

The Present State of Antineoplaston Research (1)

Stanislaw R. Burzynski

Antineoplastons work as molecular switches, which regulate expression of genes *p53* and *p21* through demethylation of promoter sequences and acetylation of histones. They also inhibit the uptake of growth-critical amino acids, such as L-glutamine and L-leucine in neoplastic cells. Phase II trials indicate efficacy of antineoplastons in low-grade glioma, brain stem glioma, high-grade glioma, adenocarcinoma of the colon, and hepatocellular carcinoma. The best results were observed in children with low-grade glioma, where 74% of patients obtained objective response, and in patients with adenocarcinoma of the colon with liver metastases whose survival rate of more than 5 years is 91% versus 39% in controls on chemotherapy. Gene array studies will explain antineoplaston-induced changes in gene expression.

Keywords: *antineoplastons; brain tumors; gliomas; colon cancer; liver cancer*

The theory of antineoplastons originated in 1976 from the theory of autonomous cybernetic systems (ACS) and studies on peptides and amino acid derivatives in human blood.¹ A cell can be compared to the ACS, which is connected with the environment through the information and energy pathways.¹ Two types of gates connect the system with the environment: receptor is for information, and feeder is for energy. After crossing these gates, both pathways travel through systems that transform and store the energy (accumulator) and information (correlator). Accumulator and correlator are connected through homeostat, which influences both of them. Finally, the ACS possesses an effector, where both pathways converge and leave the system, which is responsible for a specific reaction. The ACS can be influenced through either the energy or information pathway. Most cancer treatments, known so far, were based on a "massive destruction" principle and employed a large amount of energy and a small amount of information, which resulted in poor specificity of the treatment and numerous side effects. The ideal cancer treatment should provide a large amount of information and a small amount of energy. Such targeted treatment will primarily influence the information pathway and consist of molecular switches, which control neoplastic

DOI: 10.1177/1534735403261964

growth by "turning on" tumor suppressor genes and "turning off" oncogenes.² Experience with imatinib mesylate (Gleevec; Novartis, East Hanover, NJ) proves the validity of such an approach. The practically infinite variety of peptides and amino acid derivatives that can be formed by combinations of the 20 common amino acids indicates the possibility of the existence of a peptide- and amino acid-based system that carries information from cell to cell and inside the cell and is able to correct errors in the program for normal development.

I initiated the study of antineoplastic peptide fractions from the blood in 1967.³ The analysis of peptides and amino acids in the blood of healthy people and of patients suffering from various diseases, including cancer, revealed that there were significant deficiencies in peptide content in the serum of cancer patients. Subsequently, similar peptide fractions were found in urine, and it was postulated that they were synthesized in tissues and passed into the blood and urine. For economic reasons, urine was proposed as the main source for their isolation. The research on antineoplastic peptides from urine, by our team, resulted in the separation of urinary peptides in 119 fractions, which were further tested for their effect on the growth of neoplastic and normal cells. Fractions that inhibited the growth of neoplastic cells and did not inhibit the growth of normal cells were named antineoplastons.^{4,7} A hypothesis has been proposed that the human body possess a biochemical defense system, consisting of antineoplastons, which protects against the occurrence of abnormal cell growth. Normal cells differentiate according to the program encoded in the genes, eventually entering the stage of cellular senescence followed by apoptosis. Cancer cells, however, escape the fate of normal cells and, instead of dying, multiply endlessly.^{8,9} Growth and differentiation of the cells is regulated by influences from both outside the cells (growth factors, antineoplastons) and from inside (oncogenes, tumor

SRB is at the Burzynski Research Institute, Houston, TX. Based on a presentation at the Comprehensive Cancer Conference, 2003.

Correspondence: Stanislaw R. Burzynski, Burzynski Research Institute, 9432 Old Katy Road, Houston, TX 77055. E-mail: info@burzynskiclinic.com.

suppressor genes). Errors in the early stages of differentiation result in highly malignant neoplasms. Middle and delayed disruption of the process leads to less aggressive cancers and benign tumors. Changes in the final steps cause abnormal production of proteins and may result in autoimmune and neurodegenerative disorders.²

Aging is a continuation of a process beginning in the embryo that depends on silencing some genes when they are no longer useful. When this silencing affects tumor suppressor genes, an aging person may develop cancer.^{10,11} Silencing of gene *p53* leads to the development of many different types of cancer in the aging organism, and silencing of the *WRN* gene causes premature aging and formation of 5 different types of malignancies.¹² Methylation of promoter sequences of the genes is the main mechanism silencing both of those genes (no longer necessary for development and other genes), which are gradually turned off during the aging process.¹⁰

Cancer patients have a marked deficiency of antineoplastons in plasma. Balancing this deficiency through introduction of antineoplastons in cancer treatment may control neoplastic growth.^{7,13}

The initial studies concentrated on group A of antineoplastons with a broad spectrum of activity. Five formulations in this group, named antineoplastons A1, A2, A3, A4, and A5, consisting of peptide fractions isolated from urine, were the subjects of extensive tissue culture and animal toxicity studies and also phase I clinical studies in advanced cancer patients.¹⁴⁻²⁶ The studies revealed negligible toxicity and objective responses in difficult-to-treat cancers.^{14,24-26}

Antineoplaston A2, which contributed to the highest number of complete responses in phase I clinical studies, was elected for final purification, isolation of the active components, and structure determination. The active ingredient was identified as 3-phenylacetyl-amino-2, 6-piperidinedione and was named antineoplaston A10.²⁷ A10 has been reproduced by synthesis involving condensation of l-glutamine with phenylacetyl chloride and subsequent cyclization of phenylacetylglutamine (PG).²⁸ The metabolism of A10 in the human body yields PG, phenylacetyliso-glutamine (isoPG), and phenylacetate (PN), which were reproduced synthetically and formulated into antineoplaston A10 injections (A10-I), AS2-1, AS5, and AS-25.²⁹⁻³³ Each of these formulations was submitted for basic research and phase I clinical studies.³⁴⁻⁴⁴ A10, A10-I, and AS2-1 were selected for phase II studies. Two initial phase II studies in astrocytoma and high-grade glioma began in 1988 and 1990 and were conducted outside the investigational new drug (IND) process.^{45,46} In 20 patients, in the astrocytoma study, complete and partial responses occurred in

