THE CANCER LETTER

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ACS PLANS TO AWARD 10 NEW INSTITUTIONAL GRANTS FOR UP TO $200,000 A YEAR IN CAUSE AND PREVENTION

The new special institutional grants announced last week by the American Cancer Society eventually may support long term programs at 10 institutions with up to $200,000 a year each for research on cancer cause and prevention. Funds for the new grants will come from the ACS special donors program and will not be taken from the $44 million traditional investigator initiated grants program.

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In Brief

GUSBERG HEADS ACS, WITH SCANLON AS PRESIDENT ELECT; MDA HONORS HOLLAND, D'ANGIO, LANDING

SAUL GUSBERG, chairman of the department of obstetrics and gynecology at Mount Sinai School of Medicine, was installed as president of the American Cancer Society at the organization's annual meeting last week. He succeeds LaSalle Lefall. **EDWARD SCANLON**, director of surgical oncology at Northwestern Univ., was elected vice president and president-elect. **ENID HAUPT**, New York philanthropist; **SIDNEY WEINHOUSE**, professor emeritus of biochemistry at Temple Univ. and editor of *Cancer Research;* and **WILLET WHITMORE**, chief of the urologic cancer service at Memorial Sloan-Kettering received the Society's highest honor, the Annual National Award. . . . **HONORED** at M.D. Anderson's annual clinical conference: **JAMES HOLLAND**, chairman of the department of neoplastic diseases at Mount Sinai, who received the fourth annual Jeffrey A. Gottlieb Memorial Award; **GIULIO D'ANGIO**, director of the Children's Cancer Research Center of Philadelphia, who received the 14th annual Heath Memorial Award; and **BENJAMIN LANDING**, director of laboratories at Children's Hospital of Los Angeles, who received the Joanne Vandenberg Hill Award and delivered the William O. Russell Lectureship in Anatomical Pathology. . . . **"FROM BOTH** Ends of the Stethoscope" is a moving interview with David Peters, La Jolla physician, which was videotaped a few months before his death from cancer. Conducted by John Trombold, director of the Scripps Memorial Hospital Cancer Center, the interview offers advice both to cancer patients and their physicians. The tape is available from Cancer Center Films, 4141 Fairmount Ave., San Diego 92105, $325 to purchase, $50 to rent. . . . **"CLINICAL CYTOPATHOLOGY,"** a postgraduate course for pathologists, will be held at Johns Hopkins Hospital April 14-25. The course will include new techniques, special problems and recent applications in clinical cytopathology. Applications must be made before March 7. Contact John Frost, 610 Pathology Bldg., Johns Hopkins Hospital, Baltimore 21205. . . . **CHARLES LEMAISTRE**, president of the Univ. of Texas System Cancer Center, has been elected to a one year term as president of the Damon Runyon Walter Winchell Cancer Fund.

MOUNT SINAI GETS FIRST GRANT IN NEW ACS SPECIAL INSTITUTIONAL PROGRAM

(Continued from page 1)

The first award under the new program will be to Mount Sinai School of Medicine of the City Univ. of New York, with Irving Selikoff as the principal investigator.

ACS said the new program "will be unique in the field of cancer research in providing substantial, flexible and relatively long term support. It is planned that institutions which are recipients of these grants will be funded for periods up to five years, renewable for possible five year increments, with budgets of not more than $200,000 each year. Grant applications will be reviewed by an appropriate ad hoc special institutional grants scientific advisory committee.

The Mount Sinai grant will support establishment of an information unit to obtain and analyze information in response to questions from the public and Congress about agents in the environment suspected of causing cancer. The unit will be designed to shorten the time period of information gathering by collecting and integrating information already available among scientists and instutions worldwide, ACS said, thus making possible expert, critical evaluation of existing scientific evidence on environmental carcinogenesis in one, integrated systematic coordinated effort.

"Where existing information is found to be lacking, unsound, conflicting or difficult to interpret, epidemiological and other research will be initiated with emphasis on research on human cancer," ACS said. "For example, new studies will be launched on large industrial populations which were exposed to a suspect carcinogenic substance and compared with the general population to get a measure of the occupational risk compared with the general risk of cancer.

