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1411 ALDENHAM LANE RESTON, VIRGINIA TELEPHONE 703-471-9695

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DE-EMPHASIS OF TRADITIONAL GRANTS STILL HAUNTS SOME; RECENT NCI ACTIONS HELD UP AS EVIDENCE

The fear that NCI sooner or later will de-emphasize traditional research grants and put most or all of its extramural money into applied

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In Brief

TOP PRIORITY: KEEP RAUSCHER; PAY RAISE BILL NOT MOVING; MILLER, HOLLAND HEAD AACR, ASCO

KEEPING FRANK RAUSCHER as director of NCI and the National Cancer Program remains top priority for the Program's leading supporters. They feel that, as highly qualified as are some of those mentioned as possible replacement, none combine Rauscher's administrative ability plus the immense enthusiasm he imparts to the scientific community, the public and Congress; it would be a severe blow to the Program to lose him now. Rauscher does not want to leave, but there is no other way he can assure the education of his five children unless he can make more money. The bill raising his salary is still waiting action by Chairmen Paul Rogers and Edward Kennedy of the House and Senate Health Subcommittees. Letters or telegrams might help. Send to Rogers, Subcommittee on Public Health & Environment, Committee on Interstate & Foreign Commerce, 2125 Rayburn, Washington, D.C. 20515; to Kennedy, Subcommittee on Health, Committee on Labor & Public Welfare, 4226 Dirksen, Washington, D.C. 20510. . . . **JAMES HOLLAND**, Mt. Sinai School of Medicine, appeared fully recovered from his heart attack when he assumed the presidency of the American Society of Clinical Oncology at the Toronto meeting. He succeeds Joseph Bertino. Vincent DeVita, director of NCI's Div. of Cancer Treatment, is president-elect. . . . **ELIZABETH MILLER**, McArdle Lab-Univ. of Wisconsin, became the third woman president of the American Assn. for Cancer Research and the first woman to succeed a woman as president when she took over from Charlotte Friend. Gordon Zubrod, former DCT director and now director of the Univ. of Miami Comprehensive Cancer Center, was elected vice president and will head the organization next year. . . . **COOPERATIVE GROUPS** were jolted when it became apparent that the proposed new human research regulations would require protocol sign off by each group member's institution; NIH would not be permitted to accept a blanket sign off for the entire group. A committee of three group chairmen—Paul Carbone, Bernard Fisher and Barth Hoogstratten—plus Franco Muggia from NCI and Robert Backus from NIH are in the process of writing guidelines especially for the groups. . . . **MEMBERS** of the two cooperative groups being phased out, Western and Central, are being signed up by other groups. The Southwestern group has landed six from COG, including its chairman, William Fletcher, and vice chairman, William Wilson. Southwestern also has picked up four of Western's members.

RAUSCHER, KING ATTEMPT TO QUIET FEAR THAT THEY ARE DE-EMPHASIZING GRANTS

(Continued from page 1)

and targeted research continues to haunt many cancer investigators.

"They way it is now, Pasteur coming to NCI with an offbeat proposal wouldn't get funded," lamented one participant at the Toronto meeting of the American Assn. for Cancer Research.

Others complained, privately and at the session on "Critical Issues" that the Cancer Program is swinging ever closer to the time when investigator-initiated, grant supported research will be severely curtailed. These fears have been fueled by a number of NCI actions during the past year:

- Transfer of the Clinical Cooperative Groups from the NCI division responsible for administering most grants, the Div. of Cancer Research Resources & Centers to the Div. of Cancer Treatment. Although grants supporting the cooperative groups are not the traditional type, they do involve investigator-designed protocols. NCI insists nothing will change, except that the groups will get first crack at DCT's clinical research contracts, and duplication will be reduced.
- Transfer of the Cancer Control Grants Review Committee from DRRC to the Div. of Cancer Control & Rehabilitation. This was seen as just one more bit of evidence that the grants division was being gutted, although DRRC Director Thomas King initiated the transfer and it seemed to make sense administratively.
- Implementation of the Cancer Research Emphasis Grants. Ironically, this program was developed primarily to switch sizeable sums from contract supported research into investigator initiated grants. Some have seen it as "targeted" grants and feel it really is a contract program with another name. DRRC administers CREG for the program divisions, with peer review by regular NIH study sections, except for the diet and nutrition CREGs being hurried through the Div. of Cancer Cause & Prevention to beat the fiscal year deadline.
- The decision to fund traditional grants at only 80% of the approved amount in fiscal 1976 so that a higher percentage of competing new and renewal grants would be supported. Those at the lower end of the priority lists who are funded might appreciate that decision, but for the others, whatever the justification, it amounts to a cutback.
- The move by King and his headquarters staff from their suite in Building 31 on the NIH campus to the Westwood Building, on the other side of Bethesda. Rauscher's office and those of all other division directors except Cancer Control (located in the Blair Building, in Silver Spring) are in Building 31, along with most of the other NIH institute directors. Some have seen the move as the final cruel blow in slashing the prestige and influence of the grants division. NCI Director Frank Rauscher insists it is no such thing, that there are other staff members who need to be in daily contact with his office and with their own division directors, and that nearly all of King's staff has long been located in Westwood where they will now have easier access to him.