30%, stable disease in 50%, and progressive disease in 20%.⁴⁵ In the high-grade glioma study of 12 patients diagnosed with glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA), 36% obtained complete and partial responses, 33% obtained stable disease, and 33% had progressive disease.⁴⁶ The phase II study of AS2-1 capsules with low-dose diethylstilbestrol in hormonally refractory cancer of the prostate revealed complete and partial responses in 36%, stable disease in 50%, and progressive disease in 14%.⁹ An independent review by the National Cancer Institute (NCI) of 7 patients with brain tumors concluded that an association existed between antineoplaston administration and objective tumor regressions.⁴⁷ After this review, a phase II study was authorized by the US Food and Drug Administration (FDA) and sponsored by the NCI.⁴⁸ The study was stopped after the treatment of 6 evaluable patients diagnosed with GBM and AA when it was found that the concentration of A10-I in patients' blood during the treatment was approximately 50 times lower than optimal concentration.⁴⁹ There were no objective responses reported.⁴⁸ Since 1994, the FDA has authorized 74 phase II studies with A10, A10-I, and AS2-1, under INDs 43,742 and 22,029, in advanced malignancies. In addition, 2 phase II studies were performed at Kurume University School of Medicine under the supervision of the Japanese government. Burzynski Research Institute sponsored all the studies. The preliminary results of the studies in primary brain tumors have been previously published.⁵⁰⁻⁵²

Mechanism of Action of Antineoplastons

The current theory of the mechanism of action of antineoplastons is their function as "molecular switches," turning on tumor suppressor genes and turning off oncogenes. Such molecular switches should theoretically control cancer because they deal directly with its biochemical causes: increased activity of oncogenes and decreased expression of tumor suppressor genes. It is known that there are accelerators and brakes in the cancer process. Oncogenes are the accelerators, and normal tumor suppressor genes serve as brakes. PN and AS2-1 inhibit farnesylation of the p21^{ras} protein and cause downregulation of *Bcl-2*.^{53,54} While it causes inhibition of mevalonate 5-pyrophosphate decarboxylase, it does not affect mevalonate kinase and mevalonate 5-phosphate kinase.⁵⁵

PN and AS2-1 also activate the *p53* gene. In most cases, protein p53 activates the *p21* gene, which directs the synthesis of the p21^{WAF1/Cip1} protein. This protein activates promoter sequences of the tumor suppressor

gene *p53*, which are undergoing demethylation. DNA methylation of tumor suppressor genes is a common mechanism of silencing the genes.⁵⁶ Removal of methyl groups activates the gene, which causes malignant cells to die through apoptosis. The reaction is facilitated by the enzyme methyltransferase. Methyltransferase can complete this reaction only if it is activated by binding to proliferating cell nuclear antigen (PCNA). In normal cells, PCNA binds to the p21^{WAF1/cip1} protein, leaving methyltransferase inactive. In cancer cells, in the absence of the p21^{WAF1/cip1} protein, the reaction of methylation proceeds uninhibited.^{57,58} Induction of p21^{WAF1/cip1} suppresses human glioma cell proliferation.⁵⁹

At one of the previous comprehensive care conferences, we reported detailed data indicating that PN, which is the active ingredient of antineoplaston AS2-1, interrupts signal transmission in the *ras* pathway and activates tumor suppressor genes *p53* and *p21* through demethylation of their promoter sequences.^{53-55,57,58,60} Since the previous conference, our team has conducted research on the molecular mechanism of action of PG, the main ingredient of A10-I, and has concluded that

- PG exhibits antineoplastic activity across a wide array of cancer cell lines;
- PG inhibits the uptake of growth-critical amino acids, such as L-glutamine and L-leucine, in neoplastic cells;
- the reduction in amino acid availability may contribute to the drug's antineoplastic activity;
- human glioma (U-87) cells rapidly take up PG by a mechanism similar to facilitated diffusion;
- upon the removal of PG from the media, PG rapidly and completely effluxes from the cell; and
- PG enters cells via the stereospecific amino acid transporters for L-glutamine.⁶¹

Publications on these conclusions and supporting data are now in preparation.

Formulations of Antineoplastons

Antineoplastons are a class of 12 antitumor agents. The following synthetic antineoplaston formulations are currently being used in phase II studies.

- Antineoplaston[®] A10 capsules contain 500 mg of 3-phenylacetyl-amino-2, 6-piperidinedione.
- Antineoplaston[®] A10 injection is a mixture of the sodium salts of PG and isoPG in a 4:1 ratio. It is available in 500 mL and 1000 mL (300 mg/mL) plastic bags.
- Antineoplaston[®] AS2-1 capsules containing 500 mg of a 4:1 PN and PG.
- Antineoplaston[®] AS2-1 injection is a mixture of PN and PG in a 4:1 ratio. It is available in 250 mL (80 mg/mL) plastic bags.

Clinical Trials With Antineoplastons in the United States

All of the clinical trials in cancer in the United States are performed under FDA supervision (INDs 22,029 and 43,742). The government did not fund antineoplaston trials discussed here. Two additional trials are under way in Japan, supervised by the Japanese government. We have emphasized treatment of primary malignant brain tumors; colon, liver, esophageal, pancreatic, lung, and breast cancers; and non-Hodgkin's lymphoma.

The studies had the following common exclusion criteria: serious active infections, fever, or other serious concomitant disease that would interfere with the evaluation of the treatment drugs, (such as severe lung disease, hepatic failure, and history of cardiovascular or renal conditions that medically contraindicate administration of high dosages of sodium). Patients should have histological confirmation of the malignancy, except for cases of dangerous locations, where biopsy may entail unacceptable risk to the patient. There were no exclusions based on tumor size, multifocality, or leptomeningeal or systemic metastases except that the tumor size should be larger than 5 mm. Patients in the low-grade glioma group were excluded if less than 8 weeks had elapsed from the last dose of radiation therapy and 4 weeks from the last dose of chemotherapy (6 weeks from the last dose of nitroureas), immunotherapy, and surgery. In brain stem glioma, high-grade glioma, and GBM groups, there was a separate subgroup of patients who developed progression during standard therapy and within 4 weeks after chemotherapy (6 weeks after nitroureas) and also within 8 weeks after radiation therapy. Patients who had a Karnofsky performance status of less than 60 were excluded.