"Using computer and other communication techniques, the information unit will furnish the Society with prompt evaluations of potential environmental risks, both as needed and in anticipation of their emergence as public issues. These assessments will aid the Society in setting policy positions about potentially hazardous environmental agents. The information unit will not provide information directly to the public but rather to the Society's national staff and the Public Issues Committee of the Board of Directors."

ACS in 1971 began its support of the Environmental Cancer Research Project, a three way collaborative intramural research venture involving E. Cuyler Hammond and his staff at ACS, Selikoff and staff at Mount Sinai and Oscar Auerbach and staff at the Veterans Administration Hospital in East Orange, N.J. This collaborative research effort will continue but will become incorporated administratively into the special institutional grant award at Mount Sinai.

Hammond, who recently retired as ACS vice president for epidemiology and statistics, will join the faculty of Mount Sinai as director of an epidemiology educational program and will continue as a consultant with ACS. Lawrence Garfinkel has assumed the position of vice president for epidemiology and statistics.

There are three major components to the Mount Sinai special institutional grant—the information unit, the Hammond faculty appointment, and the three way collaborative Environmental Cancer Research Project. The current annual budget will be about $540,000, of which about $190,000 will be from the special donors fund, contributed "generously and perceptively," ACS said, by the ACS Illinois Div. for the first two years of the project. Support of the Environmental Cancer Research Project already comes from regular ACS research funds.

Frank Rauscher, ACS senior vice president for research, told ***The Cancer Letter*** that ACS hoped to award additional special institutional grants for research in nutrition and its relationship to cancer; psychosocial aspects of cancer incidence and morbidity; chemoprevention; and questions relating to minority groups and cancer.

"There are many major leads on diet and cancer that need following up," Rauscher said. "There is evidence that patients with a positive approach to cancer treatment do better. We should find out, first, if that is the case, and if so, how can we exploit positive attitudes. What is it about lifestyles that will prevent cancer? How can we modify behavior to prevent cancer? The entire area of chemoprevention is evolving fast. What can we do more than we are now about cancer in minority groups? Including training more black and chicano epidemiologists?"

There will be four or five more awards, in addition to Mount Sinai's and those suggested by Rauscher, if investigators can put together creative proposals in important subject areas. ACS offered some suggestions:

- An international agency, a branch of the World Health Organization, has identified 1,000 chemicals that cause cancer in animals and 230 of these are chemicals to which human beings are exposed. There is, thus, no dearth of leads in searching for potential carcinogenic agents in the human environment: these include polycyclic aromatic hydrocarbons, inorganic microparticles, metals, halogenated ethers, radiation, aromatic amines, and halogenated biphenyles.
- In addition to these known carcinogens, there are a large number of known substances which are not carcinogenic themselves but which can become converted to carcinogens by interacting with host factors.
- Important advances in biochemistry, enzymology, molecular biology and genetics in the past 25 years can help us understand how chemicals from the outside can change body metabolism and be changed

by it. What is needed is better scientific and intellectual integration of this information for cancer control in people.

• A general rule is that most carcinogenic substances are also mutagenic, altering permanently the genetic makeup of cells. Therefore, to shorten the time of identifying harmful agents, new methods and techniques using bacterial and other systems should be explored to test the mutagenicity and carcinogenicity of suspected agents.

• In addition, mutagenicity and teratogenicity often go together; that is, birth defects and abnormalities in embryos and fetuses caused by genetic mutations. Examination of birth defect registries may be an important source of information in identifying harmful substances to which fathers might have been exposed in occupational situations, or mothers secondarily exposed.

• Data on long term human exposures are readily at hand from the unfortunate, unknowing experiment that has been taking place, not in laboratory cages, but in the work place for the past 30 or 40 years. These data are available but have to be gathered and analyzed. Identifying agents which are not hazardous may be as important as identifying those which are harmful.

• Often the population of exposed individuals is too small to allow for reliable statistical analysis, especially if long term effects are involved. Thus, industry wide cooperation and the use of union records can provide useful information. These resources are being utilized at present at Mount Sinai and will continue to be by them and others.

Each institution with a cause and prevention grant will be required to retain a cumulative 15% of its funds so that as new agents and problems arise, ACS can request it to initiate studies immediately. Grantees will have a great degree of flexibility in following up leads, Rauscher said.

The ACS special donors program, suggested last year by Rauscher and approved by the Board of Directors, has added a new dimension to the Society's activities.