King and Rauscher attempted to quiet the fears at the AACR meeting (***The Cancer Letter***, May 7). King told how as a young investigator he was supported by a traditional grant and that he still considers those grants "the primary instrument for advancing biomedical knowledge."

Rauscher, although arguing strenuously on countless occasions for money to support funding a high percentage of approved grants, does not consider the traditional grant program as untouchable. He feels that much good basic science, some of it investigator-initiated, is being done through other mechanisms. He is particularly pressured now to beef up funding for centers.

"Our biggest problem right now is with budget limitations." he told the President's Cancer Panel last week. "Do we continue to fund RO1s (traditional grants) at a high level, or do we take dollars away from them for centers. To my surprise, several traditional grants people came to me in Toronto to suggest that more money is needed for centers—for core support, and for discretionary use of center directors.

"I think we should take a look at non-recurring, one-shot money, such as the $20 million for construction. Would it not be more important to use that money for supporting operations?"

Traditional grants have a powerful supporter in Benno Schmidt, Cancer Panel chairman. "We should be careful we don't make traditional grant support the cushion money that gets invaded for every other program when money gets tight," Schmidt said. "The contract programs and special programs tend to get more internal support. One of the things that has kept the Cancer Program with the support it has had is the diligence with which we have preserved individual, investigator-initiated, grant-supported research. Everyone has been looking for us to cut back on that. The fact that we have the data to refute [charges that traditional research has been de-emphasized] has been our salvation."

Rauscher said that since the National Cancer Advisory Board has determined it wants to fund at least 50% of new approved grants and 65% of competing renewals, "I suggested maybe we ought to take that number and divide it into the finite money we have, and pay it accordingly. I didn't have many takers."

DCT ADOPTS NEW PROCEDURES FOR REVIEW OF UNSOLICITED CONTRACT PROPOSALS

NCI's Div. of Cancer Treatment, acting on the request of its Drug Development Committee, has established procedures to more directly involve its outside advisory committees in the process of review and award of unsolicited contract proposals.

The Drug Development Committee had objected to the practice of NCI staff having almost sole discretion in determining which unsolicited proposals would be selected for review and final award.

Philip Amoruso, DCT administrative officer, presented an outline of the new procedures at the committee's recent meeting. The committee accepted them without objection. Saul Schepartz, director of the Drug Research & Development Program, said that additional changes may be recommended in the future.

The new procedure is as follows:

1. Upon receipt of an unsolicited proposal, it should be forwarded directly to the administrative officer for acknowledgement.

2. After conferring with staff, the administrative officer will refer it to an appropriate program area for review. DCT program areas are Experimental Therapeutics, Drug, R & D, Clinical Oncology and Cancer Therapy Evaluation.

3. Upon receipt of the proposal, program area staff will review for priority and relevance and present to DCT's Cancer Treatment Program Staff (senior staff members) within 45 days. The program area will provide the administrative officer with all review materials 10 days prior to the date of a CTPS meeting.

4. Simultaneously with the CTPS review, the unsolicited proposal will be assigned to two members of a contract technical review committee. The chairman and vice chairman will select these members and obtain written response of this review within 30 days.

5. An abstract, which the administrative officer will receive from the proposer at a later date must be sent to all members of the contract technical review committee. If an unsolicited proposal is assigned to either Experimental Therapeutics or Drug R & D, the abstract should be sent to the Drug Development Committee and the Developmental Therapeutics Committee, and if assigned to Clinical Oncology or Cancer Therapy EValuation, the abstract should be sent to the Combined Modality Committee and the Clinical Trials Committee. In the case where an unsolicited proposal is assigned to the Baltimore Cancer Research Center, the nature of the proposal will determine to which committee the abstract will be sent.

6. If the two committee members recommend that this be reviewed by the full committee, then a quorum will be called to conduct the technical review.

7. Following review by the full contract technical review committee, the proposal will be resubmitted to CTPS for final approval/disapproval and for establishment of priority and funding.

8. If the subcommittee of the technical contract review committee does not recommend the proposal for review by the full committee, the program area will notify the administrative officer of this action. It will not be necessary for the CTPS to review a negative action by the subcommittee unless it differs from the recommendations of the CTPS. For example, approval by the CTPS and disapproval by the subcommittee will be referred back to the CTPS for a second review.

9. If the proposal is approved by the Cancer Treatment Program Staff and the contract technical review committee, it will be forwarded to the contract officer, DCT Section, Research Contracts Branch for award as a non-competitive procurement.

10. If the unsolicited proposal is disapproved, the administrative officer will notify the principal investigator submitting the unsolicited proposal.