Response criteria were as follows: Complete response (CR) is complete disappearance of all contrast-enhancing tumors on imaging studies for at least 4 weeks without the use of corticosteroids. Partial response (PR) is more than 50% reduction in the sum of the products of the greatest perpendicular diameters of the contrast-enhanced tumors for at least 4 weeks with no appearance of new lesions on a decreasing or stable dose of corticosteroids. Objective response (OR) is either CR or PR. Minor response (MR) is at least 25% and less than 50% of tumor reduction. Stable disease (SD) is defined as less than 25% reduction and no progression. Progressive disease (PD) is a greater than 50% increase in the sum of the products of the greatest perpendicular diameters of the contrast-enhanced tumors, compared with nadir evaluation, or the appearance of new lesions. PD is often considered as a 25% increase in size of

measurable tumor. The reason for 50% PD in these studies is a long dose-escalation process, which extends to more than a month's time period, before the optimal dosage is reached. The patient may experience continuous tumor growth (more than 25%) on lower dosages and respond to the treatment at higher dosages. Some patients were not evaluable because they did not have follow-up scans, and others were not evaluable because it was too early for evaluation. Such patients were not included in the statistics. The results of our studies were compared with standard therapy. The comparison groups have a similar population of patients, as far as prognostic factors are concerned, including extent of prior resection, performance status, lag time to treatment, prior treatments, and so forth, unless stated otherwise. The patients were selected at the Burzynski Clinic (BC) from the general population of oncology patients. All consecutive patients who fulfilled entrance criteria were admitted to the trials, and remaining patients were treated with standard cancer treatment, including cytotoxic chemotherapy and immunotherapy. ORs have been confirmed by radiologists not associated with the BC. In addition, the FDA conducted an independent review of all medical records and films of patients who obtained OR.

Because of the slow enrollment and similarity among protocols, the results were integrated into clinical and statistical reports for a faster and more meaningful review of the results of treatment of low-grade glioma in children, brain stem glioma, high-grade glioma, and GBM. Protocols are listed at www.clinicaltrials.gov. The accrual for studies BT-20 and CAN-01 is completed. To complete the remaining studies, we are still accruing the following number of patients: BT-06, 31; BT-07, 2; BT-08, 25; BT-09, 16; BT-10, 27; BT-11, 10; BT-13, 30; BT-15, 15; BT-17, 29; BT-18, 26; BT-21, 11; BT-22, 34; and BT-23, 30. Some detailed reports on individual clinical studies have been published or are in preparation for publication (Table 1).

Low-Grade Glioma in Children

Protocols BT-10, BT-11, BT-13, BT-23, and CAN-01

- Nineteen evaluable children with a median age of 7 years were enrolled.
- Only 16% of patients were not previously treated, except for tumor biopsy—the remaining 84% had developed tumor recurrence after surgery, radiation, or chemotherapy.
- OR = 74%, MR = 5%, SD = 16%, PD = 5%.
- Median overall survival from diagnosis (OSD) was 6.3 years.

Table 1. Phase II Clinical Trials With Antineoplastons: Update on Efficacy, June 1, 2003 (in percentages)

Diagnosis	Objective Response	Minor Response	Stable Disease	Progressive Disease
Low-grade glioma in children	74	5	16	5
Brain stem glioma	39	11	17	33
High-grade glioma	36	7	29	29
Glioblastoma multiforme	19	9	21	51

Objective response = complete response and partial response.

- Median overall survival from start of treatment (OST) was 5.1 years with the majority of patients alive (26% deceased or status unknown).
- Maximal survival (MS) is more than 17 years.
- For progression-free survival (PFS), only 1 patient progressed after 24 months.

After completion of the study, the responses and survival will be evaluated for the following groups: (1) low-grade astrocytoma, (2) recurrent and progressive multicentric glioma, and (3) visual pathway glioma.

Low-grade gliomas in children account for more than 50% of all primary central nervous system tumors in childhood. The main conventional treatment is surgery, but in many cases, the location of the tumor can be too risky. In addition, some patients develop multiple tumors throughout the brain and spine. Unfortunately, when only the conventional treatment is employed, most patients will experience disease progression and death, except in a small number of cases in which the tumor can be totally resected. With the use of aggressive chemotherapy, only 4% of patients obtained OR; SD was 74%, and PD was 22% (Figure 1).⁶²

Case Study, Patient 1

This young man was 10 years old when diagnosed with low-grade astrocytoma in October 1997. Because his tumor grew in a difficult area of involvement, including the thalamus and brain stem, he was not a candidate for surgery and did not receive any standard treatment (Figure 2). He began treatment with antineoplastons in April 1998. His contrast-enhancing tumor decreased and finally could no longer be seen on magnetic resonance imaging (MRI) in March 1999. Repeated positron emission tomography (PET) scans did not show tumor recurrence. This patient has made a complete recovery. When he came to us for the first time, he was partially paralyzed and in a wheelchair (Figure 3).

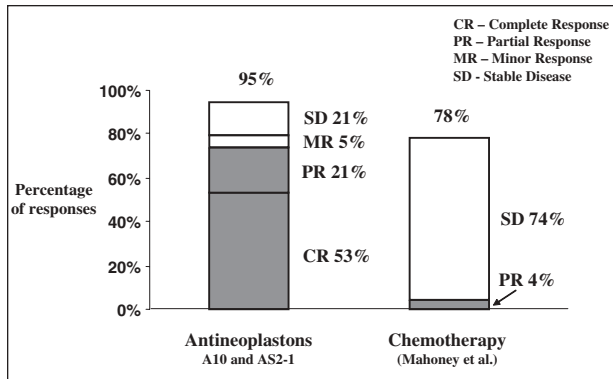


Figure 1 Phase II clinical trials with antineoplastons A10 and AS2-1 in children with low-grade glioma (as of June 1, 2003), comparison of responses. For the comparison study, see Mahoney et al.²²



Figure 3 Patient 1 before and during the treatment with antineoplastons A10 and AS2-1.

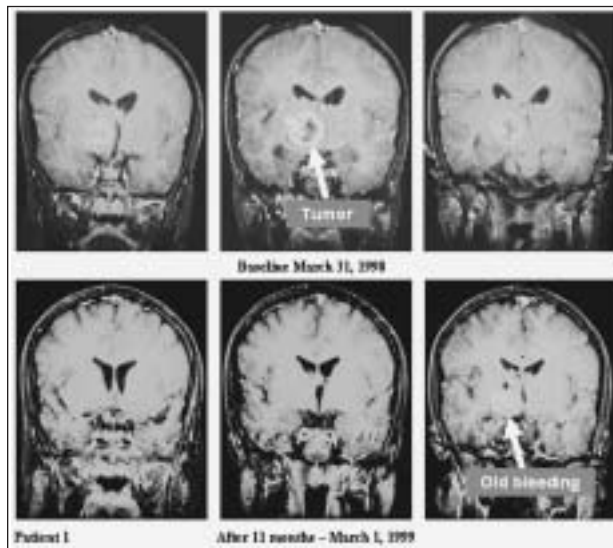


Figure 2 Treatment of low-grade glioma with antineoplastons A10 and AS2-1 in a 10-year-old male (patient 1). The patient was diagnosed in October 1997 with inoperable astrocytoma, grade 2 of thalamus and brain stem. Magnetic resonance imaging (MRI) of the head with gadolinium. Images in the upper row show a large tumor before treatment. Images in the lower row confirm disappearance of the tumor after 11 months of treatment. Residual signal on posttreatment MRIs corresponds to old hemorrhage.

