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PATHOLOGY

TEACHING HOSPITALS
WALTER REED ARMY MEDICAL CENTER
NAVAL HOSPITAL, BETHESDA
MALCOLM GROW AIR FORCE MEDICAL CENTER
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April 16, 1990

Stanislaw R. Burzynski, M.D., PH.D.
The Burzynski Research Institute
6221 Corporate Dr
Houston, Texas 77036

Dear Dr. Burzynski:

I would like to take this opportunity to express my thanks to you and your wife for the exceptionally warm attitude during our stay in Europe. I hope that this is just the beginning of a long and exciting relationship.

Upon your request, I have summarize some of my research plans for Antineoplaston AS2-1. (An official research proposal will follow; this has to go through the appropriate University channels and would take much longer to prepare). The proposed work is based on our preliminary experience with AS2-1. We could include A10 in the in-vivo studies. Also, I would like to initiate the isolation and characterization of <u>active peptides from A2</u>: It is my impression that A2 contains most active anticancer substance(s) (more active, per molar ratio, compared to AS2-1), which could induce differentiation through non-toxic novel mechanisms. Our tumor differentiation and phenotypic reversion models would be most useful in detecting such activities.

As you know, however, the Antineoplaston research in my laboratory depends totally on extramural funding. Unfortunately, I can not see the NCI fund this work in the near future. Sigma-Tau, on the other hand, provided us with a budget that will run out in a month or two. I hope that this will not mean a delay/termination of our important research, and wish you **Good Luck** in meetings with the potential supporting agencies !

With warm regards to you and Barbara,

Yours,

Dvorit

Dvorit Samid, PhD
Assistant Professor of Pathology

p.s. please send more AS2-1 (powder, ~30g).
enc.

Research Proposal

ANTINEOPLASTON AS2-1 IN CANCER PREVENTION AND THERAPY

Dvorit Samid, PhD
Department of Pathology, Uniformed Services University
of the Health Sciences. Bethesda Maryland

Antineoplastons may offer a novel alternative to current non-satisfactory cancer therapies. The limited preclinical and clinical experience with the Antineoplaston AS2-1 (sodium salts of phenylacetylglutamine and phenylacetic acid, 1:4) has been most promising, showing potent antitumor activities with minimal or no adverse effects. However, the efficacy and mechanisms of action of AS2-1 are not well characterized. We proposed to develop a comprehensive preclinical program aimed to evaluate the potential role of AS2-1 (used alone and in combination with other drugs) in cancer prevention and therapy. The following projects are proposed:

I. AS2-1 in Cancer Therapy.

A variety of in-vitro and in-vivo tumor models, involving genetically characterized human and mouse tumor cell lines, will be used to test for tumor-directed effects of AS2-1. The biological end-points to be examined are: (a) inhibition of cell proliferation; (b) induced phenotypic reversion; and, (c) terminal cell differentiation. A special emphasis will be placed on the latter since differentiation therapy is a most desired approach to fighting cancer. Our preliminary data indicates that AS2-1 may indeed be a potent differentiating agent. To determine the efficacy of the Antineoplastons under physiological conditions, in-vitro studies will be extended to include athymic mice carrying primary and metastatic human tumor cells.

The phenotypic observations will be coupled with molecular analyses focusing on alterations in gene expression (oncogenes and others), in order to determine the mechanisms of AS2-1 anti-tumor action.

II. The Efficacy of Combination Therapies.

It is well recognized that tumors are composed of heterogeneous cell populations that vary in their drug responses. Therefore, appropriate combination treatment protocols, including agents that suppress tumor growth through different mechanisms, are likely to be more effective compared to monotherapies. We plan to examine the efficacy of AS2-1 in combination with other biologicals (i.e., interferon) and with chemotherapeutic drugs, in order to realize their full anticancer potential.

(a) AS2-1 and Interferon. The pleiotropic biological response modifiers interferons (IFNs) have gained much attention in recent years due to their antiproliferative and antitumor activities. However, the clinical experience with IFN has been rather disappointing. We shall test the efficacy of IFNα (Roferon) in combination with AS2-1. Our preliminary data showed that such a combination has synergistic effect inhibiting cancerous growth, and thus

potentially a potent therapeutic approach.

(b) AS2-1 and Classical Chemotherapy. While numerous chemotherapeutic drugs can effectively suppress cancer growth, they pose a clinical problem due to the toxic, mutagenic and carcinogenic side effects. The greatest concern is the fact that the drugs may actually promote recurrences. Interestingly, our preliminary data suggests that AS2-1 can prevent tumor progression induced by some chemotherapeutic drugs. Moreover, combination treatments with AS2-1 might allow to lower doses of chemotherapeutic drugs, thus further minimizing their adverse effects. The specific drugs to be evaluated will be determined depending on the tumor types of interest (see section IV, b and c).

