'Antineoplastons'

An Unproved Cancer Therapy

Saul Green, PhD

UNORTHODOX and unproven treatments for cancer are big business in the United States.[1,2] Usually, individuals providing these treatments offer the public little or no information on their qualifications to treat cancer. Their science is generally presented in unverifiable testimonials, anecdotal material, and non–peer-reviewed magazines sold in supermarkets or publicized on television talk shows and in throwaway health-fair circulars. When reviewed, they reveal a patchwork of half-truths and scientific misinformation.

In contrast, there is no lack of published material for the patient who may be considering antineoplaston therapy. There are hundreds of papers about this therapy and its discoverer, Stanislaw R. Burzynski, MD.[3-9] They include his curriculum vitae, his list of publications, explanations of his theory of cancer and the way his treatment works, clinical information, press releases, brochures, abstracts of his speeches, reports of his research results, review articles, government reports, court opinions, legal depositions, records of public hearings, and transcripts from television talk shows.

This article reviews material on the subject of antineoplaston therapy for cancer, so that the reader can come to an informed conclusion as to the validity of the claims made for its scientific basis.

INFORMATION FROM BURZYNSKI'S PUBLICATIONS

Background and Credentials[9]

Burzynski's graduation from the Medical Academy in Lublin, Poland, in 1967 coincides with his claim to have discovered the peptides that control cancer growth in the human body, which he later named *antineoplastons*. He received a doctorate in medical science in 1968, interned at Lublin in surgery, internal medicine, pediatrics, and obstetrics and gynecology, and he then undertook a residency in internal medicine. Burzynski came to the United States in 1970 and worked as a research associate in the Department of Anesthesiology, Baylor College of Medicine, Houston, Tex, where he isolated peptides from the brain tissue of conditioned rats.[10,11] He was licensed to practice medicine in 1973, published his theory of antineoplastons in 1976,[6] and began treating patients at his newly opened Burzynski Research Institute (BRI) in 1977.[7]

Hypothesis of Antineoplastons

In 1976, Burzynski[6] proposed that since cancer was a disease of differentiation and since new cells are constantly being produced, groups of abnormal cells could constantly arise as a result of the influence of carcinogenic factors. Without a reliable mechanism for "normalizing" such erroneously developed cells, he hypothesized, the organism would not live very long. Since spontaneous regression of cancer does occur, he proposed that a normalizing mechanism must therefore exist in the body. Based on this reasoning, Burzynski suggested that the ideal approach to cancer therapy would be to direct cancer cells into normal channels of differentiation. He named those naturally occurring substances that could "normalize" cancer cells *antineoplastons*. Because the scientific literature identified peptides as molecules that carried information, he concluded that antineoplastons must be peptides. Since peptides were found in the urine, he judged urine to be the most economical source for the isolation of antineoplastons.

Antineoplaston Literature

The current antineoplaston literature contains more than 140 citations.[9] Between 1964 and 1972, there are 23 citations. Burzynski's earliest studies conducted in Poland describe methods for the isolation and quantitative measurement of peptides from mushrooms and from the blood of humans with renal disease, heart disease, and obesity. The studies conducted in the United States deal with peptides from rat brains.[10,11] The first report of an effect of peptides from human urine on cancer cells in vitro appeared in 1973.[3] A 3-year National Cancer Institute (NCI, Bethesda, Md) grant (RO-1-15056) was awarded in 1974.[4] From 1973 through 1976, Burzynski worked on methods for extracting peptides from urine, methods for their quantitative determination, and the effects of urinary peptides on isolated frog hearts and intestinal smooth muscle.[12,13] In 1976, he published one article on the effect of urinary peptides on tumor cells in vitro.[5]

Burzynski's theory of the cause and cure of cancer was published in 1976.[6] In 1977, he used a urine extract he called *antineoplaston A* to treat 21 cancer patients.[7] In 1985, Burzynski described the production of eight antineoplastons in a US patent.[8] He named them *A-1*, *A-2*, *A-3*, *A-4*, *A-5*, *A-10*, *AS 2.5*, and *AS 2.1*. He claimed A-10 was the active component present in the urinary antineoplastons and identified it as 3-N-phenylacetylaminopiperidine-2,6 dione. Two products, AS 2.5 and AS 2.1, were made from A-10. All three, A-10, AS 2.5, and AS

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2.1, could be synthesized in the laboratory. At present, over 95% of his patients are treated with synthetic preparations of antineoplastons that do not contain any material isolated from human urine (written communication, S. R. Burzynski, MD, May 1988).

Since 1977, the antineoplaston literature has covered the following: proposed mechanisms of antineoplaston interaction with DNA, effects on protein synthesis and cell division, toxic effects studies in mice, and phase-1 trials in cancer patients.

Literature[14] provided by the BRI states that research groups headed by Xu in China,[15] Muldoon and Hendry in the United States,[16-18] and Tsuda in Japan[19,20] have confirmed preclinical and clinical research results with antineoplastons. Burzynski Research Institute publications also claim that researchers at Sigma Tau, a pharmaceutical manufacturer located in Rome, Italy, feel there is evidence to justify the use of antineoplastons to treat cancer in humans.[21,22]

A CRITIQUE OF BURZYNSKI'S CLAIMS

Burzynski states that he discovered a naturally occurring biochemical anticancer surveillance system in humans in 1967.[9] Between that time and his departure for the United States, he claims[23] to have received a PhD degree in biochemistry. Professor Stanislaw Bilinski, the current chairman of the Department of General Chemistry at Lublin, who remembers Burzynski as a student (written communication, March 1987), stated the following:

> From December 15, 1966, to September 30, 1967, Burzynski worked as a scientific technical assistant in the Department of General Chemistry. He received his diploma as an MD on February 18, 1967, and a doctorate in medical sciences in 1968. To the best of my knowledge he did not do any independent research while he was at the Academy.

Burzynski's bibliography does not identify a PhD dissertation.[9]

None of the first 23 papers in Burzynski's bibliography, from 1964 through 1972,[9] deals with cancer or the effects of urinary peptides on cancer. None mentions information-carrying peptides with an ability to induce differentiation in cancer cells, and there is no published evidence that Burzynski experimentally tested his hypothesis that information-bearing peptides from urine could normalize cancer cells.

The methods used to produce and identify urinary antineoplastons described in his 1985 US patent[8] are as follows. Two thousand to 3000 liters of urine were processed in batches to produce the amount of each of the five urinary antineoplastons required for use. This huge volume of urine was collected and transported frequently from various sites around the city of Houston, where the weather is frequently hot. In reply to a letter requesting information about the precautions taken to prevent infection and contamination with bacteria and the acceleration of their growth in the urine during collection, storage, and transportation, as well as the methods used to remove bacteria, viruses, pyrogenic material, and other substances that might be present because of the medical conditions of the donors, Burzynski replied as follows (written communication, May 1988):

> I would like to explain to you that, at present (May 3, 1988), over 95% of our patients are treated with synthetic preparations of antineoplastons that do not contain any material from human urine. As far as the formulations obtained from urine are concerned, we are running multiple tests to check if we have any endotoxins in preparations during different steps of the procedure. The procedure is designed this way so that it should eliminate any proteins, including endotoxins, in the first step. Our production facilities were inspected repeatedly by the FDA [US Food and Drug Administration], and after the most recent inspection, we have in writing from the FDA that we are in full compliance with current good manufacturing procedures. Fever and chills observed in some of the patients some time after administration of the medicine are usually related to extensive tumor necrosis.

The process used for sterilization of the urine and its fractions is described in Burzynski's 1985 patent as filtration and ultrafiltration. Although the patent states that precautions were necessary to rid the raw material of contaminating microorganisms, Burzynski offers no specific information about the methods used, how often they were applied, or how successful they were. According to the FDA's guidelines for good manufacturing procedures[24] and the Health Industry Manufacturing Association's guidelines for sterilization of pharmaceutical products,[25] the use of filtration processes intended to result in sterilization of a product are effective only when the mass of bacterial contamination is low, when the conditions for regrowth of the microorganisms are tightly controlled, and only with very low or nonexistent amounts of pyrogenic endotoxins in the reagents or on the surfaces of the apparatus and glassware at the beginning of the process. Bacterial endotoxin[26] contaminates all unsterilized liquids and surfaces. It is a low–molecular-weight fatty substance, not a protein, and is not removed from solutions by ultrafiltration. Finally, the FDA will not confirm that it stated in writing that it considered the manufacturing plant at BRI to be operating in accordance with the FDA's good manufacturing guidelines (oral communication, S. Miller, FDA offices, Houston, Tex, October 1991).