- PFS 7 months = 43% of responding patients did not develop progression

After completion of the study, the results will be evaluated separately for (1) brain stem glioma, (2) recurrent and progressive intrinsic brain stem glioma and for brain stem glioma that progressed during standard therapy and within 4 weeks after chemotherapy (6 weeks after nitroureas) and 8 weeks after radiation therapy.

Brain stem gliomas are among the most difficult brain tumors to treat. Surgery usually is associated with great risk, while chemotherapy is ineffective. Therefore, radiation therapy remains the main treatment modality. Unfortunately, in most of the patients, the tumor will recur and cause death.

Case Study, Patient 2

This patient presented one of the most difficult brain stem glioma cases treated in our clinic (Figure 4). She arrived at the BC in October 1998, when she was less than 3 months old. The patient was diagnosed with a large brain stem glioma, measuring approximately 7 cm across its largest diameter. She was evaluated at City of Hope Medical Center in Duarte, California, and consulted with Beth Israel Hospital in New York. Unfortunately, there was no conventional treatment available for her. Her parents were told she would almost certainly die within 1 month. She came to us in terminal condition and began treatment immediately. After 4 months of treatment with antineoplastons, her contrast-enhancing tumor was no longer visible on MRI. She had been near death at the beginning of antineoplaston treatment and was being kept alive only by large dosages of corticosteroids. Pretreatment

Brain Stem Glioma

Protocols BT-11 and BT-22

- Eighteen evaluable patients with a median age of 11 years (15 children and 3 adults)
- No prior treatment = 33%, and recurrence after radiation or chemotherapy = 67%
- OR = 39%, MR = 11%, SD = 17%, and PD = 33%
- OSD = 13.7 months
- OST = 10.3 months
- MS = more than 7.5 years

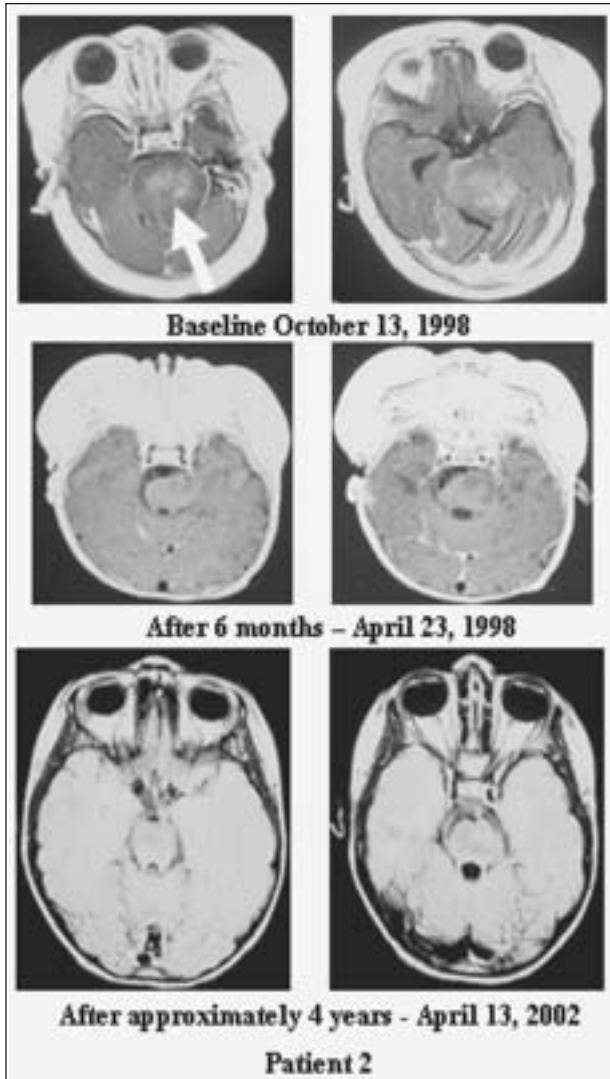


Figure 4 Treatment of progressive intrinsic diffuse brain stem glioma with antineoplastons A10 and AS2-1 in 3-month-old female (patient 2). Patient was diagnosed shortly after birth with large inoperable brain stem glioma and presented for treatment with antineoplastons in terminal condition. Magnetic resonance imaging of the head with gadolinium shows decrease and disappearance of contrast-enhancing tumor.

photographs showed the child with facial nerve paralysis and extensive swelling of the face resulting from the corticosteroids. Six months later, her symptoms decreased and disappeared. Now, 5 years from her diagnosis, she is a completely normal and healthy child (Figure 5).

A subgroup of 10 evaluable patients has been diagnosed with very difficult to treat recurrent diffuse intrinsic brain stem glioma. Among these patients, an OR was determined in 50%, MR in 30%, and PD in 20%. The longest survival (the patients are currently alive) is more than 7 years.⁵² We can compare these results to other studies. In a phase II PCNU trial, 18%

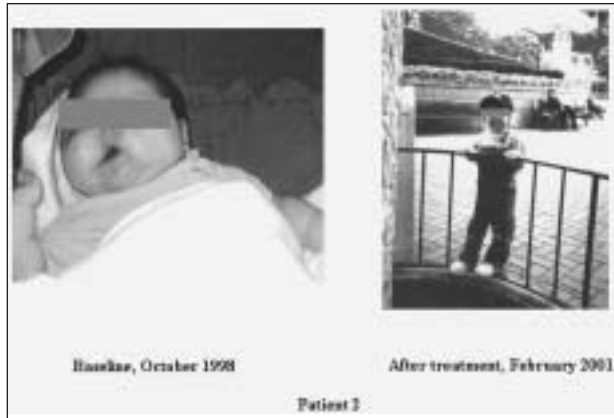


Figure 5 Patient 2 before and after the treatment with antineoplastons A10 and AS2-1.