Amoruso's proposal said, "It is important to note that unsolicited proposals are frequently received which are incomplete for review. These often take the form of a letter of inquiry as to program interest into certain research areas. In the event that this occurs, the program area, after review by the associate director, should respond directly to the principal investigator submitting the letter of inquiry. If it is felt that another program may have interest in this area of research, the letter of inquiry should be referred directly to the appropriate associate director. A copy of all correspondence either from or to the principal investigator should be sent to the administrative officer in order to maintain a central control of these inquiries and responses.

"If the program area feels that the letter of inquiry should be pursued by the principal investigator with an unsolicited proposal, the appropriate HEW instructions may be sent along with the Optional Form 60, contract pricing proposal. These two documents will assist the principal investigator with the administrative requirements for the submission of an unsolicited proposal."

The Drug Development Committee also had objected to seeing its priority ranking of contract proposals changed by staff on the basis of indirect costs. Information relating to indirect costs generally has not been made available to outside reviewers because it frequently involves privileged matters that by law must not be released to non-government personnel.

DCT Director Vincent DeVita responded by agreeing to discuss any changes in priority rankings with committee chairmen and vice chairmen before awards are made, and with the full committees if the chairmen feel that is desirable.

DeVita said that staff had been alerted to the fact that committees should receive all possible information permitted them.

Committee member Robert Goldberger said he was "still confused by what indirect costs amount to. I have the feeling I'm seeing only half the story, since decisions may be based on such costs."

Schepartz said that indirect costs include "a lot of items allocated to specific projects. The problem is, it varies from one organization to another depending on accounting procedures and organizational prac-

tices. Heat, lights, depreciation almost always are part of indirect costs. Secretarial help sometimes is direct, sometimes indirect. Determining the percentage of overhead is difficult. It varies from 50 to 100% of labor costs. With academic institutions, buildings sometimes are paid for by the state, so they don't have to be included.

"I wouldn't object to sending the total budget to the committees, but that's not in the rules," Schepartz said.

Goldberger asked if it would be possible to inform the committee "when something remarkable is in indirect costs."

"If the final determination depends on those costs, perhaps," Schepartz said.

AACR SCIENTIFIC SESSIONS INCLUDED REPORTS ON BASIC, CLINICAL RESEARCH

The scientific sessions at the 67th annual meeting of American Assn. for Cancer Research included papers on a wide variety of basic and clinical investigation. Reports on a few of those presentations are included here (also see Abstracts, following).

- **Induction of Oncornavirus Antigens by Herpes Simplex Virus** – Cathy Reed, Fred Rapp

It has been discovered that, in several animal populations, members of the oncornavirus and herpesvirus groups are responsible for naturally occurring cancers. Other viruses have been shown to transform animal cells in culture, but have not been detected under natural conditions.

Herpes simplex viruses are commonly manifested in human populations in two forms: herpes simplex virus type 1 is generally isolated from oral lesions (cold sores), and herpes simplex virus type 2 is often isolated from genital lesions. Other herpesviruses include the Epstein-Barr virus, which has been linked with Burkitt's lymphoma and with nasopharyngeal carcinoma, and cytomegalovirus, which shares many properties of the oncogenic herpesviruses and has been associated with several human cancers.

Because the genetic function of type-C oncornaviruses is often found attached to the chromosomes of cells, these viruses share with the herpes simplex viruses the property of latency within the host and are of special interest to viral oncologists. The interaction between these viruses within the same cell has been examined by several groups and evidence has been obtained that the oncornavirus may sometimes interfere with the destructive property of the herpesvirus, and that this may lead to transformation of normal cells to malignancy by the herpesvirus.

This action may also occur in reverse. Oncornavirus DNA is present within each cell of some species. This DNA is normally not expressed as ribonucleic acid or protein; when herpes simplex virus is introduced into the cell, however, activation may occur.

This interaction may result in expression of oncogenic functions of the oncornavirus which lead to cell transformation.

To examine this interaction between the viruses, investigators at the Hershey Medical Center superinfected two mouse cell lines containing type-C oncornavirus with herpes simplex virus type 2. A specific protein antigen of the oncornavirus was expressed in the infected cells but not in uninfected normal cells. Infection with a human cytomegalovirus also induced this protein. These results suggest that human herpesviruses can activate expression of oncornavirus information in mouse cells. The question of whether oncogenic sequences which are present in the oncornavirus DNA are also stimulated by herpesvirus infection is now being studied.

- **Identification of a New Group of Potent Inducers of Differentiation in Murine Erythroleukemia Cells** – Roberta Reuben, Richard Wife, Ronald Breslow, Richard Rifkind and Paul A. Marks

Murine erythroleukemia cells were originally isolated from the spleen of a mouse which was infected with a leukemia virus and are now maintained in the laboratory by culturing the cells in flasks. The virus infection caused these cells to be blocked in their normal development into red blood cells and at the same time endowed them with the capacity for uninhibited cell growth. When grown in culture, these leukemia cells double their number in less than a day and will continue to multiply indefinitely as long as they are fed. The erythroleukemia cells resemble cells in the early stages in the development of red blood cells, and like those precursor cells, do not synthesize hemoglobin. When the cells are gorwn in the presence of any one of a number of drugs having a charge separation, such as dimethyl sulfoxide, a portion of the cells will develop along a pathway leading to red blood cells. The "induced" cells synthesize hemoglobin and become red in color. These developing cells are becoming specialized for a particular function, and like all specialized cells, they lose their capacity for uninhibited cell growth. They are no longer leukemic in nature.