In addition to investigator initiated grants and professional and public education efforts, supported for the most part through the annual ACS spring fund raising drive, the special donors program enables Rauscher to go after sizeable contributions from foundations, corporations and wealthy individuals for specific projects.

Rauscher has challenged the scientific community, saying in effect: Show me some scientifically sound, good solid proposals, perhaps to fill some unique voids. If it passes review, I'll get the money for it.

"I realize I'm putting myself on the spot," Rauscher said, "but it's worth a try."

The Research Development Program is a successful effort supported by special donors. ACS has awarded 80 grants and averaged 58 days from receipt of application to funding. "The success of this program is due entirely to the people who have agreed to review a grant within four weeks after they are asked," Rauscher said.

Interferon clinical trials is another special donors project. ACS kicked it off with a commitment of $2 million and recently received a legacy from an estate for an additional $1.8 million to be spent on the program. Most of it will be used to buy more interferon.

Rauscher feels there is a variety of opportunities for special donor projects on a geographic basis, with the contributions retained in a state or community for such things as improvements or facilities at centers, epidemiological research related to local cancer incidence, support of outstanding research teams and cancer control programs when their federal dollars dry up, and many others.

The challenge has been issued. Those wishing to take it up may contact Rauscher at ACS, 777 Third Ave., New York 10017.

DCT BOARD SUBCOMMITTEE SUGGESTIONS FOR BRM RESEARCH, PRODUCTION LISTED

Recommendations for a Biological Response Modifiers Program developed by the Mihich subcommittee of the NCI Div. of Cancer Treatment Board of Scientific Counselors (*The Cancer Letter*, Nov. 9) grouped its suggestions into five major areas—interferons, thymosins, chemoprevention, production of tumor necrosis factor, and definition of distinctive cell surface antigens of human cancers and development of immunogenic antigen preparations.

The subcommittee's recommendations for interferons follows (with some editing):

The interferon project should be divided into three major groups—clinical trials and related studies, preclinical research and development, and production.

1. Clinical trials and related studies. Each BRM should be developed clinically through modified phase 1 and then phase 2 trials. Clinical interferon trials carried out to date independent of the DCT BRM Program have been performed with doses and regimens which were selected empirically, based on the availability of the agent. It is therefore important that optimal doses and regimens for interferons be identified through appropriately designed phase 1 trials. As with all other BRM, phase 1 trials with interferon should include not only the tests for toxicity but also tests to monitor modification of biologic responses so that optimal doses may be selected both in terms of toxicity and biologic response modification. Although it is customary for phase 2 trials to follow and be dependent on the results obtained in phase 1 trials, in the case of interferons the phase 2 trials are already being performed independently of this program. It is recommended that phase 1

studies be carried out as soon as possible because the antitumor action of interferon has already been shown to be encouraging even with doses derived empirically, and it seems urgent to define optimal doses for rigorously designed phase 2 trials.

Phase 1 trials. Ideally there should be a minimum of two and a maximum of three trials with each of three interferons which are available, namely human leukocyte interferon (HuLeIF), human lymphoblast interferon (HuLy IF), and human fibroblast interferon (HuFi IF). Each trial should include 50 patients for a total of 100 to 150 patients for each type of IF. Based on current experience with HuLe IF, it is suggested that trials be started at a dose of 3 x 10^6 units per patient per day. Escalation of dose may be rapid, at least for HuLe IF, since based on current experience toxicity is unlikely to be observed at low doses. A total of 100 x 10^6 units is estimated to be required per patient, 5000 x 10^6 units per 50 patient trial, and 15,000 x 10^6 unit total for the three phase 1 trials with each kind of interferon. It must be emphasized that this estimate is reasonable for planning the program but may have to be changed drastically in case unexpectedly high doses turn out to be well tolerated, or unexpectedly prolonged treatments required. Also, it may become necessary to give initially more emphasis to studies with one interferon type, should overall costs become prohibitive.

During the phase 1 trials measurements will be required not only of the toxicity and pharmacology of the agent but also of biologic response modification. The only type of measurement of biologic response modification that appears practical for IF is a measurement of the effects on immune responses and especially on natural killer (NK) activity. Possible age-related differences in effects must also be taken into consideration. Pharmacological studies should evaluate the blood kinetics of IF by appropriate measurements in vitro with bioassays for antiviral and cytostatic effects.

