanoylhydrazone in P388 sensitive and adriamycin resistant cells. Similarly, we have studied the tissue distribution, the metabolism and disposition of these compounds in vivo in mice and in rabbits. These studies show that the N-alkylated anthracycline derivatives are converted to the parent drugs to some degree; however, they appear to act directly without metabolic conversion. Their pharmacokinetics are drastically altered by the N-alkylation.

Clinical pharmacology research has concentrated on drug-drug interactions and has shown that substances such as dimethylsulfoxide and tetrahydrocannabinol have varying effects on the pharmacokinetics of anticancer agents such as cyclophosphamide and adriamycin. Drug-drug interactions between vindesine and cis dichlorodiammine platinum show no significant interaction. In the study of the disposition of anthracyclines, we had the opportunity to utilize adriamycin treated patients with ascites and find that the ascites is an important reservior for adriamycin disposition. However, this accumulation in the ascitic fluid does not appear to affect plasma CxT or elimination kinetics of adriamycin.

To advance our clinical pharmacology efforts, we have developed new technology for the assay of GC mass spectroscopic analysis of trimethylsilylated sugar residues of the anthracycline agents. This method is an excellent general procedure for the analysis of the sugar of mono- and di-alkylated analogs which are applicable to newly developed anthracycline analogs. In addition, we have developed methodology for the analysis of 6-thioguanine and its metabolites through reversed phase high pressure liquid chromatography and have shown new and unidentified metabolites in plasma which we are currently investigating.

Our work on cellular control mechanisms has compared the susceptibility of HL-60 cells and normal human myeloid progenitor cells to growth inhibition by thymidine. We are investigating the mechanism by which thymidine inhibits cell growth. We determined that thymidine affects more than just the deoxyribonucleotide synthesis Our investigation of deoxyguanosine shows that this agent augments the activity of cytosine arabinoside against HL-60 and K562 cloning efficiency.

Studies on the surface membrane identification of malignant and nonmalignant cell lines led to new methodology for the specific labeling of membrane proteins and glycoproteins. The labeling is accomplished with iodine-125 or tritium, through either lactoperoxidase-hydrogen peroxide or iodogen or periodate-sodium borotritiate methodology. Through polyacrylamide gel chromatography and electrophoresis, we have developed two demensional autoradiograph patterns of specifically labeled proteins that show approximately 25 groups of multiple spots for cell types. This methodology is being utilized in examining human malignant and normal cells and tissue cultured normal and malignant cells.

Macrophages act in the dissemination of lymphoma contrary to what is expected. Cells that are most susceptible to macrophages show the most rapid growth as local tumors at transplantation times; and survival time of mice is shortest in this group. These findings suggest that macrophages may actually enhance tumor dissemination rather than contribute to host resistance. The mechanism of this process is unclear at present but is reproducible and universal in its effect. Our

immunotherapy investigations of patients with acute myelogenous leukemia treated with neuramindase modified allogeneic myelblasts show that the lymphokine is produced in tissue culture from these patients' lymphocytes and myeloblasts. This lymphokine contains activity that stimulates proliferation of myeloblasts in liquid suspension culture as studied by thymidine uptake and cell count. The mixed lymphocyte culture from a normal donor also contains such activity in the supernatant. We are utilizing the lymphokines produced in in vitro culture and have maintained a myeloblast culture from one AML patient in liquid suspension for several months. Our attempts to purify myeloblast stimulating factor from lymphokine has revealed a substance that has a molecular weight of between 10,000 and 40,000 and is undergoing further purification for the development of immunologic systems.

In summary, the Laboratory of Clinical Biochemistry continues to maintain both a wide spectrum of scientific research interests which interdigitate with clinically relevant problems as well as developing and maintaining intensive and highly specific research projects in the mechanism of drug action, mechanism of cellular growth development and differentiation, and the study of clinical pharmacology of anticancer agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06012-11 LCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology of Antineoplatic and Other Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. L. Gutierrez	IPA	LCB, NCI
	M. V. Gee	Chemist	LCB, NCI
	R. D. Friedman	Chemist	LCB, NCI
	F. E. Chou	Visiting Scientist	LCB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	4.3	0.8

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The free radical forms of adriamycin, daunorubicin, adriamycinol, and daunorubicinol show interconversion to a second asymmetric form which indicates a change in physical state. Hyperfine structure indicates electron density on the A and B Rings. Kinetics of free radical conversion suggest the free radical is obligatory to glycosidic cleavage. Liver carbonyl reductases show substrate and product stereospecificity. Actinomycin D and its analogs show free radical spectra when reduced by chemical means.

Project Description:
In our research program to determine the biochemical mechanism of anticancer agent cytotoxicity, we have studied the kinetics of free radical formation. Anthracycline antibiotics adriamycin, daunorubicin, and their alcohol metabolites adriamycinol and daunorubicinol are all activated to free radical states by the catalysis of NADPH cytochrome P450 reductase or xanthine oxidase. Under anaerobic conditions the free radical signals were detected without any lag period by means of electron paramagnetic resonance (EPR) spectrometry. During the incubation period while the first signal disappeared, a second EPR signal emerged. This second signal was generated from the product, free radical deoxyaglycones. Depending on the conditions of the reaction buffer mixture, the line details of both the initial and second signal are different. In a totally aqueous buffer system, the g values and line widths of the initial signals of the anthracyclines are similar (g=2.0036 and width=2.8 gauss). The second signal profile exhibited asymmetry and g anisotopy ($g_{\perp}$ =2.0052 and $g_{\parallel}$ =2.0023). Neither signal displayed hyperfine structure lines. When buffer-solvent combinations were manipulated, the initial signal gave hyperfine lines showing the same g value. While the second signal appeared, the asymmetry profile was less obvious and hyperfine structure of 40 lines appeared with a g value the same as the parent compound (g=2.0036). Preliminary analysis of the hyperfine structure shows localization of the free electron in the A and B rings. Similar findings were determined by electrochemically generated anthracycline free radicals. We conclude that three factors affect the EPR signal profiles, 1) the solubility of the anthracyclines; 2) the availability of protons; and 3) the life span of the free radicals. These results show that both glycoside and aglycone forms of the anthracyclines generate free radicals.

The adriamycin free radical anion concentrations have been correlated with the production of adriamycin aglycone metabolite in a reaction catalyzed by NADPH-cytochrome c reductase. The aglycone product is isolated by TLC and quantified by fluorometry. The free radical species is detected by electron spin resonance (ESR) spectroscopy and quantified by double integrations. As the concentration of adriamycin increases, a concomitant increase in aglycone levels and free radical concentrations occurs. There is a substrate inhibition in the reaction which does not allow for calculating the usual Km, Vmax parameters. Inhibition studies show that in aerobic solutions the period before the free radical can be detected is lengthened as the inhibitor concentration is increased. The degree of inhibition in aerobic and anaerobic solutions is concentration dependent with complete inhibition reached at 0.6 mM Vit K3, 2 mM Vit E, and 320 mM DMPO (5-5'-dimethyl-1-pyrroline-1-oxide). While no free radicals were detected at the latter concentrations of Vit E and DMPO, aglycone was produced up to 10% of the amount of the uninhibited reaction. Judged by the oxidation of NADPH, Vit K3 is a better substrate for this enzyme than adriamycin. Vit E and DMPO are not substrates. These experiments point to an obligatory free radical intermediate in the metabolism of adriamycin. They further suggest that ViT E and DMPO act as free radical scavangers of the adriamycin free radical while Vit K3 competes successfuly for the reducing power of NADPH.

Xenobiotic carbonyl reductases have been isolated from rabbit liver by ammonium sulfate fractionation and isoelectric focusing. Although these enzymes are very heterogeneous, most of the carbonyl reduction of oxisuran, 3,7-dimethyl-1-(5-oxyhexyl)-xanthine, metyrapone, and daunorubicin (pH 6.o) was accomplished by two distinct enzymes of pI 4.84 and 4.98. Other reductases with lesser activities

toward these same substrates also occurred at higher pI values. Also resolved were several forms of enzymes that reduced daunorubicin (pH 8.5) (previously identified as aldehyde reductase), naloxone and naltrexone (dihydromorphinone reductases), and the model compounds, p-nitrobenzaldehyde and p-nitroacetophenone. The hydrogen stereospecificity of each of the rabbit liver carbonyl reductases, as well as rat liver aldehyde reductase, was determined by reducing the carbonyl substrates with A- and b-labeled (4-^{3}H)NADPH and examining transfer of label to alcohol products and retention of label in the resulting oxidized cofactors. All of the oxisuran, metyrapone and daunorubicin (pH 6.0) reductases displayed B-hydrogen stereospecificity. Some enzymes that reduce 3,7-dimethyl-1-(5-oxyhexyl)-xanthine, p-nitroacetophenone and p-nitrobenzaldehyde were also B-stereospecific while other forms of these same reductases were exclusively A-stereospecific. Apparent deuterium isotope effects of A- and B-labeled (4-^{2}H) NADPH with daunorubicin (pH 6.0) reductases, daunroubicin (pH 8.5) reductase and naloxone reductases confirm the above hydrogen stereospecificity assignments. The results confirm the hydrogen specificity of aldehyde reductases as A-stereospecific ketone reductases appear to represent exceptions to the generalization that enzymes which catalyze the same reaction have the same stereospecificity. Finally, the binding of rat liver aldehyde reductase to NADPH produced a red shift in the cofactor 340 nm absorbance maximum which is opposite to that predicted on the basis of its hydrogen stereospecificity.

Chemical reduction of actinomycin D, 2-amino-3-phenoxazone and 1,2,4-trichloro-7-nitro-phenoxazone were investigated by spectrophotometric methods to probe structural changes during the time course of the reaction. In order to detect free radical intermediates, electron spin resonance spectroscopy was utilized. The reduction of actinomycin D with $NaBH_4$ showed the gradual decrease of maximum absorption at 452 nm and increased optical absorption at 362 nm and generated a free radical signal with a broad width. A similar optical absorption spectral pattern for the reduction of 2-amino-3-phenoxazone occurred whereas the reduction of 1,2,4-trichloro-7-nitro-phenoxazone gave a complex absorption pattern during the reaction. In contrast to actinomycin D, the reduction of these analogs with $NaBH_4$ gave hyperfine free radical spectra. Several other reducing agents were investigated with 1,2,4-trichloro-7-nitro-phenoxazone.

Publications:

Felsted, R.L. and Bachur, N.R.: Ketone Reductases. In Jacoby, W.B. (Ed.): Enzymatic Basis of Detoxication, Vol. 1. New York, Academic Press, Inc., 1980, pp. 281-293.