We have attempted to find a drug that would be a more effective agent by causing all the cells in the culture to develop in this manner, that is to become cells specialized for the synthesis of hemoglobin, and thereby "terminally differentiate," or cease to multiply, after attaining their specialized function. Other drugs studied to date affect only a portion of the population and the unaffected remainder continues to multiply. The group of compounds we have synthesized and tested for activity, the bisacetamides, are active at relatively low concentrations and cause the entire population of cells to develop along a pathway leading to red blood cells. Every cell in the culture synthesizes hemoglobin and after four days ceases to multiply. By seven days all the erythroleukemia cells are dead.

These drugs, the bisacetamides, are currently being

tested by the Mason Research Institute for antitumor and antileukemia activity.

- **Prognostic Value of Antibodies to Membrane Antigens of Melanoma Cells** – K. Irie, R.F. Irie, and D.L. Morton

The antibody response to tumor antigens during postoperative immunotherapy appears to correlate with prognosis for patients with malignant melanoma.

Correlations between clinical course and the antibody response in cancer patients have been search for over the years, but without significant success. The difficulty in this type of study was the elimination of all the factors unrelated to the cancer. We were able to minimize these factors in our investigation. By indirect membrane immunofluorescence, two types of antibody, IgG and IgM, in the serum of melanoma patients were measured against tumor antigens on the membranes of tissue-cultured melanoma cells from another melanoma patient. Samples of blood were drawn from stage II melanoma patients before starting immunotherapy and during immunotherapy, but after surgical resection of their tumors. The 60 patients seen at the Div. of Surgical Oncology, UCLA, were grouped according to the type of postoperative treatment–22 who had cultured melanoma cell vaccine with BCG, 22 who were treated with BCG alone, and 16 who were followed but had no further treatment. The experimental nature of the study and its possible complications were explained to each patient and informed consent was obtained prior to entrance into the study.

Prior to the initiation of immunotherapy, there were no significant differences in the three groups in the amount of antibody to tumor antigens. After immunotherapy was started, there was a significant increase in group 1, the tumor cell vaccination group, but not in the BCG or no further treatment groups. However, at this stage of our investigation, we could not identify the amount of antibody needed to assure a favorable prognosis for the patient. When we analyzed the proportion of increase in antibody response in all three groups of stage II melanoma patients, we could define a marked difference for prognosis between the positive responders and the negative responders. Of 32 positive IgG responders, 28 were free of disease compared to 12 of 28 negative IgG responders. All 18 IgM responders were disease free, but only 22 of 42 negative IgM responders had no evidence of disease. All told, 34 of 38 patients who displayed an increase in either IgG or IgM antibody remained free of their disease at one year in contrast to only 6 of the 22 IgG and IgM negative patients.

The results of our investigation suggest that there is a close correlation between antibody response and a favorable prognosis for stage II melanoma patients. Based upon this study, we are studying immunotherapeutic methods to increase the antibody to tumor antigens in cancer patients.

- **Co-Carcinogenic Activity of Nicotine** – F.G. Bock

A study undertaken to estimate the effect of nicotine on the carcinogenic activity of cigarette smoke condensate solutions at Roswell Park has demonstrated that low to moderate concentrations of nicotine act as important co-carcinogenic stimuli when applied to the dorsal skin of mice.

The first induction of cancer in mouse skin using cigarette smoke condensate was reported in 1953 by Wynder et al. Although the mouse skin system differs anatomically from the human lungs, evidence thus far indicates a parallel response which can be used in attempts to control lung cancer.

Since previous experiments have indicated that an acetone solution of bonzopyrene and 12-0-tetratecanoylphorbol-13-acetate would provide tumor incidence characteristics similar to 30% cigarette smoke condensate when applied to the dorsal skin of mice, various amounts of nicotine were added to this solution in order to determine its effect on mouse skin carcinogenesis. One of many substances in tobacco smoke, nicotine by itself does not produce tumors.

At the end of 37 weeks, after female mice were painted 10 times weekly with 0.2 ml of the solutions, the tumor incidence for the various solutions was 37% from a no nicotine solution to an incidence of 80% with a nicotine solution of 6 mg/ml. Similar results were obtained when nicotine was added to solutions containing half as much of benzopyrene and 12-0-tetratecanoylphorbol-13-acetate.

- **Transfer of Tumor Immunity In Vivo Using Cytotoxic Cells Generated In Vitro in a Secondary Immune Response to Syngeneic Rat Lymphoma Cells** – Irwin Bernstein

Researchers at the Fred Hutchinson Cancer Research Center in Seattle have shown that antitumor cells can be stimulated in vitro so as to more effectively kill tumor cells in vitro and inhibit tumor growth in vivo.