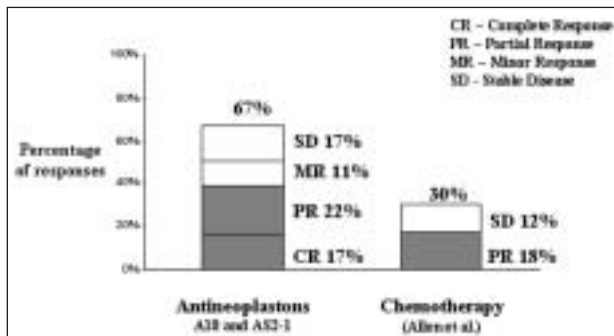


Figure 6 Phase II clinical trials with antineoplastons A10 and AS2-1 in brain stem glioma (as of June 1, 2003), comparison of responses. For the comparison study, see Allen et al.⁶³

of patients obtained PR and 12% obtained SD, compared to the total of 80% of CR, PR, and MR in our study (Figure 6).⁶³ In the study with thiotepa, in 14 cases there were no objective responses.⁶⁴ A paper by Mandell et al analyzes the results of 66 patients treated with radiation therapy and chemotherapy with cisplatin. The study involved easier to treat cases of newly diagnosed patients than those in the antineoplaston study.⁶⁵ The percentage of patients surviving at 2 years in the antineoplaston study was 42% compared to 7.1% for radiation and chemotherapy (Figure 7).

High-Grade Glioma

Protocols BT-06, BT-08, BT-09, BT-10, BT-11, BT-15, BT-17, BT-18, BT-21, BT-22, and CAN-01

- Sixty-two evaluable patients with a median age of 39 years (54 adults and 8 children)
- No prior treatment = 24%; recurrence after surgery, recurrence during and/or after radiation or chemotherapy = 76%
- OR = 36%, MR = 7%, SD = 29%, PD = 29%

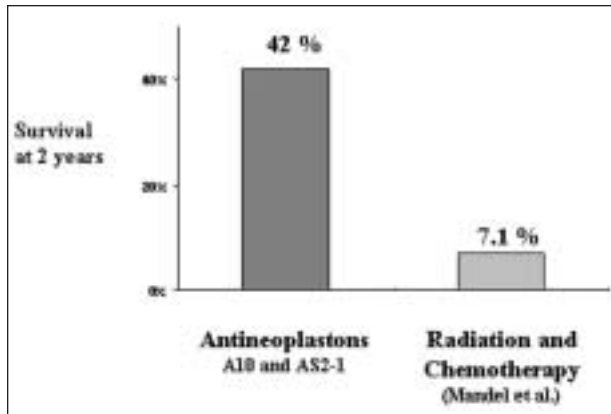


Figure 7 Phase II clinical trials with antineoplastons A10 and AS2-1 in brain stem glioma (as of June 1, 2003). Comparison of patients' survival at 2 years. For the comparison study, see Mandell et al.⁶⁵

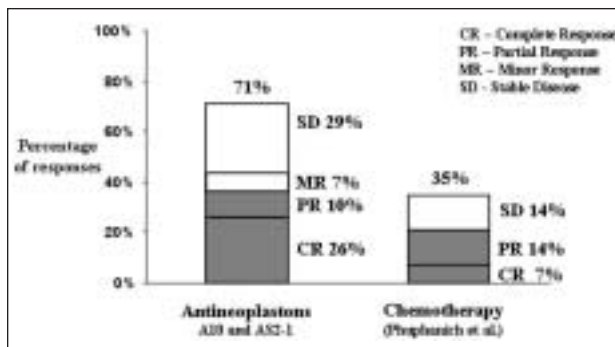


Figure 8 Phase II clinical trials with antineoplastons A10 and AS2-1 in high-grade glioma (as of June 1, 2003), comparison of responses. For the comparison study, see Phuphanich et al.⁶⁶

- OSD = 3 years
- OST = 1.15 years
- MS = more than 12.5 years
- PFS 12 months = 61% of OR patients had not progressed

After completion of the studies, the results and survival will be evaluated separately for (1) high-grade glioma in children, (2) high-grade glioma in adults, (3) AA not treated with radiation and chemotherapy, (4) AA recurrent after radiation and chemotherapy, (5) recurrent anaplastic oligodendroglioma, (6) recurrent mixed glioma, and (7) high-grade glioma that progressed during radiation and chemotherapy and within 4 weeks after chemotherapy (6 weeks after nitroureas) and within 8 weeks after radiation therapy.

High-grade gliomas are usually treated surgically, followed by radiation therapy and chemotherapy. Median survival without treatment is 17 months and with treatment may approach 3 years. For recurrent

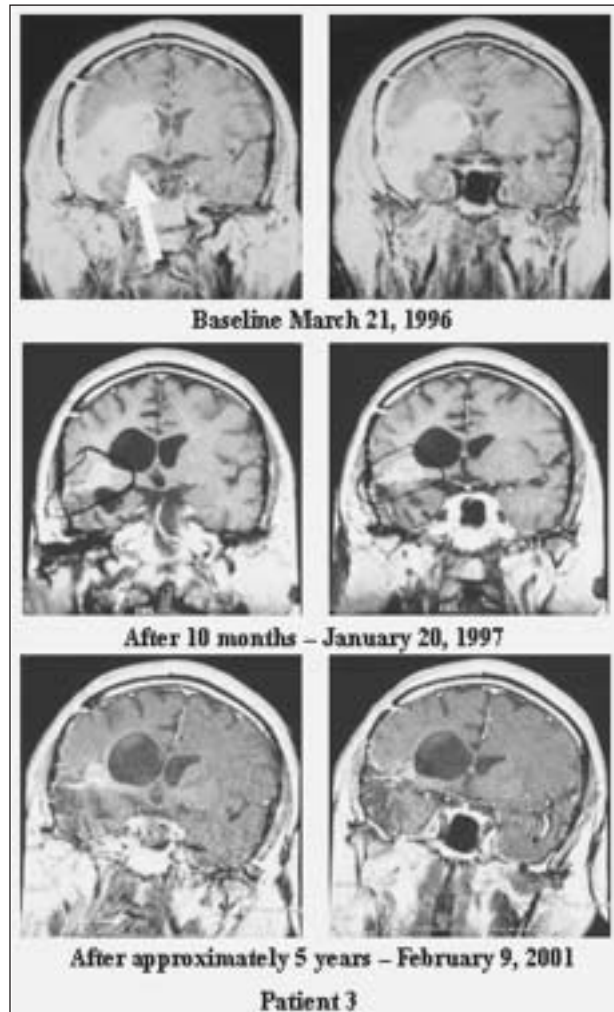


Figure 9 Treatment of high-grade glioma with antineoplastons A10 and AS2-1 (patient 3). The patient is a 54-year-old male who underwent subtotal tumor resection for high-grade glioma on February 23, 1996. After the treatment with antineoplastons A10 and AS2-1 from March 27, 1996, to August 27, 1996, his tumor gradually decreased and disappeared. Magnetic resonance imaging of the head with gadolinium shows gradual decrease and disappearance of the tumor.