III. Antineoplastons in Tumor Prevention and Maintenance Therapy.

Current advances in molecular techniques allow the detection of genetic disorders associated with predisposition to cancer. Consequently, it is now possible to identify high-risk individuals and patients (in remission) with residual disease. Inspite of these remarkable capabilities, there is no acceptable preventive treatment. Antineoplastons may play a role in cancer prevention; indeed there is some experimental evidence supporting this hypothesis. The use of AS2-1 in prevention and long-term maintenance therapy (to minimize disease relapse) is particularly attractive considering that AS2-1 has little or no adverse effects and is well tolerated by patients. The efficacy of AS2-1 will be examined using oncogene-primed premalignant cell and animal models, all of which are predisposed to cancer development.

IV. Preclinical Studies of Patient Tumor Responses.

Preclinical studies rely primarily on the use tumor cell lines, which arise from selected subpopulations of tumor cells. To obtained more clinically relevant data, it is important to evaluate the efficacy of new drugs also on authentic patient material. We plan to develop a novel in-vitro three-dimensional (3D) model, involving tumor biopsies grown on agar plugs, which precludes the selective outgrowth of tumor subpopulations. Under the 3D conditions, and in contrast to the 2D growth on plastic or glass surfaces, tumor responses to anticancer agents may mimic closely that occurring under physiological conditions. The 3D model will be used to:

(a) obtain data on tumor growth, necrosis, and cellular differentiation in treated tissues.
(b) identify those tumor types more likely to respond to treatment with AS2-1.
(c) determine the efficacy of different combination therapy protocols.
(d) study tumor responses in parallel to the clinic, in order to determine the predictive value of the 3D model. -- If so, this system could be used in the future to prescreen patients candidates for Antineoplaston therapy.

Summary. Current approaches to combat cancer rely primarily on the use of chemicals and radiation, which are themselves carcinogenic and may promote recurrences. There is, therefore, an urgent need for new effective and safe treatment modalities. The Antineoplaston AS2-1 may offer an attractive approach to cancer prevention and therapy. Our proposed studies have been designed to help realize the full therapeutic potential of AS2-1 before its wide use in the clinic.

SUMMARY of PRELIMINARY RESULTS / Dr. SAMID
(November 1989 - April 1990)

I. Phenotypic Reversion by AS2-1 of Mouse Fibrosarcoma V7T Cells

1. Profound and rapid restoration of contact inhibition of growth, and loss of anchorage independence
2. Long-term treatment may result in stable phenotypic reversion (?)
3. Effective in cells resistant to other cytokines (IFN)
4. Modulation of gene expression:
   a. inhibition of ras oncogene expression
   b. induction of collagen biosynthesis (differentiation ?)
   c. induction of 2'-5'A synthetase (autocrine IFN ?)

II. AS2-1 : Prevention of Neoplastic Transformation and Tumor Progression

1. NIH 3T3 cells — Prevention of spontaneous or oncogene-induced transformation (gene transfer studies)

2. Oncogene-primed 3T3 cells — Prevention of tumor progression by the chemotherapeutic drug 5AzadC

III. AS2-1 Induced Cell Differentiation

1. HL-60 promeyelocytic leukemia
   reduced proliferation
   monocyte/granulocyte (?) conversion (NBT-positive)
   inhibition of myc oncogene expression

2. K562 erythroleukemia
   reduced proliferation
   increased hemoglobin production

3. NIH 3T3 and C3H 10T1/2 embryonic fibroblasts
   reduced proliferation
   adipocyte conversion
   no induction of neoplastic transformation

BUDGET

I. **An Estimated 12 Month Budget Required for Developing a Comprehensive Research Program on Antineoplastons, with a prime focus on AS2-1 and A2.**

Personnel (salary plus benefits):	
2 research assistants	70,000
1 technician	25,000
1 student stipend	10,500
Supplies (reagents, animals, small equipment)	28,000
Administrative support	4,000
	$137,500

(plus 42% university overhead)

Research support is requested for five years.

II. **Immediate Minimal Budget Requirement**

This is needed in order to continue the ongoing research, so that we can bring the preliminary data to a "Publishable" stage. We will also initiate studies on the combination AS2-1 and Interferon alfa (Roferon), so that I could submit a more comprehensive proposal to Roche within one year.

Personnel (salary plus benefits):	
50% support of a research assistant	17,000
50% support of a technician	12,000
50% of a students stipend	5,000
Supplies	10,000
Administrative support	2,000
	$ 46,000

(plus 42% university overhead)