Irwin Bernstein and Peter Wright of the tumor immunology program at the center have used immune cells which initially lack both the ability to kill tumor cells in vitro and the ability to transfer tumor immunity to nonimmune recipients. When the cells are restimulated with tumor antigens in a test tube, however, the specifically immune cells increase in numbers and/or become activated. The immunity can then be effectively transferred to nonimmune recipients.

Using a lymphoma model, the researchers have immunized rats to a Gross virus-induced lymphoma designated (C58NT)D, by administering a dose of tumor cells which grow transently and then regress. Four to six weeks later the spleens of the immune animals are removed.

Untreated immune spleen cells do not kill lymphoma cells when tested in vitro by a chromium release assay. Furthermore, when these untreated spleen cells are administered systemically (by injection into the heart), the recipient animal fails to inhibit the growth

of injected tumor cells. However, if these spleen cells are cultured for a period of five days in the presence of small numbers of the lymphoma cells (which are treated with mitomycin to prevent them from dividing), they generate the capacity to kill the lymphoma cells in vitro.

When the cells are injected systemically, they can prevent the growth of a subcutaneous injection of tumor cells, including an inoculum of cells that would otherwise grow progressively and kill all nonimmune animals. The immune cells, on the other hand, will only inhibit the specific lymphoma cells and will not inhibit the growth of other tumors which possess different tumor antigens.

- **Susceptibility of BCG-Activated Peritoneal Macrophages as an In Vitro Assay of Spontaneous Neoplastic Transformation** – Robert Tucker

Research on cancer-causing agents and laboratory-grown cancer cells might be aided by a simple test studied at NCI. The test is based on destruction of cancer cells—but not normal ones—by macrophages, white blood cells that attack and destroy "foreign" material in the body.

The only reliable method at present of determining whether cells grown in the laboratory are cancerous is to inject the cells into animals and wait for a tumor to develop. Cancer cells grown in culture do not differ enough from normal cells in culture to be labeled as cancerous by any one simple test, such as microscopic examination.

Tucker found that rat, mouse and hamster cancer cells were killed by mouse macrophages activated with BCG. The cancer cells had developed spontaneously from cell lines cultured in the laboratory for a number of generations.

The activated macrophages readily killed cancer cells but not normal cells from the same embryo. In addition, the more virulent cancer cells were killed quicker than were cancer cells that produced cancer more slowly. The exact mechanism by which a macrophage destroys a cancer cell is not known and is the object of intense research.

Two groups of cultured cells studied in detail were not susceptible to destruction by macrophages during the period of transformation from noncancerous to cancerous forms. Only after the rodent cells had been cancerous for 50 days would the macrophages attack and destroy them.

- **Metastatic Brain Tumor Model With Human Gonadotrophin (HCG) Marker System** – C.R. West, A. Fjelde and S. Gailani

A hormone-producing tumor of human origin has been grown successfully both within a test tube and inside living animals at Roswell Park, giving scientists a more accurate way of measuring the efficacy of drugs.

Audrey Fjelde explained that the tumor is unique in that it is:

a) of human origin

b) can be grown and tested both in vivo and in vitro

c) produces the hormone, human gonadotrophin.

"This hormone provides us with a clearly identifiable marker system by which to measure both growth and viability of the tumor," Fjelde said. "Although there is no perfect way yet of testing efficacy of drugs designated for human use when using animal model systems, this tumor seems ideally suited for our purpose."

Many other tumors were studied unsuccessfully before finding this one. It has now been employed at Roswell Park for two years. The tumor is grown in tissue cultures and in the cheek pouches of hamsters and is frozen in ampules for preservation of stock material for future research.

The hormone produced by the tumor, human gonadotrophin, serves as a marker system facilitating the measurement of growth and viability of the tumor. It can be obtained by examination of blood and spinal fluid samples taken from the animal.

ABSTRACTS OF OUTSTANDING PAPERS PRESENTED AT ANNUAL AACR MEETING

The program committee for the 67th annual meeting of the American Assn. for Cancer Research selected 44 papers as among the outstanding ones presented at the meeting. The following abstracts are from that list, chosen from sessions on clinical investigations, biochemistry, immunology, chemical carcinogenesis, experimental chemotherapy and in vitro carcinogenesis. Others will appear in subsequent issues of ***The Cancer Letter.***

FIVE CLINICAL PATTERNS OF RECURRENCE OF CANCER OF THE BREAST – R.V. Smalley, Temple Univ. Health Sciences Center

An evaluation of the clinical pattersn of first recurrence following primary therapy for carcinoma of the breast in patients referred for cytotoxic chemotherapy (CT) was undertaken. 110 patients entering SECSG study No. 339 (CCR 57: 110, '73) and 42 consecutive patients seen in a single institution have been analyzed. All patients fit into one of five patterns. The median time to relapse following primary therapy was influenced by the clinical pattern as was the likelihood of response to CT and survival from first recurrence. The mode of spread appeared hematogenous for three and by local extension for two of the patterns as follows: a) early widespread metastases with involvement of all major organs, b) pulmonary (nodular or lymphangitic) c) bone d) ipsilateral effusions with pleural involvement, and e) local skin and node recurrence c occasional subsequent bone metastases. All except a) were dominated by, if not clinically exclusively confined, to the presenting site of recurrence. Variables at time of primary therapy influencing subsequent recurrence pattern will be sought.