Felsted, R.L. and Bachur, N.R.: Mammalian Carbonyl Reductases. In Di Carlo, F.J. (Ed.): Drug Metabolism Reviews, Vol. 11. New York, Marcel Dekker, Inc., 1980, pp. 1-60.

Felsted, R.L., Richter, D.R., Jones, D.M., and Bachur, N.R.: Isolation and characterization of rabbit liver xenobiotic carbonyl reductases. Biochem. Pharmacol. 29: 1503-1516, 1980.

Nakazawa, H., Chou. F.E., Andrews, P.A., and Bachur, N.R.: Chemical reduction of actionmycin D and phenoxazone analogues to free radicals. J. Org. Chem. 1493-1496, 1981

Reich, S.D., Steinberg, F., Bachur, N.R., Riggs, C.E., Goebel, R., and Berman, M.: Mathematical model for adriamycin (doxorubicin) pharmacokinetics. J. Cancer Chemother. Pharmacol. 3: 125-131, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06014-11 LCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. W. Schrecker	Research Chemist	LCB, NCI
	D. D. Ross	Senior Investigator	LCB, NCI
	S. A. Akman	Expert	LCB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

HL-60 cells, a human acute progranulocytic leukemia, and normal (CFUc) are growth inhibited by thymidine. Thymidine affects more than deoxyribonucleotide synthesis. Deoxyguanosine and cytosine arabinoside act synergistically to inhibit HL-60 and K562 cloning efficiency. HL-60 and K562 surface antigens are studied by specific iodine and tritium labeling. Cell surface specific, plant isolectins from phaseolus vulgaris have been confirmed to have a homo and hetero tetrameric conformation.

We compared the relative susceptibility of HL-60, a human acute progranulocytic leukemia cell line, and the normal human myeloid progenitor cell (CFU-C) to growth inhibition by thymidine . Normal human myeloid colony formation was more sensitive to thymidine than HL-60 colony formation, the former being inhibited by $\geq$ 0.5 mM thymidine, and the latter by $\geq$ 5.0 mM. Colony inhibition by thymidine was irreversible in both cases after a seven day incubation of the CFU-C in liquid medium enriched with thymidine and subsequent replating in thymidine free soft agar. Thymidine-induced inhibition of HL-60 cloning could be partially prevented by deoxycytidine, whereas normal myeloid cloning could not, suggesting that high concentration thymidine may affect processes necessary for cloning in addition to deoxyribonucleotide synthesis. HL-60 growth in liquid suspension culture could be suppressed transiently by 1.0 mM thymidine, totally by $\geq$ 5.0 mM thymidine, and rescued completely by concomitant addition of deoxycytidine. The development of resistance to 1.0 mM thymidine could not be accounted for by reduction in thymidine pool size or by reduction in thymidine kinase activity. Replating of HL-60 cells growing in the presence of 1.0 mM thymidine in liquid medium in thymidine-free soft agar produced significant colony inhibition, also suggesting that thymidine affects more than just deoxyribonucleotide synthesis, or that the clonogenic HL-60 cell represents a distinct subpopulation with more sensitive deoxyribonucleotide synthetic control mechanisms.

We investigated whether deoxyguanosine (dGuo) augments the activity of 1-beta-D-arabinofuranosylcytosine (Ara-C) against human leukemia cell lines HL-60 and K652, activity being asssayed by a reduction of in vitro soft agar cloning efficiency. Although 50 uM dGuo or 5 nM Ara-C individually did not affect cloning efficiencies, together the two agents demonstrated synergy by reducing HL-60 cloning efficiency by 40%. Similarly 10 uM dGuo plus 5 nM Ara-C reduced K562 cloning efficiency by 40%, whereas neither compound alone was effective at these concentrations. A three-hour incubation of HL-60 cells in log phase growth liquid suspension culture with 500 µM dGuo reduced cellular uptake of ^{3}H-Ara-C by 45 - 68%. ^{3}H-Ara-C 5'-triphosphate pool size was reduced proportionally. Incorporation of ^{3}H-Ara-C into acid insoluble material was reduced by 15-50% by 500 µM dGuo. The reduction in cellular uptake and phosphorylation of ^{3}H-Ara-C caused by dGuo was of larger magnitude than the reduction in incorporation of ^{3}H-Ara-C into acid insoluble material, such that dGuo increased the percentage of available cellular Ara-C 5'-triphosphate incorporated into acid insoluble cellular material by 160-225%. Cesium chloride density gradient analysis showed significant incorporation of ^{3}H-Ara-C into DNA, but none was detectable in RNA. These data suggest that dGuo promotes activity of Ara-C against human leukemic cells, despite inhibiting uptake and/or phosphorylation of Ara-C by promoting the relative availability of Ara-C 5'-triphosphate for DNA polymerase. In addition, recent evidence suggests that dGuo 5'-triphosphate may inhibit DNA polymerase; thus, the phosphorylated products of dGuo and Ara-C may synergistically inhibit DNA polymerase.

Human lymphoma and cultured human lymphoid (HL-60 and K562) cell surface. proteins and glycoproteins were specifically labeled with I-125 or H-3 by either (1) the lactoperoxidase-hydrogen peroxide; (2) the 1,3,4,6-tetrachloro3-alpha, 6-alpha-diphenylglycoluril (Iodogen); or (3) the periodate-sodium borotritiate methods. Labeled cells were extracted with 1% Triton X-100 and subjected to a first dimension isoelectric focusing on polyacrylamide gels and then a second dimension electrophoresis on an SDS polyacrylamide slab gel. After the second dimension electrophoresis, the gels were fixed and stained for protein, dried, and autoradiographed or fluorographed. Surface specific labeling was indicated by the similiarity of I-125 autoradiograph 2D patterns resulting from the two different 2D surface

specific iodine labeling methods and because the autoradiograph 2D patterns were completely non-overlapping with their respective protein stained 2D patterns. Membrane autoradiograph 2D patterns were unique for each cell type examined with approximately 25 groups of multiple spots of similar molecular weight for HL-60 and human peripheral and plural lymphoma. In contrast, K562 membrane autoradiograph 2D patterns consisted of approximately 30 individual spots of much less change heterogeneity. The results indicate that two dimensional polyacrylamide gel electrophoretic analysis may be a useful method for identifying and following changes of surface proteins and glycoproteins during cell differentiation.

The subunit compositions of individual phytohemagglutinin isolectins from red kidney bean Phaseolus vulgaris were examined by isoelectric focusing and sodium dodecyl sulfate electrophoresis on polyacrylamide gels. Isoelectric focusing reveals heterogeneous but unique and non-overlapping protein band patterns for each of the homotetrameric isoelectins, E_4 and L_4. Isoelectric focusing of the intermediate isolectins which contain both subunits (E_3L_1, E_2L_2 and E_1L_3) show all the protein bands common to isolectins E_4 or L_4 in proportions relative to their suggested subunit compositions. Polyacrylamide gel electrophoresis in a continuous sodium dodecyl sulfate buffer system gives a single protein band for all of the isolectins. In contrast, a discontinuous sodium dodecyl sulfate buffer procedure resolves isolectins E_4 and L_4 into single major protein bands of apparent molecular weights 31,700 ($\pm$ 600) and 29,900 ($\pm$ 200), respectively. Each of the intermediate isolectins contained both protein bands and their relative proportion, as determined by absorbance scanning, confirm the phytohemagglutinin isolectin subunit compositions as E_4, E_3L_1, E_2L_2, E_1L_3, and L_4.

Publications:

Felsted, R.L., Li, J., Pokrywka, G., Egorin, M.J., Spiegel, J., and Dale, R.M.K.: Comparison of Phaseolus vulgaris. Int. J. Biochem., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06016-11 LCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacodynamics of Cancer Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief	LCB, NCI
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	R. D. Friedman	Chemist	LCB, NCI
	R. E. Clawson	Laboratory Scientist	LCB, NCI
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COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.1

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Drug metabolism can be altered by biological response modifiers such as hyperthermia. Cyclophosphamide activation to alkylating species is decreased under hyperthermia both in vivo in human and in vitro with rat liver preparations. The cellular pharmacology of the amino sustituted anthracycline analogs N,N dimethyl adriamycin and N,N dimethyl daunorubicin show greater cellular uptake than for adriamycin and daunorubicin but shows similar effects on DNA and RNA synthesis. These analogs are significantly metabolized in vivo in rabbits. Adriamycin actinoylhydrazone is metabolized in vivo in rabbits yielding high drug concentrations in the lung.

Hyperthermia has been shown to modify the response of tumors to cancer chemotherapeutic agents. We have initiated a research program to study effects of such response modifying as hyperthermia to cancer chemotherapeutic pharmacodynamics. The ability of rat liver microsomes and liver slices to metabolize the antineoplastic compound cyclophosphamide was studied at 37° and at elevated temperatures comparable to those used for human systemic hyperthermic antineoplastic therapy. Temperatures above 40.5° and 41.8° inhibited cyclophosphamide metabolism by microsomes and liver slices respectively. Therefore, cyclophosphamide may not be a suitable drug for combination with systemic hyperthermia in cancer therapy.

The cellular accumulation and disposition of the anthracycline antibiotics daunorubicin (DNR) and adriamycin (ADR) were compared to those of their N,N-dimethyl derivatives. The cellular accumulation of N,N-dimethyl daunorubicin (DMD), was greater than that of N,N-dimethyl adriamycin (DMA) which was greater than the accumulation of DNR or ADR. DNR and ADR resistant P388/ADR cells accumulated less of each drug than did sensitive P388/S cells. The presence of 15% fetal bovine serum in the incubation medium did not affect the accumulation of DNR, increased the accumulation of DMD by 20-25%, and reduced that of ADR and DMA by 20-25%. Lowered temperature (0°) reduced the intracellular accumulation of all four drugs. 10 mM sodium azide did not alter the cellular accumulation of DNR or ADR but reduced the intracellular content of DMD and to a lesser extent that of DMA. The loss of all four drugs from cells placed in drug-free medium was greatly reduced at 0° compared to 37°. 10 mM azide increased the efflux of DMD but did not affect that of DNR, ADR, or DMA. Unlike intact L1210 cells, isolated nuclei accumulated more ADR than DNR and more DMA than DMD. The nuclear accumulation of DMA > ADR > DMD> DNR. Nuclear accumulation of all four drugs reached equilibrium by 10-30 min and was the same at 0° and 37°. All four drugs were lost from nuclei placed into drug-free buffer and this loss was reduced at 0°. DMD and DMA were nuclearly localized as were DNR and ADR. All four drugs produced dose-dependent inhibition of [^{3}H] uridine incorporation and DMD and DMA inhibited P388/ADR cells to the same degree as P388/S cells. DNR and ADR inhibited [^{3}H] -thymidine incorporation more than [^{3}H] -uridine incorporation, whereas DMD and DMA inhibited these processes to the same degree.