Five fractions were produced from human urine by Burzynski, A-1, A-2, A-3, A-4, and A-5. For these, five chromatograms are shown,[8] and each is said to specifically represent one fraction. The five chromatograms are nearly identical both qualitatively and quantitatively, and without the figure number assigned to each, it would be almost impossible to distinguish one from the other. Data in this patent clearly show that all five fractions have essentially the same anticancer activity and the same degree of toxicity. Although the text implies that they all contain the antineoplaston A-10, Burzynski does not offer an explanation for the basis on which he chooses any one specific fraction for treatment of a patient, or why he has never reported using fractions A-1 or A-4 to treat patients. Burzynski claimed that A-10 from urine fraction A-2 is the active factor common to all the fractions.[8] But since A-10 was not isolated from any of the other urinary fractions, there is no basis for this claim.

The method for the synthesis of A-10 is presented in Burzynski's 1985 patent. In this method, phenylacetylglutamine (PAG) is synthesized from glutamine and phenylacetyl chloride. Acidification of the solution containing the PAG converts it to the piperidine A-10 by cyclization of its glutamine moiety through removal of one molecule of water. Since Burzynski's process for producing A-10 from urine involves acidification of urine containing PAG, the PAG must be the precursor of the A-10, which he isolates from urine fraction A-2.

The antineoplastin A-10 (3-N-phenylacetylaminopiperidine-2,6 dione) is insoluble in aqueous solutions.[8] Nevertheless, Burzynski states that it is produced in the body and circulates normally in aqueous biological fluids like blood and urine. He offers no explanation of how or where this insoluble substance is made or how it gets from the blood, through the kidneys, and into the urine.

Being insoluble, A-10 is obviously not suitable for intravenous administration. Burzynski says that treatment of A-10 with sodium hydroxide and heat results in the production of the water-soluble sodium salt.[8] In a later paper,[27] Ashraf et al (Burzynski was a coathor) state that A-10 is unstable in alkali and breaks down (hydrolyzes) to yield PAG. As we have seen, this is the urinary

substance from which the A-10 was derived in the first place. Therefore, the "soluble" A-10 that Burzynski says he is using in intravenous injections and infusions is not the soluble sodium salt of A-10 but is the sodium salt of PAG. The Chinese researchers that Burzynski says confirmed his work with antineoplastons stated this fact in one of their papers.[15]

Phenylacetylglutamine is a waste product that is only found in the urine of humans.[28,29] It results from conjugation of glutamine in the liver, with the organic acid, phenylacetic acid (PA). The toxicity of PA in humans has been recognized since 1919[30] and more recently has been associated with the brain damage due to the faulty amino acid metabolism of phenylketonuria.[31-32]

Some of Burzynski's patients may be given the insoluble (authentic) A-10 by mouth.[9] Burzynski[33] has reported that insoluble A-10 that is ingested is rapidly converted to PAG by alkaline digestive juices in the small intestine. Therefore, it is PAG, and not A-10, that is absorbed into the circulation from the small intestine after insoluble A-10 is ingested. This is of special interest because experimental data in Burzynski's earlier work showed that PAG was ineffective against cancer cells.[8,34] Burzynski supported his conclusion by citing the work of Israeli researchers[35] who obtained the same results in 1977.

Two antineoplastons, AS 2.5 and AS 2.1, have been derived from A-10.[8] The antineoplaston AS 2.5 is PAG and AS 2.1 is a 4:1 mixture of PA and PAG. In 1969, Burzynski recognized that metabolically produced PA was toxic in humans and needed to be detoxified for safe excretion in the urine.[36] Since PA is a strong acid, it is not surprising that AS 2.1, which is 80% PA, should cause the death of cells in culture. In evaluating Burzynski's reported results with AS 2.1, it must also be recognized that as a strong acid, PA must be neutralized with sodium hydroxide before it is added to the culture medium. Thus, the cytotoxicity of AS 2.1 might be due as much to the high salt concentration as to the PA.[34]

In a letter written to me in May 1988, Burzynski stated that 95% of BRI's patients were being treated with synthetic A-10 or AS 2.1. The antineoplaston AS 2.5 was not mentioned. Since neither AS 2.1 nor A-10 is a peptide and neither has been shown to carry information that will induce differentiation in tumor cells in vivo, these products do not qualify as antineoplastons by Burzynski's own definition. The component that makes up 80% of AS 2.1, PA, can be purchased as an ultrapure, water-soluble powder from any chemical supply house for about $0.09 a gram.[37]

The antineoplaston A-10 is 3-N-phenylacetylaminopiperidine-2,6 dione.[8] The pharmacology literature lists at least two pharmacologically potent compounds that are also piperidine 2,6 diones. They are glutithamide and thalidomide.[38] Both drugs have been withdrawn from the pharmaceutical marketplace because both are habituating and both can cause peripheral neuropathy. The teratogenic effects of thalidomide have been documented and widely known since the 1960s.[39,40] These substances and A-10 are currently classified by the US Drug Enforcement Agency as controlled substances in the 1985 US Code of Federal Regulations, title 211, part 1308.13.[37] In spite of the striking structural similarities between A-10 and these two dangerous drugs, there is no evidence in the antineoplaston literature that testing of the potential of A-10 to induce teratogenicity or peripheral neuropathy has been carried out.

Burzynski makes a strong effort through his public information office to convince his supporters and patients that his clinical successes with the antineoplastons are being confirmed by independent researchers around the world. The average reader of his press releases has no way of knowing the truth about what is being claimed, but a critical reviewer can verify the references cited, evaluate the reported experimental results, and make inquiries of those scientists whose work is cited. For this review, whenever confirmations of Burzynski's clinical results were announced in his press releases, each research worker named was contacted, when possible.

The BRI[14] claimed that Xu and associates of the Department of Pharmacy, Shandong Medical University, Jinan, China, reported a new antitumor assay for A-10 and the effects of A-10 on cyclic AMP levels in tissues and tumors of tumor-bearing mice. Their results indicated induction of cell differentiation.

In response to my inquiry, Xu sent (in 1989) four abstracts and one published article[15] reporting that A-10 had no anticancer effect when assayed by standard animal assay methods, that using a revised assay (undescribed), some evidence of inhibition in tumor cell growth could be seen, and that some effects were observed on the cAMP in the tumors of mice that were fed A-10. An antitumor effect against S-180 tumor cells in culture was reported when soluble A-10 was added at 3.0 mg/mL. No effects were seen in vivo. Xu concluded the following: "Since soluble A-10 is really PAG, it cannot be intercalating DNA in the cells."[15]

The claim that antineoplastons work by interacting with DNA has also been examined by workers in the United States. Lehner et al,[16] Hendry et al,[17] and Muldoon et al[18] used spectroscopic analysis and stereochemical modeling studies to see whether the molecular structure of insoluble A-10 might allow it to insert between base pairs of partly unwoven strands of DNA to compete with carcinogens that intercalate DNA. Based on theoretical considerations, Hendry et al[17] concluded that insoluble A-10 could form a weak, noncovalent, reversible link between a base pair and a phosphate in DNA. Insoluble A-10 might therefore block the intercalation of some carcinogenic compounds into DNA and prevent the events that initiate cancer cell growth. But this conclusion does not support the concept that insoluble A-10 would be useful in treating an existing cancer.

Hendry et al[17] used insoluble A-10 in all their modeling studies. They did not report using soluble PAG. But as we have seen, the substance reaching the tissues is not the insoluble A-10, but PAG. Therefore Burzynski's declaration that A-10 acts as an antineoplastic agent by blocking the intercalation of DNA by carcinogenic compounds is experimentally without foundation.

To clarify the relationship between the research done at the Medical College of Georgia, Augusta, and the claims of support that Burzynski attributed to that research, Hendry and Muldoon have advised Burzynski that their work does not provide support for the use of A-10 in human subjects, and that, to their knowledge, no one at the Medical College of Georgia has ever evaluated or advocated the use of A-10 in patients (written communications, T. G. Muldoon, PhD, and L. B. Hendry, PhD, November 1988). Burzynski was instructed not to use the name of the Medical College of Georgia in any of his publications or public presentations without prior approval (written communications, C. H. Wray, MD, and L. Greenbaum, PhD, November 1988).