		MEDIAN TIME TO:		CT
PATTERN	INCIDENCE	RELAPSE	SURVIVAL	RESPONSE
a	19%	9 Mos.	4 Mos.	6%
b	12%	36 Mos.	18 Mos.	33%
c	26%	15 Mos.	29+Mos.	25%
d	16%	39 Mos.	44 Mos.	33%
e	26%	15 Mos.	27+Mos.	59%

SERIAL MONONUCLEAR CELL CYTOTOXICITY LEVELS IN BREAST CANCER PATIENTS & NORMAL CONTROLS – A.C. Levin, R.J. Massey, J. Wolter, V. Schauf & F. Deinhardt, Rush & Univ. of Illinois Medical Centers

Mononuclear cell cytotoxicity (MCC) levels of 29 breast cancer patients with metastases and 20 normal controls were followed serially

for 5-26 months using a ^{51}Cr-release assay to see if MCC levels correlated with or predicted the clinical course of the patients. Target cells were the HBT-3 and ALAB cancer-derived cell lines and control fibroblasts. Mononuclear cells prepared on Ficoll-Hypaque gradients were obtained from patients and controls at 2-7 month intervals on 3-12 occasions/individual. Patients under observation or on various therapeutic regimens were followed. MCC levels to the cancer-derived cells in controls remained relatively stable whereas levels in the patients showed marked changes. A correlation of these changes with the clinical course was only seen in 16 patients (55%). Cycled combination chemotherapy (Cytoxan-5FU-Methotrexate or Adriamycin-Vincristine) was given to 13 patients. Although a fall in MCC levels was found in 8/13 patients, it did not correspond to the clinical response to treatment. These studies show that breast cancer patients with metastases have different patterns of MCC from normal controls but that these patterns do not correlate with the clinical status of the patients and cannot be used to predict the clinical course.

IMMUNOTHERAPY IN ACUTE MYELOCYTIC LEUKEMIA WITH NEURAMINIDASE (N'ASE) TREATED ALLOGENEIC MYELOBLASTS WITH OR WITHOUT MER – J.G. Bekesi, J.F. Holland, J. Cuttner, R. Silver, M. Coleman, C. Jarowski, & V. Vinceguerra, Mt. Sinai School of Medicine & New York Hospital

We previously reported successful chemoimmunotherapy with N'ase treated leukemic cells and MER in the cure of DBA/2 mice with L1210 leukemia and AKR mice with spontaneous leukemia. These data led to clinical trial in AML using N'ase treated allogeneic myeloblasts with or without MER. 53 patients were allocated to three groups following successful remission induction with cytosine arabinoside and daunorubicin. All received cyclical maintenance chemotherapy every 28 days: N'ase treated allogeneic myeloblasts or N'ase treated myeloblasts plus MER were given to two groups. Immunological monitoring included recall antigen skin testing, T & B-lymphocyte quantification and lymphoblastogenesis. In each immunization 10^{10} N'ase treated myeloblasts were injected intradermally (i.d.) in approximately 48 sites. 1 mg. MER was divided in 5-10 i.d. sites. Median remission duration of the control group is 20 weeks. Patients receiving either form of immunotherapy have not as yet reached median remission at 78 weeks. Differences are highly significant: control group vs. N'ase treated myeloblasts, p=.0008 & control vs. cells plus MER, p=.001. Chemoimmunotherapy directly influenced the survival of AML patients. 17 of 21 patients in the chemotherapy regimen died by 90 weeks compared with five of 32 patients in the immunotherapy groups, p=.00008.

ROLE OF SOLUBLE PROTEINS IN REPLICATION OF DNA AND CHROMATIN OF ISOLATED NUCLEI – Kazuto Kajiwara, Stephen Planck and Gerald Mueller, McArdle Laboratory

Nuclei from S phase HeLa cells replicate up to 5% of their DNA when provided with 4 deoxyriboside triphasphates, ATP, Mg^{++}, 0.1 M NaCl and soluble proteins from the cytoplasm (CF). DNA replication proceeds by a discontinuous, bidirectional process from sites which were active in the living cell. Omission of CF reduces the activity up to 80% and yields short DNA chains which fail to mature properly. Fractionation of CF over DEAE and phosphocellulose reveals that the activity resides in multiple fractions which support DNA replication in early and late S phase nuclei to different extents. Binding studies indicate that up to 2% of H^3-leucine labeled CF proteins associate with the nuclei by a temperature dependent process during the DNA replication interval. Electrophoresis of the associated proteins in SDS-acrylamide gels reveals this association to be non-random. Chromatin from CF or histone labeled nuclei was fixed with formalin and sedimented in CsCl-guanidinium HC1 density gradients. Labeled proteins of CF were clearly concentrated in the lighter density chromatin fractions. Labeled histones were distributed randomly. The data suggest that protein to protein interactions underly the role of CF in DNA replication in isolated nuclei and that a type of chromatin is made in the nuclear system.