The disposition, metabolism, and excretion of adriamycin octanoylhydrazone (OctAdr) were studied after i.v. administration to Balb/C mice and New Zealand white rabbits and were compared to similar studies of adriamycin (Adr). In mice, concentrations of OctAdr-derived fluorescence were initially greatest in lung with very low concentrations detectable in kidney, liver, and spleen. Little drug fluorescence was found in brain, heart, or skeletal muscle. Adriamycin had the highest drug fluorescence concentration in the kidney, with progressively lower but easily detectable concentrations in liver, heart, lung, spleen, and skeletal muscle. Drug fluorescence was lost much more rapidly from tissues of mice injected with OctAdr than from tissues of mice injected with Adr. At all times post injection, Adr was the major fluorescent drug species recovered from livers and kidneys of mice treated with OctAdr or Adr, although substantial amounts of unaltered OctAdr were recovered from the former group. In rabbits, plasma concentrations of OctAdr-derived fluorescence declined rapidly but then remained relatively constant from 120 to 480 minutes post injection. No OctAdr-derived fluorescence was observed in urine. During the 8 hours post-injection, biliary excretion of OctAdr represented approximately 25% of the administered dose. At all times after injection, OctAdr was the major biliary fluorescent species with Adr and adriamycinol (Adrol) the second and third most prominent respectively. Eight hours after OctAdr administration, lung contained the highest concentration of drug

related fluorescence with progressively lower amounts in liver, kidney, duodenum, and heart. Brain, skeletal muscle, and spleen contained little or no OctAdr-derived fluorescence. Adr and OctAdr were the major fluorescent species in all tissues. Small amounts of Adrol were detected in all tissues, but aglycones were present only in liver.

The tissue distribution, metabolism, plasma concentrations, and biliary and urinary excretion of N,N-dimethyladriamycin (DMA) were studied after iv administration of BALB/c mice and New Zealand white rabbits. In mice, concentrations of both DMD- and DMA-derived fluorescence were initially greatest in lung and kidney with lower concentrations in liver, heart, and skeletal muscles, respectively. Little drug fluorescence was found in the brain. Drug fluorescence in the spleen increased during the first 8 hr to become the highest of all tissue concentrations. Peak tissue fluorescence concentrations in DMD-and DMA-treated mice were similar to values previously reported for daunorubicin and adriamycin. DMD-derived ced fluorescence was lost from all tissues except the spleen at approximately the same rate. Similarly, DMA-related fluorescence disappeared from all tissues except the spleen at approximately the same rate. However, the loss of DMA-derived fluorescence was 3-5times slower than the loss of DMD-derived fluorescence. Also the persistence of drug fluorescence in animals treated with either DMD or DMA was greater than values previously reported for their respective parent compounds. In mice, DMD was rapidly converted to a more polar metabolite, observed in liver and kidney, whereas DMA was not metabolized to any significant degree. In rabbits, plasma concentrations of DMD-derived fluorescence declined rapidly but rose progressively from 0.5 to 8 hr after injection. Plasma concentrations of DMA-derived fluorescence declined rapidly during the first 15 min after injection and then declined very slowly. No DMD-related fluorescence was observed in urine, and the urinary excretion of DMA-related fluorescence represented much less than 1% of the administered dose. Biliary excretion of DMD and DMA represent 4% and 5.5%, respectively, of the administered. dose. DMD did not appear in bile, but a fluorescent metabolite, presumed to be N-monomethyldaunorubicinol, appeared, followed by increasingly large amounts of more polar metabolites, presumed to be conjugates. Some unaltered DMA was present in bile soon after injection, but was rapdily replaced by a single, more polar metabolite, presumed to be N,N-dimethly-adriamycinol (DMA_2). No N-monomethyladriamycinol or conjugated forms of DMA were detected. Eight hours after DMD administration, kidney and lung contained the highest concentration of drug-related fluorescence with progressively lower amounts in spleen, duodenum, liver, heart, skeletal muscle, and brain. N-monomethyldaunorubicinol was the major fluorescent species in all tissues. The liver contained significant amounts of deoxydaunorubicinol aglycone. Eight hours after DMA administration, kidney and lung had the highest concentration of drug-related fluorescence with progressively lower amounts in spleen, liver, duodenum, and heart. No drug was detected in brain or skeletal muscle. DMA was the major fluorescent species in all tissues except liver. All tissues contained significant concentrations of DMA_2 as well as adriamycin.

Publications:

Egorin, M.J., Clawson, R.E., Ross, L.A., and Bachur, N.R.: The cellular pharmacology of N,N-dimethyldaunorubicin and N,N-dimethyladriamycin. Cancer Res. 40:1928-1933, 1980.

Egorin, M.J., Clawson, R.E., Ross, L.A., Chou, F.E., Andrews, P.A., and Bachur, N.R.: Disposition of N,N-dimethyldaunorubicin and N,N-dimethyladriamycin in rabbits and mice. Drug Metab. Disp. 8:353-362, 1980.

Clawson, R.E., Egorin, M.J., Fox, B.M., Ross, L.A., and Bachur, N.R.: Hyperthermic modification of cyclophosphamide metabolism in rat hepatic microsomes and liver slices. Life Sci. 28:1133-1137, 1980.

Egorin, M.J., Clawson, R.E., Ross, L.A. and Bachur, N.R.: Cellular pharmacology of N,N-dimethyldaunouribin and N,N-dimethyladriamycin. Cancer Res. 40:1928-1933, 1980.

Egorin, M.J., Clawson, R.E., Ross, L.A., Chou, F.E., Andrews, P.A., and Bachur, N.R.: Disposition and metabolism of adriamycin octanoylhydrazone (NSC 233853) in mice and rabbits. Drug Metab. Disp. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06241-11 LCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic and Other Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our research program investigating drug-drug interactions related to cancer chemotherapeutic agents has shown that tetrahydrocannabinol administered to patients in therapeutic doses does not significantly affect cyclophosphamide or adriamycin pharmacokinetics. Similarly, dimethylsulfoxide does not affect cyclophosphamide plasma pharmacokinetics in lung tumor patients but does affect 24 hour cumulative urinary excretion. Vindesine and cis dichlorodiammine platinum were studied and shown not to affect their respective pharmacokinetics or excretion. Methods have been developed to analyze the glycosidic portions of anthracycline antibiotic molecules as well as 6- thioguanine and metabolites. A new metabolite of 6-thioguanine has been observed in human urine. Ascites fluid is an important reservoir for adriamycin in patients.

Project Description:
The search for anticancer anthracycline antibiotics with diminished toxic side effects has led to the development of compounds with alterations on the glycosidic portion of the molecule. The N,N-dialkylated analogues have proven promising in reducing the dose-limiting cardiotoxicity of the anthracyclines. In order to elucidate the structural changes on the sugar residue of anthracycline metabolites found during animal studies, an isobutane chemical ionization GC/MS analysis of the trimethylsilylated sugar residues was devised. Parent drugs or purified isolated biliary and urinary metabolites from animals are hydrolyzed with 0.2 N HCl for 30 min. at 100°C. The generated aglycones are removed by ethyl acetate extraction. Ethanol is added to the remaining water layer and the solution dried with a nitrogen jet. The residue is dissolved in trimethylsilylimidazole/pyridine (1:4) and aliquots are injected onto an OV-17 column. The column temperature is programmed, after a 5 min. hold, from 120° to 250° at 10°/min. Electron impact analysis of the derivatized sugars gave spectra displaying extensive fragmentation and no parent ions. Isobutane CI provided spectra with strong quasi-molecular ions $(M+H)^+$ and limited fragmentation. GC analysis indicated the presence of the α-anomer in all analogues studied. The natural β-anomer which was presumed to be the major peak eluted first. In all cases the later eluting α-anomer gave identical although more intense fragments than the β-anomer. Daunosamine (0.1, 9.5 min.), dimethyldaunosamine (9.0, 9.5 min.), monobenzyldaunosamine (17.1, 17.8 min.), and dibenzyldaunosamine (23.8, 25.7, 27.2) have been analyzed by this methd. Common major ions were seen at $(M+H)^+$, $(M-89)^+$, and $(M+H - CH_2CHOTMS)^+$. Daunosamine has been detected from as low as 5.7 nmol of daunorubicin. This method presents a general procedure for the analysis of N-mono and di-alkylated daunosamine analogues and may be applicable to epimeric or acetylated analogues.

Methods have been described for analysis of 6-thioguanine (6TG) and metabolites in plasma, but none have used reversed-phase HPLC. 6TG and metabolites were extracted from human plasma at 50-100% efficiency by cold 2N perchloric acid (1:1). Neutralized extracts were chromatographed on a μ-Bondapak C_{18} column by two separate isocratic conditions. 6TG, 6-thiouric acid, 6-thioguanosine, and 6-thioxanthine were analyzed with 0.01 M Na acetate, pH 3.5/10% methanol as the mobile phase and were detected at 340 nm. 6-methyl TG and three unknown metabolites were eluted with Na acetate/25% methanol and were detected at 310 nm. External standard calibration was used for quantitation. The 6TG detection limit was 0.8 nmol/ml. In six patients (1-1.2 gm 6TG/m^2 i.v.), 6TG concentration peaked from 61-118 nmol/ml (95.6 ± 23.0, mean ± S.D.) and decayed biexponentially, with initial t 1/2 of 3 hr and terminal t 1/2 of 5.9 hr. 6-thiouric acid, 6 methyl TG, 6-thioguanosine, 6-thioxanthine, and three major unidentified metabolites were also in plasma. The three unknowns were extracted with ethyl acetate from alkalinized pooled plasma extracts and were purified by HPLC. UV spectra of these three metabolites showed absorption maxima at 320; 310; and 310 and 265 nm respectively. Structural elucidation is being pursued.

Preliminary studies have shown that dimethylsulfoxide (DMSO) can increase penetration of and potentiate the biological effect of several drugs including the antitumor activity of cyclophosphamide (CTX). To determine if combination chemotherapy with CTX given with DMSO is tolerable and might produce enhanced antitumor activity in patients with squamous cell carcinoma of the lung, 14 patients were treated with five liters of a 5 or 6% DMSO solution orally over three days and 1500 mg/m^2 of CTX intravenously as a 60 minute infusion on the third day of treatment. Serial blood and urine samples were collected to assess the pharmacokinetics of

CTX. Courses were repeated every three to four weeks. No antitumor responses were observed. The plasma pharmacokinetics of CTX in this study are similar to previously reported results for CTX alone, but the 24 hour cumulative urinary excretion of cyclophosphamide in our study is much lower than previously reported. Further studies in tumors more responsive to CTX may be warranted.