tumors, median survival is reduced to 8 months.⁶⁶ Ultimately, most patients will die from their tumors despite the treatment given. In one of the recent phase II studies in recurrent high-grade glioma with temozolomide and thalidomide, there were the following results: OR = 21%, SD = 14%, and PD = 65% (Figure 8).⁶⁶

Case Study, Patient 3

The patient was a 54-year-old male diagnosed with high-grade malignant glioma (mixed astrocytoma, oligodendroglioma) on February 23, 1996. At the time of diagnosis, he underwent subtotal tumor resection and did not receive any standard treatment. He was treated with antineoplastons A10 and AS2-1 from

March 27, 1996, to August 27, 1997. He had a large tumor in the right temporal lobe, which gradually decreased and disappeared (Figure 9). He has been tumor free for more than 6 years. In the treatment of high-grade gliomas, including GBM, it is not unusual to see a rapid response to treatment and a complete response within 2 to 3 months.

Case Study, Patient 4

This case is a good example of a rapid response. The patient was 41 years old when on April 18, 2000, he underwent a partial resection of a brain tumor and was diagnosed with AA. After the surgery, he received radiation therapy, which was completed in June of 2000. On December 1, 2000, he underwent a second craniotomy for tumor recurrence, which confirmed the same diagnosis. After he developed a second recurrence, he began treatment with antineoplastons A10 and AS2-1 on February 22, 2001. His follow-up MRI on April 25, 2001, did not show a contrast-enhancing tumor (Figure 10). The treatment with antineoplastons was discontinued on August 5, 2001. A follow-up PET scan on January 22, 2003, did not show any tumor recurrence (more than 3 years after his diagnosis).

Glioblastoma Multiforme

Protocols BT-06, BT-07, BT-09, BT-10, BT-18, BT-20, BT-21 and CAN-01

- Eighty evaluable patients with a median age of 46 years (72 adults and 8 children)
- All patients underwent prior surgery (tumor resection = 87.5% and biopsy only = 12.5%), 74% developed recurrence after or during radiation and chemotherapy
- OR = 19%, MR = 9%, SD = 21%, PD = 51%
- OSD = 15 months
- OST = 9 months
- MS = more than 11 years
- PFS 5 months = 53% of OR patients had not progressed

After completion of the study, the responses and survivals will be evaluated separately for GBM in children and in adults in the following groups: (1) GBM not treated with standard therapy, (2) recurrent GBM after completion of standard therapy, and (3) GBM that progressed during standard therapy and within 4 weeks after chemotherapy (6 weeks after nitroureas) and within 8 weeks after radiation therapy.

As a glioma of the highest malignancy, GBM is difficult to control by standard therapy. The conventional treatment of recurrent GBM is disappointing. Curative therapies do not exist, and median survival is

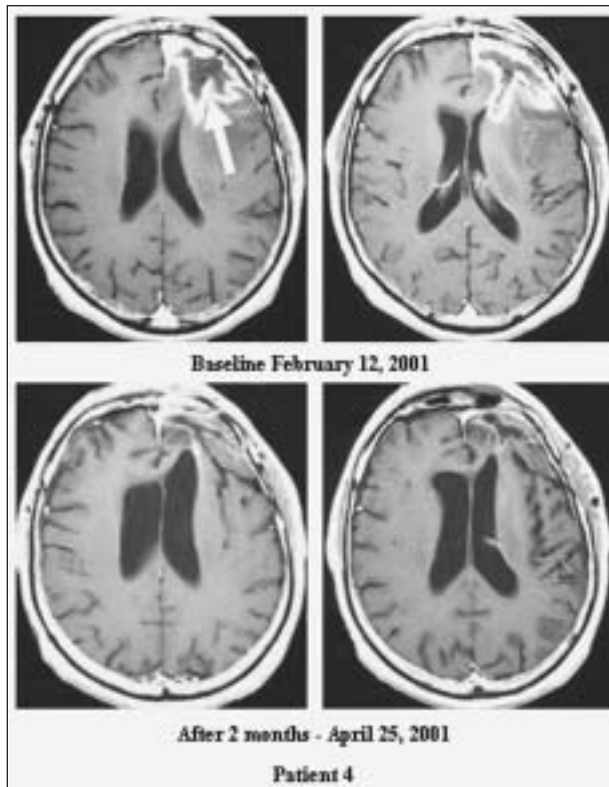


Figure 10 Rapid response to treatment with antineoplastons A10 and AS2-1 in patient with high-grade glioma (patient 4). The patient is a 41-year-old male treated with antineoplastons after his anaplastic astrocytoma progressed after partial resection, radiation therapy, and a second resection. MRI of the head with gadolinium shows disappearance of contrast-enhancing tumor after the treatment with antineoplastons.

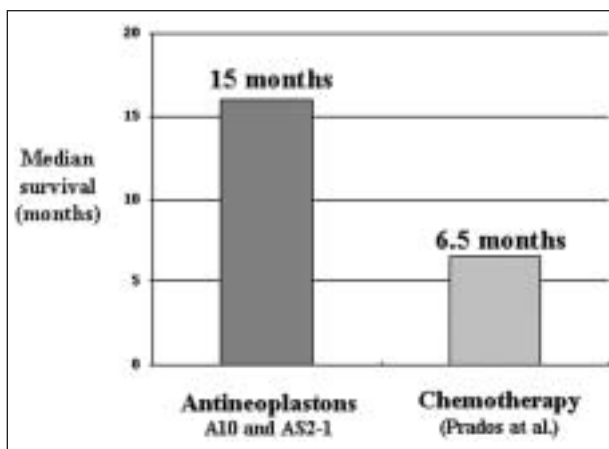


Figure 11 Phase II clinical trials with antineoplastons A10 and AS2-1 in recurrent glioblastoma multiforme (as of June 1, 2003), comparison of survival. For the comparison study, see Prados et al.⁶⁷

approximately 4 months for patients not treated. For the recurrent GBM, the median survival is 6½ months with the newest forms of chemotherapy compared to

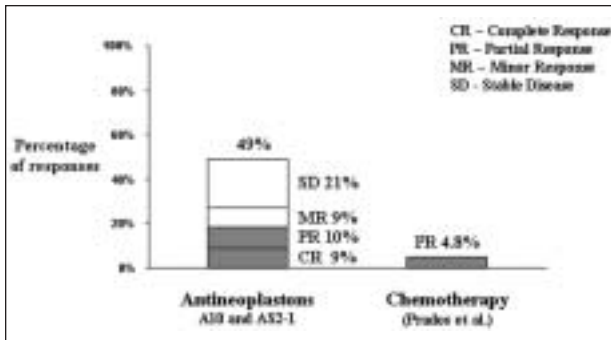


Figure 12 Phase II clinical trials with antineoplastons A10 and AS2-1 in glioblastoma multiforme (as of June 1, 2003), comparison of responses. For the comparison study, see Prados et al.⁶⁷

15 months in our study (Figure 11).⁶⁷ The percentage of objective responses (only partial responses) in patients treated with aggressive chemotherapy with blood brain disruption was only 4.8% (Figure 12).⁶⁷ Among our patients exhibiting objective responses, 1 is surviving more than 11 years, another patient for more than 9 years, and 3 additional patients for approximately 7 years.