IN VIVO PROTECTION AGAINST SYNGENEIC GROSS VIRUS-INDUCED LYMPHOMA IN RATS: COMPARISON WITH IN VITRO STUDIES OF CELL-MEDIATED IMMUNITY – Moshe Glaser, David Lavrin, and Ronald Herberman, NIH and Litton Bionetics

Spleen cells at various times after inoculation of W/Fu rats with a syngeneic Gross virus-induced lymphoma, (C58NT)D, were tested for their in vivo activity in adoptive transfer experiments and for their in vitro reactivity in a 4 hour ^{51}Cr release cytotoxicity assay and in a mixed lymphocyte-tumor cell interaction (MLTI) assay. In adoptive transfer, the best protection against tumor growth was observed with immune spleen cells taken at 30 days after tumor cell inoculation (the peak of reactivity in the MLTI assay), whereas cells taken at 10 days, (the peak in the ^{51}Cr release cytotoxicity assay) gave only partial protection. The protection detected in the adoptive transfer experiments was specific for (C58NT)D associated antigens, and this correlated well with the specificity observed in the in vitro cell-mediated immunity assays. T cells, but not complement receptor-bearing cells or macrophages, were essential for the protection against tumor growth in vivo and also for the in vitro reactivity in the ^{51}Cr release cytotoxicity and MLTI assays. These studies offer an important approach to correlate the results obtained in vitro assays, to the in vivo resista nce to tumor growth.

ISOLATION OF FOUR BENZO(a)PYRENE PHENOL METABOLITES – James Selkirk, Robert Croy and H.V. Gelboin, Chemistry Branch, NCI

High pressure liquid chromatography has allowed the separation of complex mistures of polycyclic hydrocarbon metabolites with relative ease. Utilization of a mixture of aqueous and organic solvents and gradient elution has been successful for separation of the various constituent groups of oxygenated derivatives, e.g., diols, phenols, quinones, etc.

We have developed re-cycle chromatography to re-analyze the phenol regions of the chromatographed benzo(a)pyrene metabolites. We are examining individual peaks isolated by this chromatography procedure in order to probe the possibility of additional metabolic products within the known profile. Multiple rechromatography accomplished by means of two linked Zorbax-Sil (Dupont) colums and a low-void volume six-port valve enabled continuous transferal between columns without loss of resolution. Three metabolite peaks were observed after passage through a single column, and after five passages four distinct peaks were resolved. Each of the compounds were isolated and characterized by co-chromatography and UV spectra and compared to synthetic phenol standards. These metabolites corresponded to the 3-phenol and the 9-phenol previously identified and two additional metabolites identified as 1-phenol and the 7-phenol of benzo(a)pyrene.

PREMATURE AGING OF HEMATOPOIETIC STEM CELLS SURVIVING ALKYLATING AGENTS – Leslie Botnick, Eileen Hannon, Samuel Hellman, Harvard Medical School

Hematopoietic failure is rarely the cause of death in animal or man since presumably this cell renewal system has a capacity which exceeds the normal life span. However, the proliferative capacity of the hematopoietic system may become limited when stressed by the prolonged use of cytotoxic agents. These experiments test this hypothesis. Mice are given weekly doses of Busulfan and L-Phenylalanine. Peripheral blood counts show moderate depression during drug administration but return to normal levels shortly thereafter. Serial marrow transfers into syngeneic recipient mice at 14 day intervals is a method to increase the number of divisions that the stem cell must undergo. A new transfer is initiated every five weeks. Mice treated with Busulfan and to a lesser extent L-Pam demonstrate permanent damage to the proliferative capacity of surviving stem cells as measured in serial transplants. Ten to 20 weeks following drug completion Busulfan treated marrow undergoes a maximum passage of 28. This compares to control animals that undergo 42 days of serial passage. Such data may have relevance to the use of such agents for prolonged periods in the clinic where there is a great interest in destroying subclinical micrometasis. In the adult mouse stem cells are not proliferatively active, thus other cytotoxic agents affecting only cells in cycle may be useful adjuvants without causing significant cell depletion.

MODE OF ACTION OF THE BIOREDUCTIVE ALKYLATING AGENT, 2,3-BIS(CHLOROMETHYL)-NAPHTHOQUINONE (CMNQ) – L.A. Cosby, R.S. Pardini, R. Biagini, A.J. Lin, and A.C. Sartorelli, Yale Univ. School of Medicine and Univ. of Nevada (Reno)