We studied the effect of tetrahydrocannabinol (THC) upon the pharmacokinetics of CTX and doxorubicin (ADR). Plasma THC was determined by RIA. Plasma concentrations of CTX and ADR were measured by GLC and fluorescence, respectively. RIA confirmed plasma levels of THC >20 ng/ml for patients who received THC. CTX half-life was not significantly changed with use of THC (7.7 $\pm$ 3.6 hours without vs. 5.25 $\pm$ 2.6 hours with THC). ADR half-life with THC was greater than without THC (175 $\pm$ 197 hours vs. 92 $\pm$ 92 hours, respectively). Total drug exposure as determined by areas under the curves were similar (12.4 $\pm$ 6 nM/hr without vs. 13.8 $\pm$ 4 nM/hr with THC). There preliminary data suggest that RIA is reliable for assessing THC plasma concentrations. THC induces no significant alterations of CTX or ADR pharmacokinetics.

Patients with ascites have a large third fluid space which may interfere with the normal disposition of antineoplastic drugs. Such patients may exhibit altered pharmacokinetics, producing differences in efficacy of treatment or in anticipated toxicities from those seen in patients without a third space. Two patients with sarcomas and large accumulations of ascitic fluid were studied on 3 occasions following ADR treatment. Serial plasma and ascitic fluid specimens were obtained from 0.25 hrs to 72 hrs following ADR doses of 45 mg/m^2 or 60 mg/m^2. Specimens were analyzed by two methods: 1) total anthracycline concentration and 2) HPLC. Measurable ADR concentrations were found in ascites and plasma samples. A2 was measurable in ascitic fluid in 2/3 courses and in plasma in 1/3 courses. Ascitic fluid:plasma concentration ratios for total anthracycline and ADR concentrations increased from approximately 0.25 at 1 hr following ADR administration to >1.0 by 24 hrs following drug infusion, and remained >1.0 for the duration of study (72 hrs). Plasma exposure (CxT $\pm$ SD) to total anthracyclines and ADR were 10.8 $\pm$ 10.8 nmol/hr/ml and 2.5 $\pm$ 0.6 nmol/hr/ml, respectively, and were in accord with previous results. Terminal plasma T 1/2 for total anthracyclines and ADR were 19.6 $\pm$ 7.6 hr and 12.7 $\pm$ 6.3 hr, respectively. These data indicate that ascites is an important reservoir for ADR. Such accumulation does not appear to affect plasma CxT or elimination kinetics of ADR.

Vindesine (VDS) and cis-dichlorodiammine platinum (DDP) alone or in combination have clinical antitumor activity. The possibility of drug interaction when the drugs are used in combination has not been previously studied. Plasma and urine concentrations of VDS and platinum by radioimmunoassay and atomic absorption spectroscopy, respectively were measured in patients receiving VDS 3 mg/m^2 I.V. bolus or the combination of VCS and DDP I.V. 100 mg/m^2 over six hours with mannitol diuresis. VDS pharmacokinetics were similar in all groups. DDP pharmacokinetics including free platinum were similar compared to DDP given alone indicating VDS does not interfere with protein binding of DDP. In one patient with a pleural effusion VDS accumulated in the effusion exceeding plasma concentration by 24 hours post therapy.

Publications:

Andrews, P.A., Brenner, D.E., Chou, F.E., Kubo, H., and Bachur, N.R.: Facile and definitive determination of human adriamycin and daunorubicin metabolites by high-pressure liquid chromatogrpahy. Drug Metab. Disp. 8: 152-156, 1980.

Bachur, N.R.: Cell Kinetics as Related to Cancer Treatment. In Lichtman, M.A. (Ed.): Hematology and Oncology. New York, Grune and Stratton, 1980, pp. 269-271.

LeRoy, A.F,. Wehling, M., Gormley, P., Egorin, M., Ostrow, S., Bachur, N., and Wiernik, P.: Quantitative changes in cis-dichlorodiammineplatinum (II) speciation in excreted urine with time after IV infusion in man: Methods of analysis, preliminary studies, and clinical results. Cancer Res. 64: 123-132, 1980.

Riggs, C.E., Jr., Egorin, M.J., Fuks, J.Z., Schnaper, N., Duffey, P., Colvin, O.M., Aisner, J., Wiernik, P.H., and Bachur, N.R.: Initial observations on the effects of delta-9-tetrahydrocannabinol on the plasma pharmacokinetics of cyclophosphamide and doxorubicin. J. Clin. Pharmacol., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06992-04 LCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cell Mediated Immune Response in AKR Lymphoma and Human Acute Myelogenous Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. Wiernik	Acting Assoc. Director	BCRP, DCT

COOPERATING UNITS (if any)

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Laboratory of Viral Diseases, NIAID

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

AKR lymphoma cell lines have been isolated and tested for in vitro and in vivo growth and lethality characteristics. Macrophages actually stimulate AKR tumor growth. Macrophage cytotoxicity is inhibited by phagocytosis of both nondigestible and digestible particles. Partial purification of myeloblast stimulating factor has been accomplished.

Project Description:
The role of macrophages in the dissemination of lymphoma is further studied with AKR lymphoma cell lines that exhibit different subsceptibilities to macrophage cytotoxicity in vitro. From the original cell line established in this laboratory, subcloning of lymphoma cells were done by the limiting dilution method, as well as agar cloning method. Three sublines of AKR lymphoma cells were selected, and one of them showed marked resistance to macrophage cytotoxicity. These sublines showed similar, if not identical proliferation kinetics under in vitro culture condition. When transplanted to AKR mice, however, they showed different growth rates in vivo and different lethality to the mice. Contrary to what was expected, the cells that were most susceptible to macrophages showed most rapid growth as local tumor at transplantation site, and the survival time of mice were the shortest in this group. These findings suggest that macrophages in the nonmanipulated host may actually enhance tumor growth and dissemination rather than contribute host resistance to tumor growth. This is consistent with our previous findings showing that peritoneal macrophages contribute little, if any resistance to tumor growth in vitro, despite their ability to lyse tumor cells under in vitro conditions. Caution must therefore be exercised in extrapolating the in vitro cytotoxicity assay to the immune resistance in vivo.

The AKR lymphoma cell line was further characterized with regard to virus production and its cellular lineage. The cell line we isolated was shown to produce ecotropic, xenotropic and recombinant viruses. Immunofluorescence studies showed that the cell line possesses AKR type thy-1, 1 antigen. The macrophage mediated cytotoxicity against AKR lymphoma cell line was markedly enhanced in the presence of monoclonal anti-Thy-1, 1 antibody but not anti-Thy-1, 2 antibody.

Macrophage cytotoxicity against tumor cells is inhibited by silica particles. It was generally believed that this is due to the autolysis of macrophages upon ingesting the nondigestible silica particle. We have shown that phagocytosis mediated inhibition of tumor cell cytotoxicity could be related to phagocytosis rather than autolysis of macrophages. Macrophages that have phagocytized digestible particles such as opsonized candida, C. parvum, and hemolysin treated sheep RBC showed less than 50% of their cytolytic activities. Pretreatment of macrophages with azide or deoxyglucose did not cause such inhibition, suggesting that cellular metabolic energy depletion was not the cause of phagocytosis mediated inhibition of cytolytic activity of macrophages. Further investigation is in process to elucidate the mechanism of this phenomenon.

Immunotherapy of patients with AML in our current protocol employs the neuraminidase treated allogeneic myeloblasts. We have studied the effect of such immunotherapy on the patients' immune system. We have previously shown that the natural killer cell activities of patients receiving immunotherapy against myeloid cell lines HL-60 and K562 did not differ significantly from control patients receiving chemotherapy only. We have further investigated the consequence of mixed lymphocyte-tumor cell culture in vitro, an in vitro counterpart of in vivo immunotherapy. It was shown that the interactions between the patients' lymphocytes and the myeloblasts that they received did not generate specific cytotoxic T cells or nonspecific natural killer cells. The culture supernatant, which contains T-cell elaborated lymphokines were studied in terms of their effect on the growth of myeloblasts. It was shown that the lymphokine contains activity that stimulates the proliferation of myeloblasts in liquid suspension culture, as studied by thymidine uptake and cell count. The mixed lymphocyte culture from normal donor

also contains such activity in supernatant. The question is thus raised as to whether the effect of immunotherapy if any, is mediated by lymphokine rather than cellular mechanisms.

Using the lymphokine produced by in vitro culture, it was possible to maintain a myeloblast culture from an AML patient in liquid suspension for several months. Because of stimulated cell division in vitro, cytogenetic studies were carried out in a large number of myeloblasts from this patient. It was demonstrated that the karyotype of this patient's leukemic cell was normal but 7% of cells carried chromosome 21 trisomy. The remission marrow was similarly stimulated with lymphokine and the population of cells that carried 21 trisomy disappeared. We plan to study the marrow when the patient relapses in the future to see if the trisomy clone will become the main component.

Attempts are made to purify myeloblast stimulating factor(s) from lymphkine. Preliminary results revealed that the substance has a molecular weight between 10,000 to 40,000 and precipitated in 40-60% ammonium sulfate fraction. Further purification is in process utilizing preparative slab gel isoelectrofocusing technique. Our goal is to isolate the pure factor and to produce antibody which can then be used to detect the possible presence and the level of such factor in AML patients.

SUMMARY REPORT

LABORATORY OF MOLECULAR BIOLOGY

October 1, 1980 - September 30, 1981

The investigators of the Laboratory of Molecular Biology have applied many sophisticated modern research techniques in approaches to solutions of basic problems in oncology. For example, in attempts to clarify the mechanisms by which a normal eucaryotic cell becomes potentially malignant, we have focused attention on the biology of herpes simplex virus. Using recombinant DNA, viral gene sequences in human lymphoblastoid cells are being identified and characterized. The K562 cell line is a highly differentiated and transformed cell line that may contain DNA sequences similar to that of the thymidine kinase (TK) gene of the herpes viruses. In order to investigate the extent of the viral DNA sequences present in these cells, large quantities of the viral gene were obtained by cloning an endo R. Bam HI DNA fragment of the virus in E. coli using the plasmid pBR322 as vector. The viral DNA sequence has been nick translated and is being used as a probe to search for complementary DNA sequences in various clones of K562 cells. In close collaboration with members of the Clinical Oncology Branch and the Clinical Biochemistry Branch, similar studies are underway using leucocytes of patients with leukemia and lymphoma.