The BRI[21,22] also claimed the following:

We are happy to report that by the end of this year, at least three countries outside of the US will be conducting clinical trials with the Burzynski treatment. In March 1990, Dr H. Tsuda, of Kurume, Japan, will be presenting 1989 clinical trial results with antineoplastons to the 9th International Symposium on Future Trends in Geneva, Switzerland. In Poland, six different clinical trials are beginning this year under the supervision of the Institute for Drug Research and Control, Warsaw. They will be using naturally occurring chemicals called antineoplastons to

treat patients with brain cancer, non-Hodgkins lymphomas, prostate cancer, and breast cancer. Researchers from Sigma Tau, Italy's largest pharmaceutical firm, are in Ireland finalizing preparations for clinical trials. The researchers at Sigma Tau feel there is adequate scientific evidence to justify the use of antineoplastons to treat cancer. Millions of dollars have been earmarked for antineoplaston research and development as the company prepares to conduct preclinical and clinical studies in specific European countries to evaluate four of Dr Burzynski's formulations.

The following individuals responded to my inquiries regarding the BRI's claim: H. Tsuda, MD; T. Sugimura, MD; C. Trevisani, MD; and A. Danysz, MD.

Tsuda, from the Kurume University School of Medicine, Japan, wrote the following on October 9, 1990: "Regarding your questions to our clinical investigation, we are afraid you have to wait until we publish the data." On January 7, 1991, Tsuda wrote the following: "We have not published any results of our clinical investigation on antineoplastons. You have to wait for our publication. We do not think that you are going to pick up any biological effect of antineoplaston A-10 in our study."

Sugimura, president of the National Cancer Center, Tokyo, Japan, wrote, on July 24, 1990: "I am afraid that antineoplaston A10 has no popularity in our country."

Trevisani, medical director of Sigma Tau Pharmaceuticals Inc, Rome, Italy, wrote, on May 22, 1991:

> Dr Burzynski was informed on January 31, 1991, that Sigma Tau did not intend to proceed with the development of the antineoplastons. . . . We have studied antineoplaston A-10 and AS 2.1; both compounds were supplied by Dr Burzynski. . . . We have tested AS 2.1 and A-10 in several in vitro experiments on human and murine tumor cell lines. In addition, we have studied the percentage of survival and the mean survival time of tumor-bearing conventional mice and the effect in nude mice transplanted with human colon carcinoma. On the basis of these results, the project has been discontinued and more extensive testing or clinical trials have not been planned. These results have not been published. Dr Burzynski was notified of these results and our decision to discontinue the project on January 31, 1991.

Danysz, director of the Institute of Drug Research and Control, Warsaw, Poland, wrote, on July 8, 1991: "Antineoplastons may be investigated in some clinics in Poland, but the Institute of Drug Research and Control is not supervising any of these studies."

RESULTS OF INDEPENDENT TESTING OF ANTINEOPLASTONS

In 1983 and 1985, at the request of the Bureau of Prescription Drugs of Health and Welfare, Canada, the NCI conducted tests of A-2 and A-5. The tests were done at the Southern Research Institute, Birmingham, Ala, using the P 388 leukemia in mice as the tumor target. The results showed that those doses that were high enough to produce toxic effects in the mice were not effective in inhibiting the growth of the tumor or in killing it (written communications, N. H. Greenberg, PhD, November 1983, and J. M. Venditti, PhD, March 1985).

In 1990, the NCI carried out a series of tests using antineoplaston A-10 against a standard panel of tumors that included different cell lines from tumors of the following classes: leukemia, non–small-cell lung cancer, small-cell lung cancer, colon cancer, cancer of the central nervous system, melanoma, ovarian cancer, and renal cancer. The chief of the Drug Synthesis and Chemistry Branch of the NCI reported the following: "The drug exhibited neither growth inhibition nor cytotoxicity at the dose levels tested" (written communication, V. L. Narayanan, PhD, July 1990).

These test results, along with those reported from the in vitro and in vivo trials carried out at Sigma Tau Pharmaceuticals Inc are compelling evidence of the lack of efficacy of antineoplastons against experimental cancer.

CONCLUSION

This article reviews the claims made by Burzynski in support of his theory that an antineoplastic biochemical surveillance system exists in humans. Burzynski's own literature is the basis for the conclusions reached herein.

The rationale upon which the existence of antineoplastons was postulated is as follows: (1) cancer cells are constantly produced in the body, but not everyone develops cancer; (2) cancer that exists in people can regress spontaneously, indicating the presence of a "normalizing" mechanism; (3) cancer is a disease of cell differentiation and certain information-carrying chemicals can induce differentiation; (4) peptides are chemicals that can carry information that can trigger biochemical reactions in cells; and (5) peptides with antineoplastic activity, antineoplastons, are found in human urine and therefore urine is the best place to look for naturally occurring antineoplastic substances.

None of Burzynski's publications between 1964 and 1990 contain objective experimental evidence supporting the postulate that a naturally occurring antineoplastic biochemical surveillance system exists in humans. The so-called five urinary antineoplastons (A-1 to A-5) have not been shown to be chemically, biologically, or pharmacologically distinct from each other, and none has been proven to have antineoplastic activity against experimental cancer. Only one urine fraction (A-2) has been used to produce antineoplaston A-10. The admitted insolubility of A-10 makes it physiologically incompatible with aqueous body fluids, so it cannot be part of the normal anticancer system that Burzynski postulates circulates throughout the body.

The process that Burzynski says "solubilizes A-10" does not convert it to the sodium salt but hydrolyzes it to PAG, now named AS 2.5, which is not an information-carrying peptide. Interestingly, Burzynski does not cite AS 2.5 as an antineoplaston in his most recent *Patients Information Brochure*. The antineoplastin AS 2.1 also contains no information-carrying peptides but is a mixture of synthetic PAG and PA.

None of the independent tests carried out with antineoplastons in experimental tumor systems have shown anticancer activity.

These considerations lead to the conclusion that the treatment for cancer with substances called antineoplastons actually involves the use of two simple commercially available organic chemical compounds, PA and PAG, which are marketed under the names A-10, AS 2.1, and AS 2.5. None is a peptide, none has been shown to "normalize" tumor cells, none has been shown to actually intercalate DNA, and none has been proven to be active against cancer in experimental tumor test systems.

Since the manuscript was accepted for publication, the NCI announced that on October 4, 1991, an NCI site-visit team, headed by M. J. Hawkings, MD, visited the BRI, where they reviewed a best-case series of seven patients prepared for them by Burzynski. The team did not independently contact the patients or the physicians who previously treated them. Based on their review, the NCI has decided to conduct four independent phase-2 clinical trials on patients with glioblastoma multiforme, anaplastic astrocytoma, pediatric brain tumors, and low-grade astrocytoma using antineoplastons A-10 and AS 2.1 (written communications, BRI Inc, December 1991, and M. J. Hawkings, MD, chief, Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, February 1992).

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Green concludes that "[n]one of the independent tests carried out with antineoplastons in experimental tumor systems have shown anticancer activity." However, following a site visit to Japan by the Food and Drug Administration (FDA) to review the University of Kurume Medical School's independent tests documenting antineoplaston antitumor activity in animals, the FDA approved our Investigational New Drug application for antineoplaston A-10 on March 16, 1989.

It is of special interest that Green omits the official statement of the NCI following their site visit to our Institute: "The National Cancer Institute reviewed 7 cases of primary brain tumors that were treated by Dr Burzynski with antineoplastons A-10 and AS 2.1 and concluded that antitumor responses occurred." (written communication, NCI, Office of Cancer Communications, January 6, 1992).

Green asserts that there is no published evidence that antineoplastons can induce differentiation in cancer cells. To the contrary, there are many, including published studies authored by several researchers from the NCI.[3,4]

Green states that A-10 is insoluble in aqueous solutions (incorrectly citing one of my patents, which is silent on this). The solubility of A-10 in water is within the range of solubility of amino acids—more soluble, in fact, than tyrosine and tryptophan. Many important biological substances, such as steroid hormones, have a solubility much lower than A-10.

Contrary to Green's statement, "In 1969, Burzynski recognized that metabolically produced PA [phenylacetic acid] was toxic in humans," the publication referenced by Green does not even mention PA. Green seems unaware that since 1980 the sodium salt of PA, phenylacetate, has become an investigational new drug approved for human use by the FDA and has already been established as safe and effective in the treatment of hyperammonemia.[5] It is important to note that phenylacetate has been successfully used in the treatment of children a few months to a year old.[5]

Stanislaw Burzynski, PhD, MD
Houston, Tex

Dr Burzynski is chairman of the board, chief executive officer, and majority stockholder of Burzynski Research Institute, Inc, which has the exclusive license for antineoplastons in the United States.