Case Study, Patient 5

This 26-year-old female was diagnosed with GBM and underwent subtotal resection of the tumor on July 28, 1994. She received initial treatment with radiation therapy and chemotherapy with BCNU. In January of 1995, due to tumor recurrence, she received chemotherapy with procarbazine. After the second recurrence of her tumor, she received treatment with antineoplastons A10 and AS2-1 from April 2, 1996, to July 29, 1996. The tumor decreased and was no longer seen on repeated MRIs. She survived and is now in good health more than 9 years from tumor diagnosis (Figure 13).

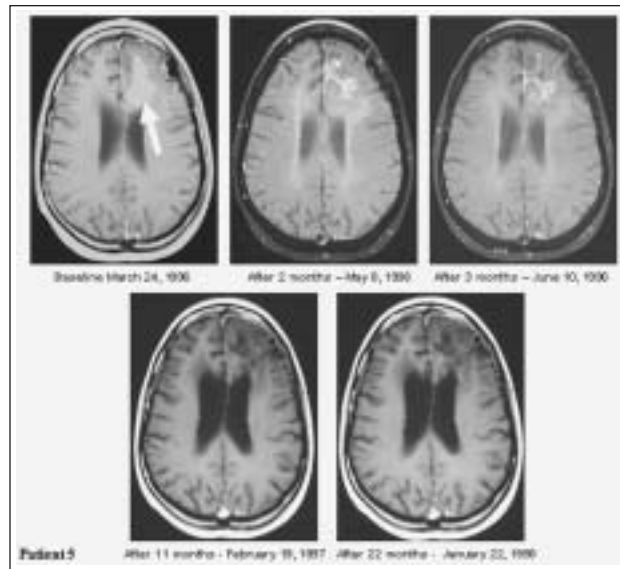


Figure 13 Treatment of glioblastoma multiforme with antineoplastons A10 and AS2-1 in a 27-year-old female (patient 5). The patient was diagnosed with glioblastoma multiforme on July 28, 1994. She started antineoplastons A10 and AS2-1 on April 2, 1996, after tumor recurrence postsubtotal resection, radiation therapy, and 2 types of chemotherapy. Magnetic resonance imaging of the head with gadolinium shows gradual decrease and disappearance of the tumor. This patient's survival is more than 9 years.

Clinical Trials With Antineoplastons in Japan

Colon Cancer

According to preliminary results of the clinical trials conducted at the University of Kurume Medical School in Japan, A10 and AS2-1 given to patients with adenocarcinoma of the colon with metastases to the liver in conjunction with chemotherapy increased more than 5-year survival to 91%, compared with 39% in the control group treated with chemotherapy alone (Figure 14).⁶⁸ The study was randomized and compared the results of treatment of 19 patients who received hepatic arterial infusion (HAI) of chemotherapeutics with antineoplastons and 56 patients who received HAI chemotherapy alone. HAI

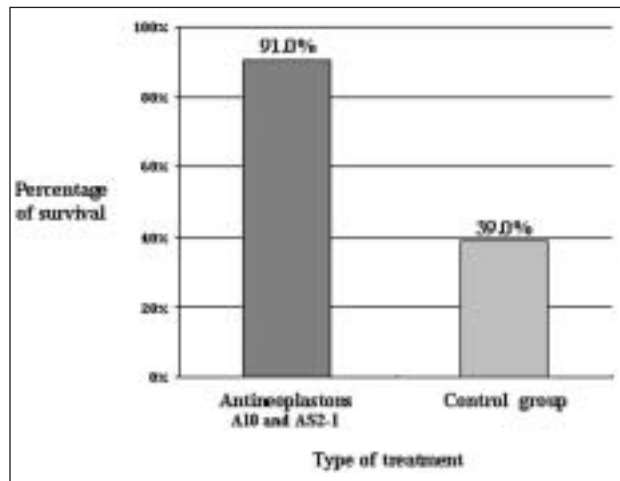


Figure 14 Phase II clinical trials with antineoplastons A10 and AS2-1 in adenocarcinoma of the colon with liver metastases in University of Kurume Medical School of Japan. Comparison of survival, antineoplastons in conjunction with chemotherapy versus chemotherapy alone.

chemotherapy improved 5-year survival rate from 32% to 39% compared to standard chemotherapy.

Liver Cancer

The same medical center described follow-up of 10 patients who had antineoplaston treatment during the

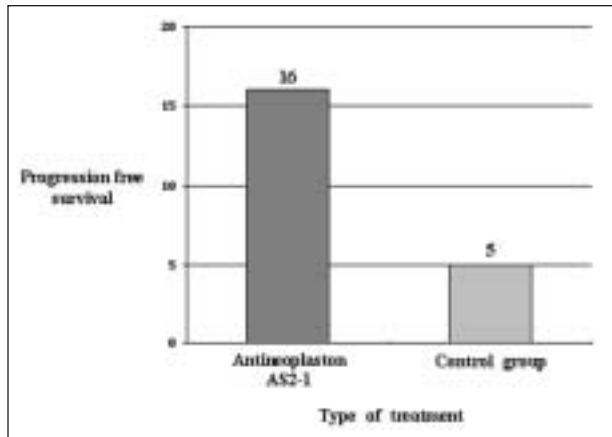


Figure 15 Phase II clinical trials with antineoplastons in hepatocellular carcinoma at the University of Kurume Medical School of Japan. Time to recurrence in patients given antineoplaston AS2-1 after standard chemotherapy compared to control group.