In associated biochemical studies, a new DNA relaxing enzyme or topoisomerase that copurified with the DNA polymerase induced by herpes simplex virus throughout a multi-step purification scheme has been isolated and characterized. The role of the viral topoisomerase to catenate or decatenate the replicative intermediates of the complex structure of viral DNA is under analysis.

In examinations of the causative agents of mouse leukemia, we have continued our studies on the effects of core proteins from Rauscher Leukemia Virus (RLV) on DNA synthesis. We have found that viral p30 exists as two distinct species having molecular weights of 30,000 and 60,000. The two species, which probably represent monomer and dimer forms, are antigenically identical and have the same isoelectric points. Both species bind to reverse transcriptase (RT), but only the heavier one could modulate RT activity. Tightly associated with the heavier p30 fraction are ATPase and protein phospho-kinase activities. RT-gag protein complexes extracted from RLV particles display ATPase and protein kinase activity as well. The relationship between these activities and the ability of p30 to regulate the activity of RT is under investigation.

The existence of complexes with ATPase and phospho-kinase activity in RLV particles and the extraction of similar complexes from retroviral related tumors suggests that these complexes may serve as markers for retroviral infection. This possibility is currently being explored using cells from patients with acute leukemia.

In conjunction with members of the Clinical Oncology Branch, important advances have been made in the direct separation and visualization of nuclease activities from patient lymphocytes. Individual nuclease activities from peripheral leukocyte subpopulations are separated by isoelectric focusing in a disc gel. In order to distinguish and further resolve individual enzyme activities, a slab gel containing polynucleotide is then cast on the side of the disc gel. Electrophoretic conditions for the second dimension are designed to minimize interaction between the nucleases and the substrate. No denaturing agents such as urea or SDS are necessary in either dimension. Results

obtained using polynucleotide-polyacrylamide gel electrophoresis are superior to those determined using classical techniques that quantitate enzyme activity in that the substrate-in-gel procedure allows the simultaneous detection and analysis of many nucleases using such crude samples as serum or supernatants from sonicated cell preparations. More than 15 nucleases were resolved from human cells and microheterogeneity of charged enzymes was found. Cell subpopulations from donors with chronic myelogenous leukemia exhibit large increases in several nuclease activities relative to amounts detected in equivalent cell subpopulations from normal donors. Other abnormal nuclease activity patterns are observed in donors with acute myelogenous leukemia.

The new methodology we have developed has been used to demonstrate that abnormal DNase expression appears to correlate with cellular immaturity since induction of differentiation (HL-60 cells and murine erythroleukemia cells in tissue culture) results in DNase expression undergoing significant change as the cellular morphology becomes more mature.

Using our sensitive gel techniques, we have found that interferon has a strong effect on DNase expression in cells grown in tissue culture. Although interferon does not bring about a change of abnormal DNase expression towards normal expression in cells capable of being induced to differentiate (murine erythroleukemia), IF induces a 2-4 fold augmentation in DNase expression. In addition, interferon increases DNase expression in the human cell line, HeLa S3. These results may be related to the antiviral effect induced by interferon. However, they do suggest that therapeutic effects by interferon may not be due to pushing the neoplastic cells past a hypothetical differentiation barrier.

Evidence obtained by others suggests that myeloid leukemia represents a disturbance in the process of granulocyte differentiation, which results in the accumulation of proliferatively active, immature cells. In many instances, it has been possible to stimulate more advanced differentiation through the experimental application of certain physiological or artificial substances (differentiation inducers). In attempts to define alternative modes of therapy for human myeloid leukemia, we are exploring certain possibilities using the established human myeloid leukemia cell line HL-60. For example, the phenotype of the HL-60 cells is being deliberately altered by developing drug-resistant variant cultures. So far, cultures have been developed, and cloned, which are specifically resistant to dimethyl sulfoxide, retinoic acid, 5-bromodeoxyuridine, and 6-thioguanine - all of which can act as differentiation inducers and which can be used for selection in tissue culture. All of the variants have been demonstrated to have altered phenotypic properties, which are being defined in terms of cytologic characteristics, cell surface components, certain cellular enzyme activities, growth in semi-solid media (cloning), and response to specific growth and differentiation effectors. Concerning the mechanism of action of inducers of myeloid differentiation, the variant cell lines, discussed above, should be of great value in dissecting out epiphenomena from those specifically related to the process of differentiation itself. So far, we have found that certain changes in cell membrane phospholipids are altered during the process of differentiation and, based on these analyses, it has in some instances, been possible to experimentally predict which anologues may have an effect on cell differentiation.

Although the above experiments are based on an established culture cell line, the investigative procedures should be transferrable to the study of fresh leukemic specimens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06242-11 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Poly(A) Induced RNase Inhibition by Metals or Polyamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Short lengths (18 residues) of poly(A), covalently linked to the 3'-termini of Escherichia coli 5S rRNA, induce powerful inhibitions (38-87%) of the activities of RNases (ribonucleases) from Citrobacter sp., Enterobacter sp., bovine pancreas, human spleen and human plasma. As the polypurine chain length is extended, enzyme activity declines. Furthermore, poly(A) sequences, present only on a small subpopulation of RNA, and accounting for less than 1% of total RNA, serve to protect all RNA, polyadenylated or not, from enzyme-catalysed degradation. The quantity of 3'-terminal adenylic acid residues, relative to the amount of substrate, determines enzyme activity. The exact distribution of a fixed amount of poly(A) residues on the 3'-termini of substrate molecules is unimportant in this respect. Comparison of the efficacies of inhibition of RNase activity, by using linked poly(A) and similar quantities of free poly(A), revealed that although the free polypurine inhibits RNase activity, covalent linkage of poly(A) to RNA is more advantageous to the stability of an RNA substrate. However, the ratio of inhibited activities obtained by using linked or free poly(A) may change considerably with alterations in either substrate concentration or polyadenylic acid segment length.

By using substrate linked to inhibitor in the form of 5S [^{3}H]rRNA · $(A)_n$, it was possible to show that, as the average number of adenylic acid residues per polymer molecule becomes larger, increases in the inhibition of RNase activities are realized. The inverse relationship between activity and poly(A) segment length is similar for many enzymes and suggests a general phenomenon. A change in the number (n) of 3'-terminal adenylic acid residues will induce quite different changes in RNase activity, depending on the value of n. In all cases, the greatest decreases in enzyme activity are found as n is increased from 0 to approximately 40 residues per molecule.

Polyadenylated RNA as well as a variety of non-polyadenylated substrates were incubated with different RNases. In each case the poly(A) segment inhibited the activity of the enzyme under study, thus protecting from hydrolysis about the same proportion of non-polyadenylated as well as polyadenylated substrates. The polyadenylated segments could constitute as little as 0.2% of all the RNA present and yet render very efficient protection. The mechanism, therefore, of poly(A) stabilization of RNA, by virtue of the inhibition of RNase activity, offers a function for poly(A) that does not require that every molecule be polyadenylated.

The addition of low concentrations of spermidine reversed inhibition by significant amounts in every case. However, the hydrolysis of polyadenylated and non-polyadenylated substrates may be affected to different extents by the presence of the polyamine.

The distribution of poly(A) segments on the 3'-termini of RNA plays little or no role in determining the amount of inhibition of RNase activity attained. If the total concenttration of poly(A) relative to that of substrate was the same, irrespective of the distribution of the poly(A) segments on substrate molecules, similar inhibitions of enzyme activity could be achieved. The total quantity of poly(A) present then, relative to that of available substrate, far outweighs in importance the exact distribution of these homopolymeric segments on the termini of RNA.

The covalent linkage of poly(A) to 5S [^{3}H]rRNA does have an important effect on the inhibitory prowess of the polypurine. For RNases from human spleen or bovine pancreas, poly(A) linked to RNA was a superior inhibitor compared with the same quantity of free homopolymer.

With respect to RNase A, a mechanism more general than that of competitive inhibition was found to apply to inhibitions of its activity caused by either form of poly(A). Our results suggest that use of poly(A) linked to substrate favors an enzyme-inhibitor-substrate complex that turns over to product much slower than if similar quantities of homopolymeric poly(A) are employed.

Publications:

Karpetsky, T.P., Shriver, K. and Levy, C.C.: Poly(adenylic acid) in small amounts, free or covalently linked to substrate, protects RNA from hydrolysis by ribonuclease. Biochem. J. 193, 311-324, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06980-04 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of the Physiological Importance of Reverse Transcriptase-P30 Interaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI
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	C. Levy	Chief	LMB, NCI
	K. Chang	Chief	LCBGY, NCI
	R. Friedman	Chief	LEP, NIAID
	P. Wiernik	Chief	CO, NCI

COOPERATING UNITS (if any)
Dr. D. Fish, Frederick Cancer Research Center;Dr. J. Bookout, Microbiological Associates;J. Eichberg, Southwest Foundation for Research and Education, San Antonio, Texas 78284;Dr. S. Moore and Dr. D. Wang, Rockefeller University

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An ecotropic type C retrovirus designated as D-1 MuLv and a xenotropic virus were detected in lymphoma of SJL/J mice. The ecotropic virus titer was high in mice with adenocarcinomas. The xenotropic virus, on the other hand, can be detected at 6 months of age, not earlier. Cross neutralization and competitive radioimmunoassays indicate that ecotropic virus is closely similar to AKR-type ecotropic virus and the xenotropic virus to Balb-2. Isolation of reverse transcriptase-p30 complex is in process.

An ecotropic type C retrovirus (D1-MuLV) isolated from SJL/J mice. The virus titers in the spleens, lymph nodes, and mammary tumour were high in the mice with adenocarcinoma. These results indicate persistence of high titer of virus associated with RCN and mammary tumour ofSJL/J mice. Xenotropic virus (X-MuLV) was detectable from spleens of normal SJL/J mice at ages 6 and 12 months (60% and 80%, respectively) but not at other ages. The X-MuLV isolated from SJL/J mouse embryo cell cultures treated with IdU (SJL-MEF-X-MuLV) and that isolated from a spontaneous RCN (SJL-RCN-X-MuLV) were compared with NZB-X-MuLV (NZB mouse origin), and AT124-X-MuLV (NIH Swiss mouse origin), in regard to their host range, ion and primer-template preference by reverse transcriptase, viral interference, and neutralization characteristics. Cross-neutralization and competitive radioimmunoassays showed that D1-MuLV is more closely related to AKR-MuLV and the xenotropic virus to Balb-2.

We have shown previously RT-p30 can be isolated from the thymic tissue of AKR female mice at 20 weeks of age i.e. prior to the development of leukemia and our results indicate that RT is of ecotropic origin and p30 xenotropic origin. SJL/J mice develop spontaneous Hodgekin type lymphoma and they also express ecotropic and xenotropic virus long before the development of lymphoma. Therefore, it would be interesting to screen the lymphoma tissue for the RT-p30 complex.