Phase 2 trials. Phase 2 trials with HuLeIF should be ideally carried out in patients with eight types of cancers and in bone marrow transplant recipients. The eight types of malignancies suggested for study include: myeloma, lymphoma, B cell leukemia, malignant melanoma and carcinoma of the breast, colon, lung and ovary. The particular malignancies are selected on the basis of encouraging results already observed and in an attempt to determine whether the rather common carcinomas are likely to respond to interferons. Bone marrow transplantation recipients are also suggested for study on the basis of data which suggest that interferons may have activity against leukemia, against graft versus host disease, and against viruses such as cytomegalovirus–the three principal problems in clinical marrow transplantation.

Each trial should involve 50 patients for a total of 450 patients for each IF type. Since the different types of IF may differ significantly in their activities and their pharmacology, it is necessary to study all three types before a final decision is made to select a particular IF for eventual phase 3 trials. At the current empirical dose of 3 x 10^6 units per patient per day for 84 days, nine phase 2 trials would require 112,500 x 10^6 units. On the basis of the estimate that two phase 2 dose escalation trials would be carried out initially on a total of 100 patients, approximately 33,000 x 10^6 units may be required. Thus a total of approximately 150,000 x 10^6 units of each type of human interferon may be needed for the phase 2 studies proposed. To reduce the cost somewhat it is reasonable not to test all three types of interferons against all nine clinical conditions. Instead, lymphoblastoid IF, which is likely to be identical with HuLeIF should be tested only against multiple myeloma and phase 2 trial with HuFiIF should be deferred because of its high cost and problematic pharmacokinetic properties.

Formulation and quality control development research. This type of research is directly related to the clinical development of IF and should be pursued in order to standardize the various IF preparations at different stages of purification. It is estimated that about 5,000 x 10^6 units may be required for this work.

Initial clinical studies with highly purified IF. It is important that purified preparations of IF be tested clinically as soon as possible in order to determine the therapeutic advantages that they may provide. This is especially important in view of the fact that the clinical antitumor results obtained to date have been obtained with material which is considered to be approximately only 0.1% pure. This requires considerable developmental groundwork as well as scaling up procedures that are under development in pilot research laboratory form. It is estimated that about 50,000 x 10^6 units may be required for research and for selective phase 1 to 2 trials. At the least, the research part should be initiated during the first year of the BRM program.

2. **Preclinical research and development.** Substantial information still needs to be obtained on the basic mechanism of antitumor action of IFs and on ways to improve the therapeutic usages of these agents. This research program should be implemented with support from R01 and P01 grants, and contracts as pertinent.

The mechanisms of inducer cell interactions and of induction of IF need to be clarified. The mechanisms involved in the regulation of IF production–as can now be studied at the transcriptional and translational levels–need to be explored. The phenomenon of superinduction which can help in these studies needs itself to be better understood and if possible exploited in nonfibroblast type cells, since its general exploitation could lead to higher interferon yields from lymphocytes whose interferon is

presently used experimentally in the clinic.

Studies with interferon messenger RNAs, which can be assayed with great sensitivity in biological assays involving their translation in heterologous cells, should be pursued. They may lead to the synthesis of cDNAs for interferons which will allow recombinant DNA technology to be applied towards developing methods for the industrial scale production of interferons in "bacterial factories." This is a challenging area of study and one with potential practical utility.

The complete purification of several interferons has been achieved, although not yet on a preparative scale. To achieve the latter should be an urgent goal, since it would benefit the field in several ways. The preparation of highly specific antibody would itself contribute to efficient affinity chromatography purification methods. Pure mouse interferon preparations and specific antibody would allow studies of the role of the interferon system in animal models and to clarify the part it plays in protection and recovery from diseases, either infectious or neoplastic. Pure human interferon preparations will also allow the complete characterization of the interferon molecules for their physicochemical as well as biological properties in vitro and in vivo. The deglycosylated interferon polypeptide of both the human and mouse species—which is already known to be completely biologically active—can be studied for its amino acid sequence. Or, it can be degraded enzymatically or chemically, in attempts to obtain an "active core." Knowledge of a partial or complete active sequence can also lead to an application of recombinant DNA techniques, through the deduction of which DNA sequence can code for it and the latter's nonenzymatic synthesis for insertion into a plasmid and expression in prokaryotes. Alternatively, chemical synthesis of the interferon polypeptide itself can be attempted, which could also result in low unit cost industrial production. A task of immediate urgency with regard to the above is the production of sufficient amounts of deglycosylated mouse and human interferoid, or active fragments so that their pharmacological properties can be evaluated in vivo.