1. Green S. 'Antineoplastons': an unproved cancer therapy. *JAMA*. 1992;267: 2924-2928.
2. Burzynski S. *Investigations on Amino Acids and Peptides in Blood Serum of Healthy People and Patients With Chronic Renal Insufficiency*. Lublin, Poland: Medical Academy of Lublin; 1968. Doctoral dissertation.
3. Samid D, Shack S, Sherman LT. Phenylacetate: a novel nontoxic inducer of tumor cell differentiation. *Cancer Res*. 1992;52:1988-1992.
4. Samid D, Yeh TJ, Shack S. Interferon in combination with antitumourigenic phenyl derivatives: potentiation of IFN activity in vitro. *Br J Haematol*. 1991;79(suppl 1):81-83.
5. Brusilow SW, Danney M, Waber LJ, et al. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N Engl J Med*. 1984;310:1630-1634.

Antineoplastons: The Controversy Continues

To the Editor.—As a paid consultant to those formerly in litigation against me, Dr Green[1] can scarcely be considered impartial. Not surprisingly, Green gravely misrepresents more than 20 years of research. Due to space constraints, I can address only a few of Green's misstatements, and therefore am unable to counter one of the most serious problems of the article, Green's omission of dozens of publications.

Green disputes my claim to a PhD degree in biochemistry and asserts that my bibliography does not identify a dissertation. The sworn affidavit of Professor Zdzislaw Kleinrok, president of the Medical Academy of Lublin, Poland, confirms that my PhD in biochemistry was awarded on October 16, 1968. My doctoral dissertation[2] is always listed in my bibliography.

Green refers to tests done by the National Cancer Institute (NCI) as "compelling evidence of the lack of efficacy of antineoplastons against experimental cancer." Green fails to mention a third series of NCI tests, performed in 1992 for the first time at a correct dosage level and in a proper model, which demonstrated the anticancer activity of antineoplastons A-10 and AS 2.1 (written communication, M. R. Grever, MD, acting associate director, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, 1992).

To the Editor.—As a medical writer who has investigated antineoplastons, I found that numerous assertions in the article by Dr Green[1] appear to be at odds with the documentation. He claims, for example, that "none of the independent tests . . . have shown anticancer activity." Yet his article cites three independent animal studies (references 18 through 20) from Japan and the United States regarding tumor inhibition by antineoplastons. Focusing on letters, Green ignores these published results.

Also ignored were the results of published clinical studies by Dr Burzynski (references 7 and 33). Green repeatedly misstates the work of Burzynski. Contrary to Green, I found Burzynki's 1968 doctoral dissertation in biochemistry listed in the bibliography that Green claims omits it.

The article concludes that phenylacetic acid, a component of the therapy, is not active against cancer. Yet scientists at the NCI have reported that phenylacetate is a "nontoxic

inducer of tumor cell differentiation."[2] In a note at the end, Green admits that the NCI reviewed seven cases treated with antineoplastons and plans to conduct not one but four phase II clinical trials. Would it do so without reason?

Unmentioned were the findings of the case review. According to the agency, the NCI "concluded that antitumor responses occurred."

Robert G. Houston
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1. Green S. 'Antineoplastons': an unproved cancer therapy. *JAMA.* 1992;267:2924-2928.
2. Samid D, Shack S, Sherman L. Phenylacetate: a novel nontoxic inducer of tumor cell differentiation. *Cancer Res.* 1992;52:1988-1992.

To the Editor.—Dr Green has presented a very cogent exposé of a troubling cancer therapy of unknown efficacy.

Green analyzes in exquisite detail the biochemical and experimental results associated with antineoplastons, which would raise questions about their use in any clinical setting. It is one thing for new drugs under development to be studied in a classic phase I study, having gone through preclinical trials and deemed valid to merit clinical study after careful peer review. This apparently is not the case in the antineoplaston story, which raises serious concerns as to how this type of activity is allowed to continue.

I have had personal experience with one patient, a 38-year-old woman, who developed recurrent rectal cancer involving the lower pelvis. This patient's cancer had progressed in spite of treatment with radiation and chemotherapy and was looking for alternative therapy. She found her way to the Burzynski Clinic where she was treated for nearly a year. During this time, her tumor progressed. At this point, we saw her and were asked to do a resection. We began retreatment of this patient with a fluorouracil-leucovorin combination, followed by radical resection. We did obtain palliation, but the patient succumbed to her disease some 6 months later.

Green's article does a major service as it provides information to physicians who otherwise would not be familiar with this class of unorthodox and untested therapy.

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In Reply.—The conclusions in my article were reached following a review and evaluation of the science in the material Dr Burzynski published. Rather than rebutting my conclusions, he resorts to charges of partiality because I served as scientific adviser in a litigation against him. Since my conclusions derived directly from his own statements, they would have been no different had I worked for Burzynski himself. I did not cite his other papers[1] because they were clinical reports, non–peer-reviewed speeches, or redundant reviews and as such were irrelevant for the evaluation of his science.

Burzynski's curriculum vitae[1] does not list any paper as a PhD thesis. The Medical Academy of Lublin does not offer a PhD program (written communication, A. Gandara, International Education Research Foundation, Credentials Evaluation Service, Los Angeles, Calif, June 1991), a former professor recalls that his degree in 1968 was the DMSc,[1] his first US grant (Antineoplastic peptides from urine. NCI grant CA15056, 1974) lists his second degree as a DMSc, and the Polish Ministry of Health and Social Welfare says Polish medical schools do not confer the degree of PhD (written communication, R. Nizankowski, MD, May 12, 1992).

By calling attention to Samid's work in 1991 (Burzynski's references 3 and 4), Burzynski confirms that he has been treating patients with antineoplastons for 15 years with no evidence that they "normalize" cancer cells in vivo. I did not cite Samid's work because it was published 6 months after my paper was submitted for publication. Her coauthor did write to me to emphasize that a distinction should be made between antineoplastons and phenylacetate (written communication, C. Myers, MD, NCI, June 13, 1992). Phenylacetate, he said, was a defined chemical entity, inexpensive and readily available from commercial suppliers, while antineoplastons were "mixtures" prepared by Burzynski.

The NCI announced phase II trials with AS 2.1 and A-10 based on a "best case review" during a site visit in October 1991. They presented no supporting preclinical data or phase I trial results. Burzynski's claim now, that recent NCI tests with A-10 and AS 2.1 showed anticancer activity, is erroneous. The results of the tests that were done in December 1991 were sent to Burzynski in March 1992, and reported that A-10 and AS 2.1 had no anticancer activity (written communication, S.A. Shepartz, PhD, NCI, July 9, 1992).

The 5-year hold on Burzynski's Investigational New Drug application was lifted in March 1989 because FDA site visitors to Kurume, Japan, thought A-10 might be safe in humans, not because they saw effectiveness. Three years later, Burzynski has still not carried out the trials that were approved.

Medical writer Houston has made a career of condemning all who criticize "unproven treatments," but there is no evidence that he ever critiqued the science of any of them. Because he cannot refute my conclusions about the antineoplastons, he attempts to becloud the issues with the shibboleth of bias and conspiracy. If he knows the antineoplaston literature, he knows that Tsuda (references 19 and 20) and Hendry (reference 18) have worked with Burzynski. In spite of the fact that Hendry has coauthored 12 articles with Burzynski,[1] he has not endorsed the use of antineoplastons as a treatment for cancer patients.

Houston asks, "Would NCI conduct four phase II trials without reason?" I would also like to know the scientific reason for their decision.

Saul Green, PhD
New York, NY

1. *Publications of SR Burzynski.* Houston, Tex: The Burzynski Clinic; 1990. Burzynski Research Institute promotional brochure.

CORRECTIONS

Line of Text Misplaced.—In the Clinical Cardiology article entitled "Guidelines for the Diagnosis of Rheumatic Fever: Jones Criteria, 1992 Update," published in the October 21, 1992, issue of THE JOURNAL (1992;268:2069-2073), a line of text was misplaced. The last line of the first column on page 2069 should be omitted. The third sentence in the second column on page 2069 should read as follows: "These updated guidelines also expand on the available diagnostic tools and clarify the supplemental evidence of group A streptococcal infection."

Error in Table.—An error occurred in the Original Contribution entitled "Reversing the Natural Decline in Human Fertility: An Extended Clinical Trial of Oocyte Donation to Women of Advanced Reproductive Age," published in the September 9, 1992, issue of THE JOURNAL (1992;268:1275-1279). In Table 3 on page 1278, the correct percentage of the ratio of clinical pregnancies per transfer attempt for women aged 40 years and above using donor in vitro fertilization should be 39.5% [not 34.5%].