Publications:

Eichberg, J., Kalter, S.S., Heberling, R.L., Lawlor, D.A., Morrison, J.D., Bandyopadhyay, A.K. and Levy, C.C.: In vivo activation of endogeneous virus. Proc. Soc. for Exp. Biol. Med. 166, 271-276, 1981.

Bandyopadhyay, A.K., Wang, D. and Levy, C.C.: Complexing Rauscher leukemia virus reverse transcriptase with polyspermine RNase. Biochem. J., 189, 89-93, 1980.

Bandyopadhyay, A.K., Fish, D.C. and Levy, C.C.: Existence of reverse transcriptase p30 complex in AKR mice after a high incidence of spontaneous lymphocytic leukemia. J. Gen. Virol. 52, 281-293, 1980.

Bandyopadhyay,A.K. and Levy, C.C.: Type-specific interaction between reverse transcriptase and p30. J. Gen. Virol., in press, 1981.

Chang, K.S.S., Hogan, S.A., Bandyopadhyay, A.K., Levy, C.C., Kno, A.F.: Ecotropic and xenotropic type C retroviruses associated with reticulum cell neoplasm. J. Gen. Virol., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06984-04 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Reverse Transcriptase and Viral Protein Interaction in Relation to DNA Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T. Karpetsky	Sr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI
	A. Bandyopadhyay	Sr. Investigator	LMB, NCI
	R. Gallagher	Sr. Investigator	LMB, NCI

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It was shown previously that two core proteins of Rauscher leukemia virus, reverse transcriptase (RT) and p30 interact with each other and form a complex with enhanced DNA synthesis. When, however, RT was interacted with another core protein p12, two molecular size of complexes can be seen: one with high molecular weight and another low molecular weight. High molecular weight RT-p12 does not interact with p30. In contrast, low molecular RT-p12 interacts with p30 and forms a large molecular weight complex RT-p12-p30. Such a complex is capable of increasing the synthesis of DNA with a greater fidelity than RT alone. We now have included p10 in our studies. We hypothesize that low molecular weight RT-p12 may be a building block that, upon binding p30 and p10 forms a large DNA synthesizing apparatus.

We have previously shown that Rauscher leukemia virus reverse transcriptase can specifically bind with its own major internal protein p30 and form a large molecular aggregate. Such a complex enhances DNA synthesis.

P12 can also bind with reverse transcriptase and stimulate DNA synthesis several fold over reverse transcriptase alone. When RT-p12 mixtures were subjected to glycerol gradient centrifugation, it revealed two kinds of RT-p12 complex: one has a molecular weight of 170,000 and the other one 90,000. These two complexes differ on their binding ability with p30. The high molecular weight RT-p12 complex is unable to bind with p30. In contrast, 90,000 mw RT-p12 can easily form a large molecular weight (molecular weight greater than 300,000) complex of RT-p12-p30. This complex of RT-p12-p30 can synthesize DNA several times faster than the reverse transcriptase alone.

When DNA synthesized by the individual complex was compared the best fidelity was seen in DNA synthesized by RT-p12-p30 complex. The lowest fidelity was recorded in DNA synthesized by reverse transcriptase alone. RT-p30 and RT-p12 have better fidelity in DNA synthesis than RT alone, but it was still several fold less than that formed by the RT-p12-p30 complex.

Studies have been extended to p10. We have shown that p10 can stimulate the activity of purified reverse-transcriptase (RT) as a linear function of p10 concentration. However, in the presence of p30 the stimulation is several fold greater than would be expected from a simple addition of the effects of p10 and p30. The ability of p10 to stimulate RT does not appear to be related to p10 ability to bind to nucleic acids. These studies suggest a cooperative interaction between the gag proteins and RT to modulate RT activity. This further argues for a role of the gag proteins in the synthesis of retroviral DNA.

Publications:

Meric, A., Sargent, S., Purtell, M., Bandyopadhyay,A.K., and Levy, C.C.: Effect of virus-specific proteins of Rauscher leukemia virus on reverse transcriptase: Interaction between the different structural forms of p30 and reverse transcriptase. J. Biol. Chem. 255, 8523-8528, 1980

Purtell, M., Meric, A., Bandyopadhyay, A.K. and Levy, C.C.: Multiprotein complexes of Rauscher leukemia virus. J. Biol. Chem., in press, 1981.

Purtell, M., Meric, A. and Levy, C.C.: Effects of Rauscher leukemia virus p10 on the polymerase activity of reverse transcriptase. Biochem. and Biophys. Res. Comm., 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09101-03 LMB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of DNases in Normal and Leukemic Leukocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Brown	Staff Fellow	LMB, NCI
Other:	T. Karpetsky	Sr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI
	P. Wiernik	Chief	CO, NCI

COOPERATING UNITS (if any)

Clinical Oncology Branch, BCRP

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NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polyacrylamide gels containing DNA and RNA are used to resolve, visualize and characterize individual DNase and RNase activities derived from peripheral leukocyte subpopulations. Electrophoretic conditions are chosen to allow the free migration of the nuclease activities in the absence of protein denaturants and in the absence of substrate hydrolysis. Following electrophoresis, incubation under appropriate conditions and staining for intact substrate reveal the presence of the enzymes. Donors with CML appear to express greater overall activity and several new activities as compared to normal donors. Fergusson plot analysis reveals that the highly augmented activities in some samples from donors with CML probably all belong to a charge train of one polypeptide species. Donors with AML fall into 2 main groups: those with essentially normal nuclease expression, and those with new activities. The degree of abnormality in this latter group often correlates inversely with prognosis.

The DNases and RNases within mammalian cells have been very poorly characterized. A number of studies have attempted to use RNases as cancer markers. It has been reported that there is an alkaline DNase present in normal lymphocytes that is not present in lymphocytes of disease patients.

We are investigating the nucleases present in leukocytes from normal individuals and from donors with a variety of leukemias to determine whether these intracellular enzymes can serve as disease markers. Leukocytes are used as study tissue because they are easily obtained, highly purified in large amounts, and fractionated into subpopulations. We have prepared extracts from various cellular fractions. We are using these extracts and one and two polynucleotide-polyacrylamide activity gels (incorporating a wide variety of substrates) to determine the number of DNases present, intracellular activity and substrate specificity of the individual nucleases, cellular localizations, cofactors or modifiers involved, and whether any alteraction in the DNases occurs as a consequence of leukemia and consequent treatment. We are following a number of patients before therapy, during therapy, and during remission, relapse and retreatment.

Publications:

Karpetsky, T.P., Brown, G.E., McFarland, E., Rahman, A., Rictor, K., Roth, W., Haroth, M.S., Ansher, A., Duffey, P. and Levy, C.C.: The detection of human intracellular deoxyribonuclease activities using one dimensional polynucleotide-polyacrylamide gel electrophoresis. Electrophoresis, in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09125-02 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Interferon Mechanism of Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Brown	Staff Fellow	LMB, NCI
Other:	C.C. Levy	Chief	LMB, NCI
	R. Leavitt	Sr. Investigator	COB, NCI
	P. Wiernik	Chief	COB, NCI

COOPERATING UNITS (if any)
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NCI, NIH, Baltimore, Maryland 21201

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0.5	0.25	0.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of interferon on DNase and RNase expression in Friend erythroleukemia cells and in other cells in tissue culture is being investigated. Interferon causes equal 2-4 fold increases in 5 major intracellular DNase activities. The pattern of expression in these cells in the presence or absence of interferon closely resembles the DNase expression in peripheral leukocytes from human donors with CML. Induction of differentiation in Friend cells results in reversion of the "abnormal DNase pattern" to a pattern that closely resembles that observed in normal mouse or human peripheral leukocytes. Initial experiments indicate that DNase and RNase expression may serve as a marker for the differentiation status of these cells. Preliminary results suggest that changes in DNases may indicate whether or not the induction of differentiation affects the interferon induced state in these cells, whether interferon affects the DMSO induced differentiation of these cells and whether the DNases are involved in the process of differentiation and in the antiviral state. Studies on the effects of interferon therapy for malignancy in humans on nuclease expression in leukocytes are underway in collaboration with the Clinical Oncology Branch.

Interferon inhibits the replication of both RNA and DNA viruses. At least part of the inhibition may be due to the induction of an endoribonuclease which requires ATP and double-stranded RNA for activation and which uses single-stranded RNA as the substrate. We are extending these initial observations. Our 1 and 2 polynucleotide-polyacrylamide activity gel assay systems allow the easy detection and quantitation of individual nuclease activities and requires small amounts of starting materials.

We are preparing extracts from cells exposed to varying amounts of interferon for varying times. We are using cells that either can not be induced to differentiate, e.g., HeLa, or can be induced to differentiate, e.g., HL 60 and Friend erythroleukemia cells. Aliquots of these extracts (10^4 to 10^5 cells per gel) are electrophoresed into the gels either directly or following incubation with potential nuclease activating or inactivating agents. Each extract is put onto a number of gels containing a variety of substrates. Following incubation and staining, the gels are scanned with a dual beam microdensitometer to quantitate nuclease expression.

The effect of induction of differentiation upon the interferon induced changes in nuclease expression (and visa versa) are being investigated. The kinetics of the interferon induced antiviral state are being compared with the kinetics of changes in nuclease expression.

Publications:

Brown, G.E., Simon, E.H., Chung, C. Interferon Production by individual L cells. J. Gen. Virol. 47, 171-182, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09128-02 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Synthesis of Infectious RLV DNA From Purified Viral Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Purtell	Sr. Investigator	LMB, NCI
Other:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI
	A. Meric	Jr. Investigator	LMB, NCI
	P. Keiser	Jr. Investigator	LMB, NCI
	A. Andriuk	Jr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although in the mammalian virion reverse transcriptase (RT) can replicate the genome, after purification from the viral core or gag proteins it loses this ability. This suggests that the gag proteins are important in insuring proper functioning of RT. Therefore, we pursued the hypothesis that in the intact virion there exists a DNA synthesizing apparatus consisting of RT and gag proteins organized in a specific manner. We have succeeded in uncovering certain gag protein-RT interactions utilizing both purified proteins and virion cores from Rauscher leukemia virus (RLV) and hope that soon we may be able to synthesize full length DNA from highly purified viral components.

We have previously shown that both p12 and p30 can interact with reverse-transcriptase (RT) to form high molecular weight complexes of DNA which have increased polymerase activity and produce a DNA product of greater fidelity to the RNA template than does purified RT alone. We now have observed that p30 exists as two separate species of 30,000 and 60,000 daltons. Although both species can bind to RT, only the heavier one can stimulate RT activity. An ATPase is tightly associated with this heavier fraction. The relationship between the associated ATPase and the ability of only the 60,000 dalton p30 fraction to modulate the activity of RT is being pursued.