Various human interferons need to be studied with regard to their possible tissue specificities, knowledge of which would have immediate clinical applicability. The presence of a specific receptor for IF on target cells should be verified by the isolation of such molecules and expanded through a study of its binding characteristics.

The experience at the subcellular, molecular level of the various interferon activities need to be pursued. New gene products have already been identified in interferon treated cells expressing an antiviral state. So far, however, it is still not understood how cells can direct the interferon-induced transcription and translation-inhibitory machinery specifically against virus specified gene products. Such studies must continue since one cannot assume at this time that the antiviral and antitumor effects of interferons are mediated by entirely different mechanisms. Among interferon activities directly observable on cells in vitro, the growth inhibitory effect may be directly related to the antitumor effect observable in vivo. It is still poorly understood, even with regard to the changes it brings about in the cells' growth cycle.

Of particular relevance and interest to the BRMP are the many immunomodulatory effects of interferons, both suppressive or enhancing, depending on the circumstances, of the B and T cell mediated immune response. In addition, enhancing effects on NK cell and macrophage functions have been reported in vitro, in animals and in man as a result of interferon treatment, and within a context that suggests that such effects may be responsible for interferon-induced but host mediated antitumor activity. All the actions of interferons on the immune system need to be studied intensively in vitro and in vivo and understood in relation to an organism's defense against neoplastic disease, so that they may be exploited to imbalance the overall response to therapeutic advantage. The lines of investigation mentioned above, and others that should be pursued as seen by the scientific community at large, may often best be carried out with human IF, but many also have to be carried out with mouse IF in the mouse, in order to develop concepts that may later be verified in humans.

3. **Production.** The large scale production of all three types of interferons has now become feasible. Briefly, production can be implemented as follows: assuming that 2 x 10^6 units of blood could be used annually for HuLeIF production by 20 to 20 production centers, about 500,000 x 10^6 units could be produced if necessary. The production of HuLyIF can be considered virtually unlimited after appropriate scaling up of operations has occurred based as it is on the utilization of continuous lymphoblastoid cell lines in culture. The production of HuFiIF could be scaled up according to need since it is also based on the use of cell culture lines. It was estimated that, based on laboratory scale production experience, HuLeIF could cost $20 per 10^6 unit or less. Costs of the other two types of IF are difficult to estimate at this time due to the need for further procedural development.

Estimated needs. Based on the proposals mentioned above, it is likely that it would be necessary to obtain about 270,000 x 10^6 units of each type of human IF. These would be used roughtly as follows: phase 1, 15,000 x 10^6; phase 2, 150,000 x 10^6; formulations, etc., 5,000 x 10^6; purification and phase 1, 50,000 x 10^6; other research, mainly sequencing, 50,000 x 10^6.

At the hypothetical maximum average cost of $45

per 10^6 units, this would amount to approximately $12,150,000 for each type of interferon. Since it will be impossible to allocate the entire amount of $36,450,000 to the interferon production portion of the BRM program, plans have been scaled down; thus the IF production is now more appropriately related to the other important parts of the BRM program during the initial phases of its development when all the central features of the program must

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Samuel Barron, chairman of the microbiology department at the Univ. of Texas Medical Branch in Galveston, reported at a recent New York Academy of Sciences meeting on interferon that immune interferon in mice and human cells in vitro potentiated may provide a 100 fold increase in antitumor activity over leukocyte inteferon.