A 1995 Critical Scientific Assessment of the ***Journal Of The American Medical Association's*** article:

'Antineoplastons': An Unproved Cancer Therapy, written by Saul Green, PhD

(JAMA, June 3, 1992—vol 267 No. 21; pg 2924-2927)

This is a document drafted by an independent scientist who worked for the United States government hired in the 1990s to independently study the toxicity and efficacy of Antineoplastons. It was written in 1995. Some of the information is dated, but serves as an independent rebuttle to JAMA's 1992 article "Antineoplastons: An Unproved Cancer Therapy"

This scientist wishes to remain anonymous.

For more information on the documentary film "Burzynski, the Movie" visit: www.burzynskimovie.com

For more information on Burzynski's clinic, visit: www.burzynskiclinic.com

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1. Purpose and intent of Dr. Green's article on Antineoplastons

Green's statement	Burzynski's rebuttal	Independent Assessment
"This article reviews material on the subject of antineoplaston therapy for cancer, so that the reader can come to an informed conclusion as to the validity of the claims made for its scientific basis." "Since my conclusions derived from his own statements, they would have been no different had I worked for Burzynski himself. I did not cite his other papers because they were clinical reports, non-peer-reviewed speeches, or redundant reviews and as such were irrelevant for the evaluation of his science."	"As a paid consultant to those formerly in litigation against me, Dr. Green can scarcely be considered impartial...Green gravely misrepresent more than 20 years of research...(including) (1) severe distortion of the literature reviewed, (2) selective omission of the most relevant and recent literature, (3) reliance upon comments of subordinates completely contradicted by written documents of their superiors, and (4) a grave misunderstanding of some of the basic science involved."	Dr. Burzynski was correct in his rebuttal points 1,2, and 4. I have not commented on the third one because it is not pertaining to science. In addition, it is my opinion that clinical findings are more important than laboratory studies since preclinical studies are used to help understand clinical efficacy of the drugs and their findings cannot supersede the human reality. Dr. Green clearly was blind to the clinical findings in the best case series conducted by the NCI experts and other results published by Dr. Burzynski. Secondly, Dr. Green also contradicts himself when he states that non-peer-reviewed articles are not relevant for review, while all the publications by Dr. Burzynski that he quotes or misquotes are mostly non-peer-reviewed. Why is he contradicting himself? What is his rationale for doing the commentary for JAMA? One explanation may be that he did not review the literature with a pre-set research methodology, clearly laying out his approach to the literature, peer-reviewed or not. Moreover, in his 1993 reply to Burzynski's rebuttal, Dr. Green did not change any of his views in light of the fact that several more studies were published by groups independent of Burzynski, showing anticancer and differentiation-inducing ability of phenylacetate, the major ingredient of Antineoplaston AS2-1, after the publication of his commentary in JAMA. Indeed, Dr. Green did not give a well-informed conclusion, which may mislead the readers to wrongly judge Antineoplastons.

Independent Assessment

2. Antineoplaston A10 is an artifact of urine acidification

Green's statement	Burzynski's rebuttal	Assessment
"Phenylacetylglutamine is synthesized from glutamine and phenylacetyl chloride. Acidification of the solution containing the PAG converts it to the piperidine A-10 by cyclization of its glutamine moiety through removal of one molecule of water. Since Burzynski's process for producing A-10 from urine involves acidification of urine containing PAG, the PAG must be the precursor of the A-10, which he isolates from urine fraction A-2."	Contrary to Green's assertion, the actual process of A10 synthesis requires not only acidification, but also a high temperature of 160 C for an extended period of time in order to cyclise the molecule of PAG.	If the cyclization of PAG requires high heat, it is thermal dynamically impossible at room temperature to achieve that reaction. Under that premise, Antineoplaston A-10 cannot be an artifact due to acidification of the urine.

3. Water-solubility and urine origin of Antineoplaston A10

Green's statement	Burzynski's rebuttal	Assessment
"The antineoplaston A-10 is insoluble in aqueous solutions... (However,) He (Burzynski) offers no explanation of how and where this insoluble substance is made or how it gets from the blood, through the kidneys, and into the urine....The admitted insolubility of A-10 makes it physically incompatible with aqueous body fluids, so it cannot be part of the normal anticancer system that Burzynski postulates circulates throughout the body."	Green mistakenly concludes that A10 is insoluble in aqueous solutions..In support of this assertion, Green cites one of my U.S. patents, but nowhere in this patent is A10 described as insoluble in aqueous solutions. The solubility of A10 in water is within the range of solubility of amino acids - more soluble in fact than tyrosine and tryptophan. Many important biological substances, such as steroid hormone, have solubility lower than A10.	Antineoplaston A10 has limited water solubility, but it is not water insoluble. Dr. Burzynski provides adequate explanation on the water-solubility of Antineoplaston A10 in his rebuttal. Plasma levels of rats within 2 to 3 hours of oral administration of 150 mg/kg of A10 rose to about 0.7 mM, indicating certain water-solubility of A10. Moreover, limited water solubility of antineoplastic agents, such as retinoic acid, does not exclude them from being anticancer agents. However, Dr. Burzynski did not address the urine origin of A-10 put forth by Dr. Green.

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4. Conversion of Antineoplaston A10 to phenylacetylglutamine (PAG)

Green's statement	Burzynski's rebuttal	Assessment
"Being insoluble, A10 is obviously not suitable for intravenous administration... treatment of A10 with sodium hydroxide and heat results in the production of the water soluble sodium salts. In a later paper, Ashraf et al state that A10 is unstable in alkali and breaks down to yield PAG. As we have seen, this is the urinary substance from which the A10 was derived in the first place. Therefore, the soluble A10 that Burzynski says he is using is not the soluble sodium salt of A10 but is the sodium salt of PAG. The Chinese researchers that Burzynski says confirmed his work with antineoplastons stated this fact in one of their papers." "Some of Burzynski's patients may be given the insoluble (authentic) A-10 by mouth. Burzynski has reported that insoluble A-10 that is ingested is rapidly converted to PAG by alkaline digestive juices in the small intestine. Therefore, it is PAG, and not A-10, that is absorbed into the circulation from the small intestine after insoluble A-10 is ingested."	During pharmacokinetic studies of A10 administered orally, there was no substantial hydrolysis in simulated gastric juice, about 30%of A10 was hydrolyzed when exposed to simulated pancreatic juice for 3 hours. Two products of hydrolysis were identified as PAG and phenylacetyl-isoglutamine (isoPAG) . The ratio of these two compounds was similar to the ratio of the products obtained during alkaline hydrolysis of A10 equal to 4:1. A decision was made to produce a formulation of antineoplaston A10 injections, 100 mg/ml as a 4:1 mixture of sodium salts of PAG and isoPAG. Green refers to the same article, but distorts the truth saying that A10 is a sodium salt of only PAG. Subsequently, Green alleges that Chinese researchers came to the same conclusion. In fact, however, these researchers concluded that "Antineoplaston A10 injection is a mixture of sodium salts of PAG and isoPAG."	Dr. Burzynski's rebuttal was correct and Dr. Green's quotation was a total misinterpretation and misrepresentation. Moreover, Antineoplaston A-10 does not convert to PAG when ingested by animals as demonstrated in an animal study . However, A-10 did convert to certain extent to PAG and isoPAG in simulated pancreatic juices. This indicates that in vitro data are not applicable to the in vivo situation. Dr. Green neglects to include the in vivo findings in his assessment.

For Dr. Burzynski he can show the plasma profile of A-10 and solubilized A-10 (PAG + isoPAG) in patients following treatment with either formulation. The HPLC profile of A-10, PAG and isoPAG should be presented to further demonstrate their differences in chemical structures and plasma concentrations in patients. This would show to the jury that Dr. Green is an incompetent scientist.