We have also previously described RT-p12-p30 complexes which can be extracted from Rauscher leukemia viral particles. These also have an associated ATPase, suggesting further that this ATPase may be important for the functioning of RT.

The polymerase activity of purified RT and of RT gag protein complexes formed either in vitro or extracted from viral particles was studied further using globin messenger RNA and Rauscher leukemia viral RNA as templates. All complexes and purified reverse-transcriptase fully replicated the globin template. Similiarly they all produced DNA product of approximately 3000 nucleotides from the viral RNA. This suggests that although the gag protein may be important for the functioning of RT, their presence is not sufficient to allow RT to fully replicate the genomic viral RNA.

Publications:

Meric, A., Purtell, M., Bandyopadhyay, A.K. and Levy, C.C.: Analysis of DNA strands synthesized in vitro by Rauscher leukemia viral reverse-transcriptase in the presence of core proteins p12 and p30. J. Virol., in press, 1981

Meric, A., Purtell, M., Bandyopadhyay, A.K. and Levy, C.C. Characterization of an ATPase activity associated with p30 from Rauscher leukemia virus: A possible role in DNA replication. J. Biol. Chem., in press, 1981.

Purtell, M., Meric, A., Bandyopadhyay, A.K. and Levy, C.C.: DNA synthesis by a multi-protein complex from Rauscher leukemia virus. J. Biol. Chem., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09129-02 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Nucleases as Differentiation Markers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Brown	Staff Fellow	LMB, NCI
Other:	T. Karpetsky	Sr. Investigator	LMB, NCI
	R. Gallagher	Sr. Investigator	LMB, NCI
	R. Leavitt	Sr. Investigator	COB, NCI

COOPERATING UNITS (if any)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

HL 60 cells are being treated with agents that induce differentiation towards either granulocyte or monocytic populations. The uninduced and induced population are fractionated using a one gravity cell separator (LACS) into subpopulations of narrow maturity ranges. The more mature the cells, the more their nuclease patterns resemble those of normal mature granulocytes. Mouse peritoneal macrophages from a variety of mouse strains are being elicited by a variety of agents and activated by BCG. Upon treatment, nuclease activity is greatly augmented, and there is a change in enzyme content indicative of low molecular weight charge modifications. These changes parallel the elicited and activated statue of these cells as measured by traditional methods. Currently, this study is being extended to other rodent groups to determine the universitality of our findings and to allow the investigation of macrophages from other body sites.

This study is designed to biochemically characterize macrophage and HL60 nucleases, test the hypothesis that nuclease activities may be markers of differentiation and to investigate the role of nucleases in the process of differentiation. HL60 cells and mouse peritoneal macrophages are being stimulated to differentiate by a variety of agents. In some cases the differentiation process is being perturbed by interferon. The intracellular nuclease activities will be correlated with the state of differentiation and with the treatment.

HL60 cells exhibit many of the properties of the leukemia cells (APGL) from which they were derived. These cells exhibit DNase activities not found in cells from normal donors. These extra activities resemble those found in leukocytes obtained from CML patients.

Mouse peritoneal macrophages exhibit a complicated pattern of nuclease activities. This pattern does not resemble those expressed by mouse peripheral blood leukocytes. Nuclease activities increase quantitatively and qualitatively upon peritoneal injection of substances known to induce elicitation or activation of macrophages. Macrophages from other body sites, i.e. alveolar macrophages and Kupfer cells, are also obtainable. Many highly inbred mouse strains, some with genetic defects in macrophage function, are under examination.

Cell subpopulations are isolated by density gradient centrifugation, by 1 gravity sedimentation into ficoll gradients, and by fluorscence and scatter activated cell sorting. Cell free extracts from whole cells and from subcellular fractions are assayed using 1 and 2 dimensional polynucleotide-polyacrylamide gel electrophoresis systems. The enzymes are being characterized as to temperature, pH and ionic strength optima, buffer requirements, size and charge and post translational modification.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09133-02 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Herpes Simplex Viruses in Malignant Transformation of Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Biswal	Sr. Investigator	LMB, NCI
Other:	C.C. Levy	Chief	LMB, NCI
	D. Ross	Clinical Associate	CO, NCI
	S. Akman	Clinical Associate	CO, NCI

COOPERATING UNITS (if any)
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NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.2	0.9

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the molecular mechanism by which Herpes viruses transform normal human cells to their malignant state we have attempted to identify and characterize the viral gene sequences in human lymphoblastoid cell lines and in leukocytes from patients with leukemia and lymphomas. We have cloned specific viral DNA sequences in E. coli and have used these DNA sequences as probes to search for complementary DNA in various lymphoblastoid cells. A cloned DNA sequence encompassing the viral thymidine kinase gene appears to be present in certain lymphoblastoid cells. Current experiments are in progress to transcribe and translate the cloned viral DNA in vitro, characterize the protein products and analyze if these proteins are present in lymphoblastoid cells and related tissues from patients with various leukemias and lymphomas. We have also discovered a new topoisomerase that copurified with the DNA polymerase induced by herpes simplex virus. The role of the viral topoisomerase to catenate and decatenate the replicative intermediates of the viral DNA is now being analyzed.

We continue to study the molecular biology of the herpes simplex virus (HSV) as the model system to understand the mechanism by which a normal eucaryotic cell becomes a potentially malignant one. These studies can be divided into two parts. The first part deals with the identification and characterization of viral gene sequences in human blastoid cells by using recombinant DNA technology and the second part of the project involves the analysis of the role of DNA polymerases and topoisomerases in the replication of the complex viral DNA.

1. Identification and characterization of HSV-DNA sequences in human blastoid cell line (K562) and in leucocytes from patients with leukemia.

The K562 cell line is a highly differentiated and transformed cell line that may contain DNA sequences similar to that of the thymidine kinase (TK) gene of the herpes viruses. To investigate the extent of the viral DNA sequences present in these cells and the role of such DNA sequences in cell transformation and differentiation we have conducted the following experiments.

(a) TK gene of the virus was amplified by cloning a endo R. Bam Hl DNA fragment of the virus in E. coli using the plasmid pBR 322 as vector. The viral DNA sequence was nick translated and was used as a probe to search for complementary DNA sequences in various clones of K562 as well as in the DNAs from leucocytes of patients with leukemia and lymphomas.

(b) Other viral DNA fragments adjacent to the viral TK gene are now being cloned to investigate if any viral DNA sequences are present in the K562 cells.

(c) Current efforts are directed to

(1) isolate the cellular DNA sequences complementary to the viral DNA sequences

(2) amplify the cellular DNA in E. coli and

(3) determine the protein(s) the cellular DNA sequences can specify so that they can be used as diagnostic reagents in the clinics.

2. Discovery of a new topoisomerase associated with HSV-1 induced DNA polymerase.

A new DNA relaxing enzyme that copurified with the DNA polymerase induced by herpes simplex virus throughout a multi-purification scheme has been isolated and characterized. DNA-relaxing enzymes belong to a class of enzymes known as DNA topoisomerases which change the topology of a DNA without changing its primary and secondary structures.

The viral DNA topoisomerase had the same sedimentation coefficient as the viral DNA polymerase, required high salt, Mg^{++} and rATP for optimal activity

and was inhibited by antisera against virus infected cells. Current efforts are aimed at analysis of the functions of the viral DNA polymerase and topoisomerase in the replication of the viral DNA.

Publications:

Biswal, N., Feldan, P. and Levy, C. A topoisomerase activity associated with the DNA polymerase induced by herpes simplex virus. Nature, in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09147-01 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Polyamine Effects on Ribonuclease Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Sr. Investigator	LMB, NCI
Other:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Certain polyamines, or compounds containing polyamine substructures, mediate reversal of poly(A) induced inhibition of RNase activity. Effective compounds contain three amino groups, at least two of which are charged and are separated from the others by no less than three carbon atoms. Spermidine and 9-aminoacridines, which contain substituted propyl- or butyl-amino moieties at the 9-amino position and which bear two positive charges per molecule, are efficacious at low concentrations (5 μM). a decrease in effectiveness is associated with the removal of one aromatic ring from the 9-aminoacridines. However, the resulting 4-aminoquinolines, unlike the acridines, do not inhibit enzyme activity when present in concentrations above 30 μM. Relocating the diamino side chain from the 4- to the 8-position of the quinoline nucleus causes a decrease in charge density to +1, with the result that such compounds are ineffective. The identity of the most effective polyamines depends on the RNase studied. The combination of variable 3'-terminal poly(A) segment length and polyamine identity and concentration constitutes a system by which RNase activities, and therefore, substrate-degradation rates, may be easily varied.

Certain general principles may be stated of the type of molecular structure a polyamine must possess to mediate the reversal of poly(A) induced inhibition of RNase activity.

(1) An increase in the molecular charge density from +3 to +4, by adding a propylamino moiety to spermidine, for example, does not alter the efficacy.

(2) A decrease in the number of amino groups per molecule from three (spermidine) to two (putrescine) is associated with a decrease in efficacy.

(3) The distance between positively charged amino groups appears to be important, since decreasing the number of methylene groups separating the amino groups below three results in compounds that are ineffective.

(4) Polyamine efficency is lowered, not abolished, on methylation of the amino segments of aliphatic polyamines, whereas conversion of these amino groups into guanidino moieties improves activity.

(5) Polyamine flexibility can be eliminated by incorporating a polyamine substructure within polynuclear heterocycles. Ethidium bromide and proflavine generally had low effectiveness in our test system, and at high concentrations inhibited RNase activity.

(6) One consequence of the aromatization of the carbon adjacent to the $-NH_2$ moiety is an alteration in the pK_a of the amino group, thus changing the charge density of the entire molecule. By selective positioning of an aromatic nitrogen atom relative to an amino side chain, pK_a values of more than 9.0 may be obtained for two of the amino groups. Compounds of this type, such as the 9-aminoacridines, caused partial or complete reversal of RNase inhibition and these analogues were generally superior in this regard to spermidine. The flexible side chain of atabrine may be shortened, and the substituents on the methylene groups or terminal amino groups changed to yield azacrine or acranil, with little change in effectiveness.

(7) 4-Aminoquinolines, such as chloroquine and plaquenil, must be used in higher concentrations than the corresponding 9-aminoacridines to reverse inhibition. An advantage is associated with the quinolines, however, in that unlike the acridines, these compounds are not inhibitory at concentrations exceeding 50 μM.

(8) The introduction of an aromatic residue into the flexible side chain of plaquenil to yield amodiaquin did not increase the effectiveness of inhibition reversal.