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get a solid start. Specifically, it is recommended that IF production be initially geared at the following scaled down plans: a) three phase 1 trials, nine phase 2 trials, formulation, purification and research and phase 1 trials with purified IF would be planned with HuLeIF; b) three phase 1 trials, one phase 2 trial and purification research would be planned with HuLyIF; c) only research would be initially carried out with HuFiIF; d) Hu Type II IF would be produced for initial research; e) mouse IF would be used to develop further studies leading to the clarification of important research questions. Consequently the IF plan outlined below is suggested (in 10^6 units):

Phase 1, 15,000 HuLe, 15,000 HuLy; phase 2, 150,000 HuLe, 15,000 HuLy; formulation, 5,000 HuLe, 500 HuLy; purification and phase 1, 50,000 HuLe; research, 50,000 HuLe, 50,000 HuLy, 25,000 HuFi, 1,000 Hu Type II, 40,000 mouse.

Should HuLe cost $10 per 10^6 units, should HuLy IF be produced at Frederick without charge to the budget of the BRMP, should HuFi IF cost a maximum of $100 per 10^6 units, should Hu Type II IF cost a maximum of $200 per 10^6 units and should mouse IF cost about $30 per 10^6 units, the above IF production proposal would cost about $6.6 million. It is therefore recommended that DCT obtain IF at about these costs if at all possible. Should this not be feasible, the Subcommittee on BRM of the DCT Board would propose to the director of DCT a further scaled down plan. It is in fact essential that funds be available for the clinical trials to be carried out with the IF produced, and that the other aspects for the multifaceted BRM program be started with adequate support at the same time as the IF components.

FURTHER CLINICAL DEVELOPMENT OF CHEMOPREVENTION

The program components suggested for initiation of a treatment effort with retinoids are discussed under the same general headings as the other BRMs but the emphasis and needs for chemoprevention are somewhat unique. The clinical trial components focus on "patients" without manifest disease; the basic research and development aspects must interface with and depend on programs already operative in other NCI divisions, and production of current or future agents is relatively advanced in the private sector and well funded through NCI. The DCT emphasis should be placed on careful interpretation of preventive agents into therapy programs where risk is predictably high. It may be necessary to model treatment combinations before large scale trials can be opened.

1. Clinical trials. Selection of patients for clinical trials with retinoids is a fundamentally unique problem, with criteria different from that used to select patients for therapy trials. Because of the nature of the activity of retinoids, patients suitable for chemoprevention protocols must be free of disease. Response can be measured in terms of time to new lesions, either de novo, recurrent or second primary.

The time periods necessary for assessment of significant delay, as well as for dose intervals, may be very long, presenting additional problems in design of these clinical studies. (It is possible that lifetime studies might be required.) There are high risk patient categories in which trials may be carried out after appropriate phase 1 biological response trials are evaluated.

Phase 1 trials with 13-cis-retinoic acid. Subjects to be included in eventual efficacy trials for prevention will be "precancer" or NED cancer patients. Subjects for the careful toxicity and biological response initial trials should be as biologically normal as possible; patients with multiple senile keratoses, psoriasis or severe acne may be available for detailed evaluation. The initial doses for phase 1 study can be chosen from the limited clinical experience to date. Single administration studies can probably begin at 0.25 mg/kg with dose escalation to tolerance as high as 10 mg/kg. This should be followed by daily dosing, at appropriate doses, probably for 14 days. The biologic followup testing for phase 1 should be continued for at least two weeks.

The phase 1 studies should include frequent assessment for kinetics and quantitative changes in relevant host parameters. These should include hematologic and liver functions, lymphoid functions including T cell activities, and surface properties of lymphocytes (HLA or Iq). Concentrations of drug in blood, urine and biopsy material when available, should also be measured. Specialized assays for T-lymphocyte colonies, colony forming units, skin fibroblast growth characteristics, or rates of PBL mutagenesis (e.g., HGPRT) may also be important.

Since phase 2 studies may include patients who have been or are simultaneously on chemotherapy,

additional phase 1 trials are needed in order to evaluate biological effects of chemopreventives in patients also receiving chemotherapy. It is suggested that retinoids, for example, may affect the T cell suppressive, marrow or epithelial toxicities, or mutagenicity of chemotherapy. These studies should be carried out at doses and schedules defined by prior phase 1 studies.