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5. Mechanism of action of Antineoplaston A10: Intercalation of DNA

Green's statement	Burzynski's rebuttal	Assessment
"Hendry et al concluded that insoluble A-10 could form a weak, noncovalent, reversible link between a base pair and a phosphate in DNA. In soluble A-10 might therefore block the intercalation of some carcinogenic compounds into DNA and prevent the events that initiate cancer cell growth. But this conclusion does not support the concept that insoluble A-10 would be useful in treating an existing cancer." In addition, "Hendry et al used insoluble A-10 in all their modeling studies. They did not report using soluble PAG. But as we have seen, the substance reaching the tissues is not the insoluble A-10, but PAG. Therefore Burzynski's declaration that A-10 acts as an antineoplastic agent by carcinogenic compounds is experimentally without foundation."	Dr. Burzynski did not respond to the issue of intercalation of DNA by A10. However, he has previously refuted the in vivo conversion of insoluble A10 to soluble A10, PAG.	Indeed, if Antineoplaston A10 can prevent carcinogenic compounds to intercalate DNA, it can be a useful chemopreventive but not chemotherapeutic agent. This interpretation is supported by experimental carcinogenesis studies. However, animal tumor transplant studies showed that A10 had chemotherapeutic effect. Thus, the theoretical basis for DNA intercalation by A10 is not consistent with the in vivo animal tumor transplant data. Alternative mechanisms may explain the observed anticancer activity of A10 in vivo.* Moreover, the anticancer activity of A10 is not due to PAG, since this conversion does not occur in rodents as previously discussed.

*Alternative mechanisms of Antineoplaston A10 action are brought forth in the next issue.

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6. Potential human toxicity of Antineoplaston A10 (phenylacetylamino-2,6-piperidinedione)

Green's statement	Burzynski's rebuttal	Assessment
Antineoplaston A-10 is 3-N-phenylacetylamino-2,6-piperidinedione. The pharmacology literature lists at least two pharmacologically potent compounds that are also piperidine- 2,6-diones. They are glutethimide and thalidomide. Both drugs have been withdrawn from the pharmaceutical marketplace because both are habituating and both can cause peripheral neuropathy.... In spite of the striking structural similarities between A10 and these two dangerous drugs, there is no evidence in the antineoplaston literature that testing of the potential of A10 to induce teratogenicity or peripheral neuropathy has been carried out."	It doesn't take more than an elementary chemical education to determine that these structures are quite different. Of course, neither glutethimide nor thalidomide contains a peptide bond. For some reason, Green fails to notice that A10 resembles the most nucleic bases, uracil and thymidine. The absence of mutagenic effects of antineoplastons A10, AS2-1, and AS2-5 has already been reported (in 1987). The study which indicated the lack of teratogenic effect of A10 was submitted to the NCI last year (1991).	Adding to Dr. Burzynski's argument is my assessment on thalidomide. Toxicological data support that thalidomide is one of the safer drugs in terms of the LC50 values in test animals with, of course, the exception of its teratogenicity. However, thalidomide is useful in treating leprosy, ulcer, host-vs-graft and conditions with elevated tumor-necrosis-factor ∀ with minimal toxicity. In addition, thalidomide is being considered by the NCI for treating glioma, hormone-refractory prostate cancer, metastatic breast cancer and Karposi sarcoma, based on the fact that thalidomide has antiangiogenic effect. A phase II clinical trial is ongoing to evaluate its efficacy in brain tumor. Thalidomide may be also useful for counteracting cachexia in AIDS patients. Judging from the state of art of thalidomide, Antineoplaston A-10 should be carefully studied as a thalidomide-like anticancer agent.* Based on Dr. Burzynski's or my argument, the potential benefit of A10 is outweighing the risk.

*Judah Folkman, M.D., a keynote speaker at the last American Society of Clinical Oncology meeting in Philadelphia, stresses that "there is great synergism between angiogenic therapy and chemotherapy". He adds that such a strategy is more effective than either therapy alone and in mice brings high levels of "permanent cures" (JNCI new, 1996). This "synergism" may be seen in the results that Dr. Burzynski has with A-10 in combination with chemotherapy in his breast cancer patients.

Dr. Burzynski should also show Dr. Michael Friedman's letter, to Dr. Bruce Chabner (NCI), showing his enthusiasm about the lipophilicity of A10 . He comments that A10 is like thalidomide and has good penetration across the blood brain barrier.

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8. Cytotoxicity of Antineoplaston AS2-1

Green's statement	Burzynski's rebuttal	Assessment
"Since PA is a strong acid, it is not surprising that AS2.1, which is 80% PA, should cause the death of cells in culture. In evaluating Burzynski's reported results with AS2.1, it must be recognized that as a strong acid, PA must be neutralized with sodium hydroxide before it is added to the culture medium. Thus, the cytotoxicity of AS2.1 might be due as much to the high salt concentration as to the PA." "Burzynski's claim now, that recent NCI test with A-10 and AS2.1 showed anticancer activity, is erroneous. The results of the tests that were done in December 1991 were sent to Burzynski in March 1992, and reported that A-10 and AS2:1 had no anticancer activity (written communication, S. A. Shepartz, Ph.D., NCI, July, 1992).	Green fails to mention a third series of NCI tests, performed in 1992 for the third time at a correct dosage level and in a proper model, which demonstrated the anticancer activity of antineoplaston A-10 and AS2.1 (written communication, M. R. Grever, M.D., acting associate director, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, 1992)	Many tumor cells, when treated with PA, express markers of differentiation without cytotoxicity. Moreover, PA and Antineoplaston AS2-1 must be neutralized before being added to the medium for cell culture work. So, tumor cells are not treated with strong acids. The acidity of PA and AS2-1 cannot be the cause for growth inhibition. In fact, sodium salts of PA and AS2-1 are slightly basic. According to Dr. Green's assertion, f the inhibition of tumor cell proliferation is then due to high salt concentration. It is unheard of that high salts can cause tumor cell differentiation. It is therefore my assessment that the strong acidity and high salt concentration of PA and AS2-1 caused tumor cell differentiation. Since both sides are using different test results and written communications to foster their argument, both sides should just show all documents of the test results that they have obtained from the NCI and the written communications from various persons for proper judgement.

*In my own studies, I have found that there is an interaction between PA and PAG, components of AS2-1. PAG, at non-inhibitory concentrations (0-10 mM), potentiated the anticancer activity of PA in cell culture.

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7. Toxicity of phenylacetate (PA)

Green's statement	Burzynski's rebuttal	Assessment
"...the toxicity of phenylacetate (PA) in human has been recognized since 1919 and more recently has been associated with the brain damage due to the faulty amino acid metabolism of phenylketonuria (PKU)."	Contrary to Green's statement, "In 1969, Burzynski recognized that metabolically produced PA was toxic to humans," the publication referenced by Green does not even mention PA. Acute and chronic toxicity studies of AS2-1 in mice, among other studies overlooked by Green, confirmed negligible toxicity. Green asserts that toxicity of PA has been associated with brain damage. However, the publications cited by him deal with the vulnerability of the immature rat brain. Moreover, Green is apparently unaware that since 1980 the sodium salt of PA has become an investigational drug approved for human use by the FDA and has already been established as safe and effective in the treatment of hyperammonemia. It is important to note that PA has been successfully used in the treatment of children a few months to a year old.	Phenylacetate is known to cause damage mainly to the fetuses and there is a particular window of time during gestation that these fetuses but not adults are especially susceptible to the toxicity of PA as demonstrated by "maternal PKU syndrome". Actually, this is the basis for use of PA for cancer treatment. Dr. Samid puts it in an elegant statement: The vulnerable fetal glial tissues resemble neoplastic glial cells in numerous molecular and biochemical aspects, including unique dependence on MVA (mevalonate) metabolism for synthesis of sterols and isoprenoids critical to cell replication and on circulating glutamine as the nitrogen donor for DNA, RNA and protein synthesis. The hypothesis underlying our studies was that PA, which is known to conjugate and deplete serum glutamine in humans and to inhibit the MVA pathway in immature brain, might attack these critical control points in malignant glioma. The efficacy of PA was demonstrated using both in vitro and in vivo tumor models. It is for certain that there is a rational thinking behind the use of phenylacetate for clinical brain tumor treatment.