CMNQ is a relatively nonreactive agent which, following reduction to a hydroquinone, generates an extremely reactive quinone methide. This requirement for bioreductive activation suggests possible activity against hypoxic neoplastic cells with high reducing potential. CMNQ has antitumor activity against AD-755 and S-180, significantly prolonging survival time of mice bearing these neoplasms. Bioreductive activation of CMNQ in isolated mitochondria has been shown to require electron transport. This agent inhibits nucleic acid biosynthesis measured by incorporation of ^3H-thymidine and ^3H-uridine into acid-insoluble material, with DNA biosynthesis being the more sensitive. This latter effect is in part due to interference by CMNQ with conversion of thymidine to deoxynucleotide forms. Treatment of S-180 cells with C^{14}-CMNQ results in long term binding of radioactivity to DNA, RNA and protein, suggesting alkylation of macromolecules. Single strand

DNA fragments are observed in alkaline sucrose gradients of cells treated with this agent. The primary mitochondrial event produced by CMNQ is uncoupling of oxidative phosphorylation, although inhibition of NADH and succinoxidase activities occurs at higher concentrations. The results indicate diverse biochemical lesions are produced by CMNQ which are consistent with alkylating potential.

TRANSFORMATION OF CULTURED C3H/10T1/2 CELLS BY ULTRAVIOLET LIGHT AND PHORBOL ESTER – S. Nandal, D.W. Brankow and Charles Heidelberger, McArdle Lab

Carcinomas are produced in rat, mouse and human skin by repeated exposure to uv light. However, there have been no reports that uv light can transform cultured cells. We now demonstrate that uv light acts as an initiator of transformation in C3H/10T1/2 cells when followed by promotion with tetradecanoyl phorbol acetate (TPA). The cells were irradiated once with 10, 25, 50 and 100 ergs/mm^2 from a uv light. No transformation was observed at any of the above doses in six weeks of culture, and no cytotoxicity at the two lowest doses. However, when the irradiated cells were cultured in a medium containing 0.1 µg/ml of TPA, starting 48 or 72 hours after the irradiation, a high frequency of transformation was produced in all cases. The percent dishes with transformed foci were: acetone, 0; TPA, 0; uv, 25 ergs/mm^2, 0; uv, 25 ergs/mm^2 + TPA, 92. When the cells were initiated with a subeffective concentration of methylcholanthrene (MCA) (0.1 µg/ml), followed by uv irradiation at different intervals, no transformation occurred. Moreover, different doses of uv irradiation followed by 0.1 µg/ml of MCA did not yield transformation. Therefore, uv light acts as a pure initiator in our system. The mechanism is under study.

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. Some listings will show the phone number of the Contract Specialist, who will respond to questions about the RFP. Contract Sections for the Cause & Prevention and Biology & Diagnosis Divisions are located at: NCI, Landow Bldg. NIH, Bethesda, Md. 20014; for the Treatment and Control Divisions at NCI, Blair Bldg., 8300 Colesville Rd., Silver Spring, Md. 20910. All requests for copies of RFPs should cite the RFP number. The deadline date shown for each listing is the final day for receipt of the completed proposal unless otherwise indicated.

RFP NCI-CP-VO-61021-67

Title: *Monitoring of biohazards containment facilities*

Deadline: *June 25*

NCI is seeking a contractor to perform the following tasks: (1) Perform site visits to research facilities involved in handling of hazardous chemicals (including carcinogens) and to virus production facilities for the purpose of evaluating compliance with specified safety standards and advise for enhancement of work environment; (2) Evaluate an existing safety and environmental program for contract viral oncology laboratories; (3) Improve the aforementioned program; (4) Provide environmental microbiology and engineering consultation to parties seeking assistance from the Office of Biohazard & Environmental Control; (5) Provide a monitoring and analytical capability for the purpose of detecting chemical carcinogens in the research environment; (6) Provide safety consultation to NCI on matters pertaining to the safe handling of chemical carcinogens in the laboratory environment.

Accomplishment of these tasks will require expertise in environmental microbiology, environmental health, virology, microbiology, immunology, mechanical engineering, technical writing, industrial hygiene, and analytical and organic chemistry. The capability of performing continual literature review for recent developments in viral oncology, biohazard risk assessment, research techniques, and environmental control will be required.

Contracting Officer: Charles Faford
Cause & Prevention
301-496-1781

SOLE SOURCE NEGOTIATIONS

Proposals are listed here for information purposes only. RFPs are not available.

Title: Study of the effects of carcinogens on the in vitro synthesis of complement compounds
Contractor: Childrens Hospital Medical Center.

Title: Mammography training
Contractor: Arkansas State Dept. of Health.

Title: Study of hormonal factors of the human and animal prostate
Contractor: Southwest Foundation.

Title: Identification of site of origin of human pancreatic adenocarcinoma
Contractor: Memorial Sloan Kettering.

CONTRACT AWARDS

Title: Cancer control assistance program for community health professionals
Contractor: Assn. of Community Cancer Centers, $65,620.

Title: Programming services in support of contract management system
Contractor: Sigma Data Computing Corp., $23,144.

Title: Phase I studies of new anticancer agents
Contractor: Sidney Farber Cancer Center, $400,185.

Title: Demonstration of cancer rehabilitation facilities and/or departments
Contractor: Memorial Hospital, New York, $453, 480.

Title: The isolation of antineoplastic agents from plants
Contractor: Univ. of Virginia, $682,476.

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