Phase 2 trials. Certain therapeutic chemopreventive trials have been initiated previously. A study on the use of retinoic acid in recurrent superficial bladder carcinomas and papillomas has been terminated. Of the first group of 17 patients to complete six months therapy, only two were free of disease at the end of the study period, a recurrence rate of 88%, compared to an expected rate of 70%. Seven of the 17 were withdrawn because of unacceptable toxicity, for the most part identifiable as hypervitaminosis A. One patient showed acceleration of disease with extravesicular involvement. Efficacy trials for high risk patient groups should be carried out at an appropriate time, but are not recommended at this time. Such trials would be initiated in high risk persons, either with underlying predisposing disease, with environmental exposures, or with risk due to therapies received. It is essential that preclinical studies can be supported so that therapy interactions, tumor recurrence and reversal of mutagenicity can be modeled.

2. **Preclinical research and development.** The development of new retinoids with improved therapeutic index is of high priority to this program, in particular the development of compounds with well defined organ specificity. Such specificity, as apparently demonstrated for 4-hydroxyphenyl retinamide and the rat mammary gland, may be of great importance in limiting the extent of any drug induced toxicity. Synthesis of retinoids is already well funded under DCCP/NCI contract mechanisms, and research in this area should be conducted in close collaboration with this division. The development of new retinoids to organ site directed clinical trials should be streamlined. The institution of common pathways for evaluation of new retinoids will aid in the rational choice of novel compounds for clinical studies.

Production. Clinical trials: Hoffmann-LaRoche has agreed to supply at no charge sufficient 13-cis-retinoic acid for phase 1 and 2 clinical studies. This is the only retinoid at present that is at the level of clinical studies.

Preclinical studies: There are several current contracts initiated by DCCP/NCI for the synthesis of novel compounds. This phase of the development is well organized. Additional funds will be required for the scale-up synthesis of compounds passing the in vitro screens.

PROPOSAL FOR THE PRODUCTION OF TUMOR NECROSIS FACTOR

The serum of BCG-infected mice treated with endotoxin contains a substance (tumor necrosis factor; TNF) which mimics the tumor-necrotizing action of endotoxin itself. TNF is not residual endotoxin, but a factor released from host cells, probably macrophages. TNF induced in the same way in rats and rabbits also causes necrosis of transplanted murine tumors. Unlike endotoxin, TNF is toxic in vitro for neoplastic murine and human cell lines but not for mouse embryo culture. TNF has striking effects on immunological reactions in vitro, some like those of endotoxin and others unlike those of endotoxin. TNF is a glycoprotein; its molecular weight is less than 70,000. Highly purified preparations do not contain lysosomal or non-lysosomal serum enzymes, interferon or prostaglandin E_1. It is proposed to produce partially purified murine TNF for further study in experimental animals and for initial clinical evaluation.

Production of TNF^+ serum. Mice are injected first with C. parvum and nine or 10 days later with endotoxin. Blood is collected 1½ hours later. Serum is separated by centrifugation, batches of serum are pooled and tested in vivo and in vitro for TNF activity.

Standard in vivo and in vitro assays for TNF.

In vivo TNF assay: (BALB/c x C57B1/6) F1 mice with intradermal seven-day transplants of sarcoma Meth A averaging 7-8 mm in diameter, are injected IV with graded doses of TNF^+ serum or TNF fractions. Twenty-four hours later, the degree of necrosis of the tumor is assessed visually as: grade - = no change; grade + = slight necrosis; grade ++ = moderate necrosis (central necrosis extending over approximately 50% of the tumor surface); grade +++ = extensive necrosis (massive necrosis leaving at most only a small rim of viable tumor tissue).

In vitro TNF assay: TNF sensitive L cells (L-S) and TNF resistant L cells (L-R) grown in culture are exposed to the TNF preparation. The TNF titer refers to the dilution of TNF^+ serum or amount of TNF active protein that results in 50% kill (determined by phase microscopy of L-S cells).

Production of partially purified TNF. Pools of TNF^+ serum are ultracentrifuged, and the top third (lipid-rich layer) containing TNF-inactive protein is discarded. The remaining serum is fractionated and the fractions are assayed for TNF activity (see above) and other properties such as pyrogenicity, enzymatic activity and immunologic activity. Considerable purification has been accomplished in this way.

Initial tests in cancer patients. In a 20 g mouse, a single injection of 100 *ug* of partially purified TNF induces necrosis of a 1 g cutaneous transplant of sarcoma Meth A, given by the intravenous as well as intratumoral route. As the ratio of body weight to tumor weight, and thus the dilution after systemic administration, in a 70 kg patient with a 1 g cutaneous metastasis is 2500 times greater than in the mouse, initial evaluation by direct injection into

cutaneous metastases is proposed. The objective will be to determine whether single or multiple intralesional injections of TNF induce necrosis and regression of injected lesions.