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9. Uselessness of phenylacetylglutamine (PAG)

Green's statement	Burzynski's rebuttal	Assessment
Phenylacetylglutamine is a waste product that is only found in the urine of humans. (E)xperimental data in Burzynski's earlier work showed that PAG was ineffective against cancer cells. Burzynski supported his conclusion by citing the work of Israeli researchers who obtained the same results in 1977*.	Apparently biochemist Green attempted to evaluate the clinical results of antineoplastons. For some reason, he mentioned only two out of 28 studies dealing with clinical results. Each of these reports show evidence of anticancer activity in the treatment of cancer patients. In reference to one paper, Green alleges that according to my study, PAG was ineffective against cancer cells, despite the fact that this same publication describes two cases of cancer which went into complete remission as a results of treatment with PAG. Further he alleges that I cited the work of Israeli researchers who obtained the same results. Contrary to that, my statement was that Israeli researchers confirmed a slight effect on the growth of murine tumors, but they did not report the use of PAG in the treatment of human cancer.	The Israeli researchers did not remark that PAG was ineffective against ascites transplants, rather they commented on other more potent analogs. In their paper, PAG at 400 mg/kg caused a 20% reduction in tumor size and two of the nine mice developed no tumors. (No statistical analysis was given.) In the original paper on PAG, Burzynski comments that "antineoplaston AS2-1 has (an) interesting antineoplastic activity in tissue culture of breast carcinomas and low acute and chronic toxicity in mice. Antineoplaston AS2-5 does not show **significant** activity (about 20% inhibition of cell proliferation at the highest dose) in tissue culture of breast carcinomas... (However,) clinical observations of patients with primary chronic renal failure indicate that such patients have a very low incidence of cancer, unlike subjects with secondary renal failure. It is possible that phenylacetylglutamine, which was shown to have a slight inhibitory effect on murine tumors, could be one of the factors which may contribute to the low incidence of cancer in patients with primary chronic renal failure." Both groups indeed showed a slight anticancer activity (~20%) of PAG. Dr. Green did not comment on the human results of PAG study, but he did cites this paper for other purposes. In my own studies, I have found the IC50 values for PAG in cultured human prostate cancer cells at 15 and 18 mM. The argument about the anticancer activity of PAG may be a dose-related issue, which can be easily resolved.

*There is a minor mistake in Dr. Green's citation with regard to the study conducted by the Israeli researchers. The title of that article is: Antitumor activity of aromatic acyl derivatives of amino acids, rather than Effect of phenyllacetylglutamine on murine tumor cells in culture. (Isr. J. Med. Sci. 13: 316-320, 1977)

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10. Anticancer activity of Antineoplastons

Green's statement	Burzynski's rebuttal	Assessment
In 1990, the NCI carried out a series of tests using antineoplaston A-10 against a standard panel of tumors that included different cell lines from tumors of the following classes: leukemia, non-small cell lung cancer, small-cell lung cancer, colon cancer, cancer of the central nervous system, melanoma, ovarian cancer, and renal cancer. The chief of the Drug Synthesis and Chemistry Branch of the NCI reported the following: "The drug exhibited neither growth inhibition nor cytotoxicity at the dose levels tested (Written communication, V. L. Narayanan, Ph.D., July, 1990)." Dr. Green concludes that "none of the independent tests carried out with antineoplastons in experimental tumor systems have shown anticancer activity."**	It is quite obvious that any drug can be found inactive if it is tested in the wrong model at the wrong dosage. Prior to the first series of tests, I informed the NCI that "I do not believe the compound will display significant activity in the prescreen P388" (written communication to V. L. Narayanan, Ph.D., NCI, June, 1984). In spite of the suspected lack of activity against P388 leukemia, the NCI tested antineoplastons in this tumor model and, as predicted, found no activity.* The NCI's second series of test in 1990 were conducted at a dosage level 10,000 times smaller than the recommended do Following a site visit to Japan by the Food and Drug Administration (FDA) to review the University of Kurume Medical School's independent tests documenting antineoplaston antitumor activity in animals, the FDA approved our investigational New Drug application for antineoplaston A10 on March 16, 1989.	Based on the assessment in my report to the OAM, it is clear that there are data supporting the anticancer activity of , A10, solubilized A10 (PAG + isoPAG), and PA in cell culture and in animal models . This conclusion is based on studies conducted by scientists independent of Dr. Burzynski (including myself). In addition, AS2-1 (PA + PAG) was shown to has anticancer activity in cell culture. I have further showed that there is an interaction between PA and PAG in vitro. There is thus experimental evidence to support the conclusion that the anticancer activity of several Antineoplastons does exist. The anticancer activity of PA, PAG and AS2-1 was also discussed above in issues 7 , 8 and 9. Further judgement can be made with the provision of documents from previous screening conducted by the NCI.

*In 1985 the NCI abandoned the P388 screen because of its insensitivity to agents active in solid tumors.

**Dr. Green concludes that these (NCI and Southern Research Institute) test results, along with those reported from the in vitro and in vivo trials carried out at Sigma Tau Pharmaceuticals Inc. Are compelling evidence of the lack of efficacy of antineoplastons against experimental cancer. I did not include this statement above. However, I like to comment on the letter from Trevisani, medical director of Sigma Tau Pharmaceuticals Inc. , that Dr. Green quotes. Nowhere in that letter did Mr. Trevisani mention that antineoplaston AS2-1 and A-10 were ineffective or had no anticancer activity. Rather, he comments that "on the basis of these results, the project has been discontinued and more extensive testing or clinical trials have not been planned." Lack of commercial interest does not necessarily mean lack of anticancer activity. Other reasons may prevent a drug company to develop drug candidates.

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11. Treating patients with antineoplastons for 15 years with no evidence

Green's statement	Burzynski's rebuttal	Assessment
By calling attention to Samid's work in 1991, Burzynski confirms that he has been treating patients with antineoplastons for 15 years with no evidence that they n"normalize" cancer cells in vivo. I did not cite Samid's work because it was published 6 months after my paper was submitted for publication. Her coauthor did write to me to emphasize that a distinction should be made between antineoplastons and phenylacetate (written communication, C Myers, MD, NCI, June 13, 1992). Phenylacetate, he said, was a defined chemical entity, inexpensive and readily available from commercial suppliers, while antineoplastons were "mixture" prepared by Burzynski.	As the research expanded we found that not only peptides, but amino acid derivatives and certain organic acids are component of the BDS (biochemical defense system)... The BDS is the system of differentiation inducers. The mechanism of action is based not on a cytotoxic effect, but on the "reprogramming" of defective cells through the induction of differentiation.	Burzynski did provide some* but not much evidence suggestive of cellular differentiation induced by various antineoplaston formulations. Phenylacetate is only one compounds of many more antineoplastons. Dr. Samid started out with Antineoplaston AS2-1 from Dr. Burzynski in 1988. Her subsequent work on PA owes a great deal to Dr. Burzynski's openness for independent confirmation. Unfortunately, Dr. Samid only mentions in her paper that she received Antineoplaston AS2-1 from BRI without publicly acknowledging Dr. Burzynski.

*Between 1988 and 1991 Dr. Burzynski published several papers related to Antineoplaston A5, describing its ability to induce differentiation in leukemic cells. In addition, work aiming at purifying active differentiation inducers was undertaken. See Dr. Burzynski's publication list for complete information. Dr. Green did not cite any of these references.

The commentator would like to enter the statement that he had worked for Dr. Samid for one and a half years at the NCI and has first hand confirmation about the provision of Antineoplaston AS2-1 from Dr. Burzynski to Dr. Samid.

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12. Antineoplastons are not information-carrying peptides

Green's statement	Burzynski's rebuttal	Assessment
"AS2-5, which is not an information-carrying peptide..The antineoplaston AS2.1 also contains no information-carrying peptides but is a mixture of synthetic PAG and PA...(T)he treatment for cancer with substances called antineoplastons actually involves the use of two simple commercially available organic compounds, PA and PAG, which are marketed under the names of A-10, AS2-1 , and AS2-5. None is a peptide.	According to the definition published in 1976, antineoplastons are substances produced by the living organism that protect it against the development of neoplastic growth by a nonimmunological process which does not significantly inhibit the growth of normal tissues. Contrary to Green's statement that I "concluded that antineoplastons must be peptides", my initial statement was "Peptides are ideal compounds to participate in the system. As the research expanded we found that not only peptides, but amino acid derivatives and certain organic acids are component of the BDS (biochemical defense system).	Dr. Burzynski's rebuttal was correct and Dr. Green misquotes Dr. Burzynski's statement on peptides as ideal compounds for carrying information to normalize malignant cells. Of course, Dr. Burzynski will need to produce further evidence that peptides are parts of his proposed BDS. Since the development of non-synthetic or mixture Antineoplastons are in their early stages it is not possible to conclusively conclude that Antineoplastons are not information-carrying peptides.