(9) A decrease in charge density below +2, which occurs on repositioning the amino side chain on the quinoline nucleus, may result in a decrease in effectiveness. Although the 9-aminoacridines and the 4-aminoquinolines carry two positive charges per molecule at neutral pH, the 8-aminoquinolines are charged only at the terminal amino group of the side chain. This decrease in charge density is probably responsible for the extremely low

efficacy of primaquine and related compounds.

(10) The simple presence of a planar aromatic structure capable of intercalation, as found in ethidium bromide or proflavine, is not a sufficient condition for high order of effectiveness.

These generalizations suggest that, for individual RNases, polyamine structural specificity exists for the reversal of poly(A)-induced inhibition. However, this specificity may change with the identity of the RNase investigated, a situation analogous to the one in which bivalent cations are used to overcome inhibition. For example, RNases from human spleen or Enterobacter require polyamines having different molecular structures compared with enzymes from bovine pancreas, human spleen or Citrobacter. Considering the latter three RNases, polyamines such as spermidine or the 9-aminoacridines were superior to the 4-aminoquinolines with respect to restoring inhibited enzyme activity. These 4-aminoquinolines, in turn, were more active than either proflavine or the 8-aminoquinoline, primaquine. On the other hand, with the human spleen enzyme, the 4-aminoquinolines and spermidine are less effective than either proflavine or primaquine. Furthermore, the use of Enterobacter RNase gave rise to a unique order of polyamine efficacy in that amodiaquin was superior to all other analogues tested, and the use of plaquenil resulted in a higher enzyme activity than that obtained with the 9-aminoacridine, acranil.

Our results suggest that, for some enzymes, the effectiveness of the polyamines cannot be explained solely on the basis of binding to polynucleotide, and that other interactions, such as the one between polyamine and RNase, may be important in regulating poly(A)-induced inhibition of RNase activity.

Publications

Karpetsky, T.P., Shriver, K., and Levy, C.C. The effect of polyamines on the poly(adenylic acid)-induced inhibition of ribonuclease activity. Biochem. J. 193: 325-337, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09148-01 LMB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Nucleases and Two-Dimensional Polynucleotide-polyacrylamide Gel Electrophoresis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Karpetsky	Sr. Investigator	LMB, NCI
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	P. Wiernik	Chief	CO, NCI
	G. Brown	Sr. Investigator	LMB, NCI

COOPERATING UNITS (if any)

Clinical Oncology Branch, BCRP

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NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Individual nuclease activities from peripheral leukocyte subpopulations are visualized using two-dimensional gel electrophoresis with a polynucleotide in the second dimension. Proteins in cell-free extracts are separated by isoelectric focusing in a disc gel. To further resolve individual enzyme activities, a slab gel containing polynucleotide is cast on the side of the disc gel. Electrophoretic conditions for the second dimension minimize interaction between the nucleases and the substrate. No denaturing agents such as urea or SDS are necessary in either dimension. After electrophoresis, the presence of nucleases is revealed by incubation under conditions that allow substrate hydrolysis, followed by staining for polynucleotides. This procedure allows the simultaneous detection and analysis of a variety of nucleases without the necessity of multiple electrophoresis systems. Cell subpopulations from donors with chronic myelogenous leukemia exhibit differences in nuclease activities compared with those detected in equivalent cell subpopulations from normal donors. One-dimensional polynucleotide-polyacrylamide gel electrophoresis was used to examine the charge and size characteristics of the nucleases. At least some of the activities result from increased charge heterogeneity of a more limited family of nucleases.

Samples from human and animal sources were examined for multiple nuclease activities using polynucleotide-polyacrylamide gel electrophoresis. Narrow gels (2 x 90mm) allowed the routine detection of multiple nucleases from the unfractionated intracellular contents of 3×10^4 to 10^6 mouse macrophages or human lymphocytes and granulocytes. Gels (4 to 9%T, 2.5%C) were prepared containing 0.03% calf thymus DNA. Polynucleotide was not present in the stacking segment. Electrophoresis was accomplished using conditions under which the migrating nucleases did not hydrolyze the substrate. Subsequently, the gels were incubated in buffer solutions designed to activate nuclease activity and, finally, the unhydrolyzed DNA within the gels was stained with Pyronin Y. The resulting reddish-purple gels had narrow clear regions that corresponded to nucleolytic activities. It is important to note that no denaturing agents such as urea or SDS were necessary for the visualization of nucleases in the presence of other cellular proteins. Results obtained using polynucleotide-polyacrylamide gel electrophoresis are superior to those determined using classical techniques that quantitate enzyme activity in that the substrate-in-gel procedure allows the simultaneous detection and analysis of many nucleases using such crude samples as serum or supernatants from sonicated cell preparations. For example, incubation of gels in buffers of different identity, pH, or ionic strength enables simultaneous profiles of multiple enzyme activities to be obtained from these samples. Individual activities were cut as thin segments from one gel prior to incubation, placed atop other gels and rerun to determine whether or not multiple banding patterns arose from one activity via equilibrium among polymeric forms. Estimates of differences among nuclease ionic charges and molecular weights were obtained by plotting the logarithm of individual nuclease R_fs as a function of acrylamide percentage using gels containing a fixed quantity of DNA. Nuclease isoelectric points were determined using a two-dimensional procedure. After isoelectric focusing in a disc gel, a slab containing DNA was cast on the side of the disc. Subsequent electrophoresis, incubation and staining revealed the positions of nucleases in the slab and pIs were directly measured from the known pH gradient in the disc. Results obtained from the above techiques indicated that the DNases present in normal and thioglycolate induced mouse macrophages were distinct. Also, more than 15 nucleases were resolved from human cells and microheterogeneity of charged enzymes was found.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09149-01 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Drug-resistant, variants of cultured human myelogenous leukemia cells (HL-60).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Gallagher	Sr. Investigator	LMB, NCI
Other:	S. Fischkoff	Clinical Associate	LMB, NCI
	N. Biswal	Sr. Investigator	LMB, NCI
	R. Feldsted	Sr. Investigator	LCB, NCI
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COOPERATING UNITS (if any)
Laboratory of Clinical Biochemistry BCRP

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to explore the linkage between the genetic disturbance and phenotypic expression of human myelogenous leukemia, variant cultures of HL-60 cells have been developed and cloned. These variants were chosen on one or more of the following bases: (1) selectability in tissue culture for utilization in cloning and somatic cell hybrid techniques, (2) possible association with specific chromosomal gene loci which might be quantitatively measurable, and (3) probable effect on specific differentiation and/or replicative properties of the leukemic cells. Variants resistant to 5-bromo-2-deoxyuridine, 6-thioguanine, dimethylsulfoxide, retinoic acid and oubain have been developed. Measurable differences have been demonstrated in growth characteristics in suspension and viscous culture media, in responsiveness to specific differentiation inducers, in enzymatic composition in cytogenetic constitution, and in surface membrane biochemistry.

Control, early passage (< p20) HL-60 cells are exposed to various types of drugs in doses less than required to maximally induce differentiation or to produce high cytotoxic kill rates in a single exposure. Drug-resistant variants are then developed by gradually increasing the drug concentration to that which would produce cell death and/or differentiation in previously-unexposed, control cells. This method contrasts to that normally used to develop drug-resistant mutants due to simple DNA base mutations and was chosen because in other model systems this approach has been demonstrated to produce more dramatic genetic disturbances, e.g., gene amplification. Liquid nitrogen reference freezes at various points in the development of the variants are carefully preserved. In the presence and absence of various inducers, the variants are analyzed for growth rates in liquid culture media (both serum-containing and serum-free) and for cloning efficiency in semi-solid media (1% methyl cellulose and 0.4% agarose). Variant and control cells are tested in liquid and semi-solid medium assay for the secretion of and response to possible effector molecules (stimulators or inhibitors) produced by control and variant-cells. In specific instances, the variants are assayed for relavant enzyme activities, e.g., thymidine kinase, hypoxantine-guanine phosphorilbosyl transferase, membrane-bound Na^{+}-K^{+} ATPase. Cytogenetic studies by standard giemsa and by special quinacrine and R-banding techniques are performed to determine if there have been cytogenetic alterations, particularly in chromosomes which might be associated with genes affecting specific enzyme activities and granulocyte differentiation. Cell surface membrane characteristics are being determined by both endogenous and exogenous radiolabelling, followed by chromotographic or electrophoretic procedures, by measurement of binding of specific ligands, e.g., retinoic acid, chemotactic tripeptide, etc., by binding of selected monoclonal antibodies, and by detection of selected complement receptors. In ancillary studies, specific nuclease and histone protease activities are being measured as a function of cell growth and differentiation. In addition, experiments are in progress to determine if specific drug-resistant or altered differentiative properties can be transferred by DNA or gene transfer techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09151-01 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Importance of Phospholipid Metabolism in the Differentiation of HL-60 Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Fischkoff	Clinical Associate	LMB, NCI
Other:	R. Gallagher	Sr. Investigator	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phospholipids can be shown to undergo marked shifts in composition during the process of myeloid differentiation. It is currently unknown if this represents an epiphenomenon or is of crucial mechanistic importance. The promyelocytic leukemia line, HL-60 is being used to study this question. We have utilized a range of predicted lipid intermediates and antimetabolities to dissect the contribution of lipids to the complex set of biochemical changes that occur with differentiation, particularly the role of phospholipid methylation and arachidonic acid release.

Previous efforts to define the role of phospholipid changes in the process of differentiation have been purely descriptive. In order to attempt mechanistic studies, a system was developed which permits a selective alteration of membrane phospholipids. There is a high level of probability that all other observed biochemical changes will be secondary. Initially HL-60 promy-elocytic leukemia cells were established in a completely lipid-free medium containing no serum or lipid supplements. The inducibility kinetics and markers of differentiation are no different in this medium than in more traditional serum containing preparations. We were then confident that serum lipoproteins will not interact with our cells. It was then possible to set up a series of experiments to define completely the synthetic arm of phospholipid metabolism. We chose to measure the incorporation of radiolabelled methionine, choline, ethanolamine, and glycerol into membrane lipids. Also, by using a standard 2 dimensional thin-layer chromatography system, individual classes of phospholipids may be quantitated. This technique also allows localization of incorporated labelled arachidonic acid by a similar technique followed by autoradiography. As a large number of seemingly unrelated compounds are all able to cause differentiation of HL-60 cells, we hope to establish common mechanisms by this type of analysis. As a second approach, we have begun to screen a number of drugs such as biotin and choline analogs, which have a relatively selective effect on lipid metabolism, for their ability to alter the differentiative properties of the HL-60 cells as measured by standard histochemical techniques.