Risks. Mouse serum may contain lymphocytic choriomeningitis virus and Senai virus. In rare instances, these viruses have caused mild self-limited upper respiratory infections in man. Even if the serum contained these viruses at the start, it is highly unlikely that they were not eliminated by the purification procedure which includes ultracentrifugation and chromatography.

Partially purified TNF is not pyrogen-free. As a dose of 100 *ug* will not exceed the pyrogenic equivalent of 0.002 *ug* endotoxin, however, fever is not likely to occur at that dose. Beginning at that starting point, tolerance of higher doses can be safely determined.

Estimated need. For more extensive therapeutic tests in the mouse, for studies of mechanism of action, and for initial clinical evaluation, 5 g of partially purified TNF are required as a first step.

Estimated cost of production. The cost of technical labor, supplies and animal maintenance for producing 5g of partially purified TNF is estimated at $170,000. The cost of equipment depends on what is already available in the production laboratory. The cost of professional supervision, again dependent on the circumstances of the production laboratory, has to be added.

The subcommittee's recommendations for research with thymosins, augmenting agents and cell surface antigens will appear in subsequent issues of ***The Cancer Letter.***

NCI CONTRACT AWARDS

Title: In vivo screening program, five year contract
Contractor: Battelle Memorial Institute, Columbus, $4,988,245.

Title: Cancer Immunotherapy: Animal models for treatment of minimal residual systemic tumor
Contractor: Pennsylvania State Univ., $231,492.

Title: Production of aged rats, modification
Contractor: Harlan Industries, Indianapolis, $237,403.

Title: Short training course for biosafety officers on the practices of the control of biohazards in the research laboratory
Contractor: Johns Hopkins Univ., $230,815.

Title: Maintain an animal holding facility and provide attendant research services
Contractor: Cor Bel Laboratories, Rockville, Md., $902,000.

Title: Immunologic assessment of high risk cancer families, continuation
Contractor: Litton Bionetics, $50,069.

Title: Holding facility for small laboratory animals, continuation
Contractor: Litton Bionetics, $51,434.

Title: Induction and control of MuTV expression in mouse mammary preneoplastic tissues
Contractor: Univ. of California (Davis), $558,540.

Title: Preparation of antisera to oncogenic and potentially oncogenic viruses, continuation
Contractor: Huntingdon Research Center, Brookslandville, Md., $95,968.

RFPs AVAILABLE

Requests for proposal described here pertain to contracts planned for award by the National Cancer Institute, unless otherwise noted. Write to the Contracting Officer or Contract Specialist for copies of the RFP, citing the RFP number. Some listings will show the phone number of the Contract Specialist, who will respond to questions. Listings identify the respective sections of the Research Contracts Branch which are issuing the RFPs. Address requests to the contract officer or specialist named, NCI Research Contracts Branch, the appropriate section, as follows:
Biology & Diagnosis Section and Biological Carcinogenesis & Field Studies Section—Landow Building, Bethesda, Md. 20205; Control & Rehabilitation Section, Chemical & Physical Carcinogenesis Section, Treatment Section, Office of the Director Section—Blair Building, Silver Spring, Md. 20910. Deadline date shown for each listing is the final day for receipt of the completed proposal unless otherwise indicated.

RFP N01-CP-05604

Title: *Carcinogenicity and toxicity studies in laboratory animals*
Deadline: *Jan. 25, 1980*

The Carcinogenesis Testing Program, NCI, is interested in obtaining proposals to obtain toxicological and biochemical data in chemicals, in addition to carcinogenicity data, which would aid in the prediction of the potential carcinogenic risk of chemicals to man from carcinogenicity studies in rodents.

The experimental protocol will involve two major tasks: Series A—Task I—subchronic phase and Task II—chronic phase; series B—Task I—subchronic phase plus special studies and Task II—chronic phase plus special studies.

Responders may apply for consideration for series A (both tasks), series B (both tasks), or series B (task I only).

Contract Specialist: Ursula Evans
Carcinogenesis
301-427-8764

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