A 1995 Critical Scientific Assessment of the ***Journal Of The American Medical Association's*** article:

'Antineoplastons': An Unproved Cancer Therapy, written by Saul Green, PhD

(JAMA, June 3, 1992—vol 267 No. 21; pg 2924-2927)

13. Conclusion on the scientific validity of antineoplastons

Green's statement	Burzynski's rebuttal	Assessment
None of Burzynski's publications between 1964 and 1990 contain objective experimental evidence supporting the postulate that a naturally occurring antineoplastic biochemical surveillance system exists in humans. The so-called five urinary antineoplastons (A1 to A5) have not been shown to chemically, biologically, or pharmacologically distinct from each other, and none has been proven to have antineoplastic activity against experimental cancer. Only one urine fraction (A2) has been used to produce antineoplaston A10. The admitted insolubility of A10 makes it physiologically incompatible with aqueous body fluids, so it cannot be part of the normal anticancer system that Burzynski postulates circulates throughout the body. The process that Burzynski says "solubilize A10" does not convert it to sodium salt but hydrolyzes it to PAG, now named AS2-5, which is not an information-carrying peptide... The antineoplaston AS2-1 also contains no information-carrying peptides but is a mixture of synthetic PAG and PA. None of the independent tests carried out with antineoplastons in experimental tumor systems have shown anticancer activity. These considerations lead to the conclusion that the treatment of cancer with substances called antineoplastons actually involves use of two simple commercially available organic chemical compounds , PA and PAG, which are marketed under the names A-10, AS2.1 and AS2.5. None is a peptide, none has been shown to "normalize" tumor cells, none has been shown to actually intercalate DNA, and none has been proven to be active against cancer in experimental tumor test systems.	Antineoplastons are an investigational cancer therapy, in the process of approval in a number of countries throughout the world. Permission to conduct clinical trials with antineoplastons has been given in the U.S., Germany, Japan, Poland, and Czecho-Slovakia. On March 16, 1989, permission was given by the FDA to proceed with a Phase II clinical trial in advanced breast cancer using Antineoplaston A10 capsules. This spring the NCI will conduct four Phase II clinical trials in brain tumors using antineoplastons A10 and AS2-1. Would this be happening if Saul Green's conclusion were correct... Either the government agencies and researchers of the U.S., Germany, Poland and Czecho-Slovakia know something Saul Green doesn't know, or Green chooses not to acknowledge all the research available. As the reader will see, this lack of acknowledgment is a pattern common to his review of the literature of antineoplastons.... How did such an article, by an author with such grave conflicts of interest, ever pass peer review?	It is not true that several of the Antineoplastons mentioned by Dr. Green has no anticancer activity in experimental models. Based on my laboratory experience and assessment* of other studies conducted by scientists independent of Dr. Burzynski, it is fair to say that there is justification to say that certain synthetic Antineoplastons do have anticancer activity in experimental models. This conclusion is also providing grounds for their use for cancer treatment in human subjects. In addition, since the synthetic Antineoplastons consist only a small fraction of Antineoplastons it is not possible to prove or disapprove the existence of a chemo-surveillance system as proposed by Dr. Burzynski. In particular, peptides that have antineoplastic activity must be purified and identified to support his theory.

*My summary report is available for review upon request.

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14. Burzynski's credentials

Green's statement	Burzynski's rebuttal	Assessment
Professor Stanislaw Bilinski, the current chairman of the Department of General Chemistry at Lublin, who remembers Burzynski as a student (written communication, March 1987), stated the following: "From December 15, 1966, to September 30, 1967, Burzynski worked as a scientific technical assistant in the Department of General Chemistry. He received his diploma as an MD on February 18, 1967, and a doctorate in medical sciences in 1968. To the best of my knowledge he did not do any independent research while he was at the academy." Burzynski's bibliography does not identify a PhD dissertation. Burzynski's curriculum vitae does not list any paper as a PhD thesis. The Medical Academy of Lublin does not offer a PhD program (written communication, A. Gandara, International Education Research Foundation, Credentials Evaluation Service, Los Angeles, CA, June 1991), a former professor recalls that his degree in 1968 was the DMSc, and the Polish Ministry of Health and Social Welfare says Polish medical schools do no confer the degree of PhD (written communication, R. Nizankowski, MD, May 12, 1992)	Green disputes my claim to a Ph.D. degree in biochemistry and asserts my bibliography does not identify a dissertation. He further questions whether I did independent research in the Department of General Chemistry at the Medical Academy in Lublin, Poland. The informant Green quotes from Lublin was never a Chairman of the Department during the term of my employment. He was better known as the second in command of the local communist party. In contradiction to Green's allegations, my doctoral dissertation is always listed in my bibliography. Furthermore, the sworn statement and affidavit of Professor Zdzislaw Kleinrok, President, Medical Academy of Lublin states: "As President of the Medical Academy of Lublin, Poland, I am the person in charge of all transcripts and records of former medical students. Dr. Stanislaw Burzynski graduated with Distinction and received a diploma of Medical Academy Doctor on June 30, 1967 from our Medical Academy. Our records also reflect that ... on October 16, 1968, Dr. Burzynski also received a Ph.D. degree from the Medical Academy in Lublin, Poland for his studies in biochemistry." (Written communication, May 25, 1990)	Interestingly, the dispute over a Ph.D. degree may be due to language translational difficulty or a semantic triviality ,because even Professor Stanislaw Bilinski, the contact person of Dr. Green, stated that Burzynski received "a doctorate in medical sciences in 1968" in addition to his MD. From the curriculum vitae of Dr. Burzynski or publication list, it is evident that he was actively involved in research while he was in medical school and after his graduation.

15. Other minor arguments and counter-arguments

Green's statement	Assessment
To clarify the relationship between the research done at the Medical College of Georgia, Augusta, and the claims of support that Burzynski attributed to that research, Hendry and Muldoon have advised Burzynski that their work does not provide support for the use for A-10 in human subjects, and that, to their knowledge, no one at the Medical College of Georgia has ever evaluated or advocated the use of A-10 in patients (written communications, T. G. Muldoon, Ph.D., and L. B. Hendry, Ph.D., November 1988). Burzynski was instructed not to use the name of the Medical College of Georgia in any of his publications or public presentations without prior approval (written communications, C. H. Wray, MD., and L.. Greenbaum, Ph.D., November 1988)	Dr. Burzynski did not respond to this point. Granted that animal or test tube studies can not be used to advocate clinical use of Antineoplaston A10. However, these publications demonstrate the attempt on Burzynski's part to enquire about the mechanism of action of Antineoplaston A10. More interesting is the fact that Dr. Hendry has obtained patent for the use of Antineoplaston A10 for psychiatric treatment because of its inhibitory effect on mixed function oxidases. In addition, he has found several more potent hydroxy analogs of Antineoplaston A10 as potential antiestrogenic compounds. (U.S. patents # 4,705, 796 and 5,238,947)

Green's statement	Burzynski's rebuttal	Comment
Tsuda, from the Kurume University School of Medicine, Japan, wrote the following on October 9, 1990: "Regarding your questions to our clinical investigation, we are afraid you have to wait until we publish the data." On January 7, 1991, Tsuda wrote the following: "We have not published any results of our clinical investigation on antineoplastons. You have to wait for our publication. We do not think that you are going to pick up any biological effect of antineoplaston A-10 in our study." Sugimura, president of the National Cancer Center, Tokyo, Japan, wrote, on July 24, 1990: "I am afraid that antineoplaston A-10 has no popularity in our country."	Green quotes a Japanese researcher (now in his third year of conducting clinical trials with antineoplastons) completely out of context: "We do not think that you are going to pick up any biological effect of antineoplaston A10 in our study." The Japanese physician's intended point was that Green, not an M.D., does not have sufficient qualifications to evaluate the biological effects of A10 in clinical studies (written communication, H. Tsuda, June, 1992).	Disregard of Burzynski's rebuttal and Tsuda's communication, the Japanese group has finally published their results from both experimental and clinical studies. Their conclusion is in support of Dr. Burzynski and demonstrates antineoplastic activity of Antineoplastons (more than just A10). For references see the publication list by independent scientist.

Danysz, director of the Institute of Drug Research and Control, Warsaw, Poland, wrote, on July 8, 1991: "Antineoplastons may be investigated in some clinics in Poland, but the Institute of Drug Research and Control is not supervising any of these studies."	Professor Danysz wrote to me December 22, 1989: "On behalf of the Polish Ministry of Health and Social Welfare, as well as myself as Director of the Institute for Drug Research and Control, I have the honor to thank you very much for your donation of Antineoplaston A10 and AS2-1 for the treatment of breast cancer, prostate cancer and tumors of CNS."	Dr. Green is purely resorting to innuendo on this quotation. The bottom line is clinical studies are under way in Poland to evaluate the efficacy of Antineoplastons A10 and AS2-1 for various cancer with or without the supervision of the Institute of Drug Research and Control.