course of therapy for hepatocellular carcinoma (this was not a controlled study) (Figure 15). It was found that the time to recurrence was longer with AS2-1 than that without AS2-1. Patients treated with AS2-1 had time to recurrence of 16 months compared with 5 months without AS2-1. In 7 patients, recurrence-free intervals were measured with and without AS2-1 treatment. The time to recurrence was longer after adding AS2-1 to the treatment (14 months vs 5 months).^{69,70} The Japanese group has reported encouraging results in case studies of 3 patients with advanced pancreatic, lung, and breast cancers.^{71,72}

Adverse Reactions

Treatment with antineoplastons is usually free from adverse reactions. Serious adverse reactions include anemia in 1.4% and hypernatremia and fever in 0.5% of patients. Additional moderate adverse reactions include skin rash (0.76%) and slurred speech (0.9%). Most patients experience increased diuresis, which may lead to dehydration and thirst. A complete list of rare adverse reactions occurring in less than 0.5% of patients is available on request. In general, however, adverse reactions observed during treatment with antineoplastons have usually been transient and mild. Buckner et al in their phase II study of antineoplastons described reversible grade 2 or 3 neurological toxicity, consisting of transient somnolence, confusion, and exacerbation of an underlying seizure disorder.⁴⁸ Reversible grade 1 somnolence and confusion were also noted in our studies and were due to phenylacetate, which is the main ingredient of AS2-1. It is suspected that the other adverse reactions observed in our studies were due to A10. They were not observed by Buckner et al since they used approximately 50 times

lower dosages of A10 in their study. On the other hand, A10 induces diuresis, which may result in rapid elimination of AS2-1 through the kidneys, which will lower the concentration of AS2-1 in plasma and reduce the chances for higher grade toxicity observed by Buckner et al.⁴⁹

Conclusion

We have accumulated substantial data on the safety and efficacy of antineoplaston treatment in supervised clinical trials. Some of our trials are nearing conclusion, but patients are still being accrued to most. A substantial number of patients are not yet evaluable, and for some indications, the time is too short for evaluation of survival; therefore, reported statistics may change in the future. Much of the data are positive, although we sometimes see a lack of efficacy in some types of cancer. The details of all cases are reported periodically to the FDA. A majority of the patients suffered from recurrent disease after standard treatment. Such patients usually have a smaller chance to respond to the next therapeutic regimen. Most patients had been told by previous physicians that they had only a few months to live and that there were no other treatments available for them. It is still possible, however, that some of these patients have already survived through many previous treatments and may simply represent a group that has less aggressive and more easily controlled disease.⁷³

We are continuing to study the role of genes in cancer prevention and treatment. Gene array studies will explain antineoplaston-induced changes in gene expression. Other research may lead to formulations that are more powerful and faster acting or that have preventative capabilities. At this point, we remain convinced of the potentially important role of antineoplastons in the treatment of cancer.

Acknowledgments

The studies were sponsored by the Burzynski Research Institute and supervised by its Institutional Review Board (IRB). The membership of the IRB was in agreement with the FDA. The authors express their appreciation to Lucy Rorke, MD, professor of pathology and neurology, University of Pennsylvania, Children's Hospital of Philadelphia, for review of pathology slides; Dieter Schellinger, MD, professor of radiology, chief, section of neuro-radiology, Georgetown Hospital, Washington, DC; and Joshua Pleasure, MD, M. D. Anderson Cancer Center, Houston, Texas, for evaluation of MRI and PET scans. The following physicians at the Burzynski Clinic (BC) participated in the study: Robert I. Lewy, Robert Weaver, Marc Bestak, Maxwell Axler, Alonzo Peters, Benjamin

Saling, Barbara Burzynski, Tomasz Janicki, Jaroslaw Paszkowiak, Vishnu Alapati, Dmitri Davydov, Vsevolod Dolgoplov, Barbara Drynia, Andrzej Himmel, Wojciech Iwanowski, Gabor Jurida, Mohammad Khan, Eva Kubove, Grace Ormstein, Joseph Nguyen, Mohammed Radmard, Basel Salhoot, Barbara Szymkowski, and Marek Walczak. The following senior scientists (PhD), microbiologists, pharmacists, and engineers at the BRI and the BC participated in basic research: Robert Waldbillig, Majciej Klimczak, Elwira Ilkowska-Musial, Leszek Musial, Anna Baranowska, Piotr Kuligowski, Ryszard Madry, Donat Manek, Mike Mokrzycki, Andrzej Wieczorek, Anna Wisniewska, Kris Wisniewski, Irma Witkowska, Dennis Wright, and Iwona Zapadowski.

References

- Burzynski SR. Antineoplastons—history of the research (I). *Drugs Exptl Clin Res.* 1986;12(suppl 1):1-9.
- Burzynski SR. Potential of antineoplastons in diseases of old age. *Drugs Aging.* 1995;7:157-167.
- Burzynski SR. Investigations on unknown ninhydrin-reacting substances in human blood serum I. Attempts at identification of three such substances. *Experientia.* 1969;25:490-491.
- Burzynski SR. Biologically active peptides in human urine: I. Isolation of a group of medium-sized peptides. *Physiol Chem Phys.* 1973;5:437-447.
- Burzynski SR, Ungar AL, Lubanski E. Biologically active peptides in human urine: II. Effect on intestinal smooth muscle and heart. *Physiol Chem Phys.* 1974;6:457-468.
- Burzynski SR, Loo TL, Ho DH, et al. Biologically active peptides in human urine: III. Inhibitors of the growth of leukemia, osteosarcoma and HeLa cells. *Physiol Chem Phys.* 1976;8:13-22.
- Burzynski SR. Antineoplastons: biochemical defense against cancer. *Physiol Chem Phys.* 1976;8:275-279.
- Burzynski SR. Novel differentiation inducers. In: Adam D, ed. *Recent Advances in Chemotherapy.* Munich, Germany: Futramed; 1992.
- Burzynski SR, Kubove E, Burzynski B. Treatment of hormonally refractory cancer of the prostate with antineoplaston AS2-1. *Drugs Exptl Clin Res.* 1990;16:361-369.
- Burzynski SR. Gene silencing—a new theory of aging. *Med Hypotheses.* 2003;60:578-583.
- Burzynski SR. The methylation control of gene activation and silencing theory. In: Giampapa VC, ed. *The Basic Principles and Practice of Anti-aging Medicine and Age Management for the Aesthetic Surgeon and Physician.* Montclair, NJ: Giampapa Institute for Anti-aging Medical Therapy; 2003:33-34.
- van Brabant AJ, Stan R, Ellis NA. DNA helicases, genomic instability, and human genetic instability, and human genetic disease. *Ann Rev Genom Hum Genet.* 2000;1:409-459.
- Liau MC, Lee SS, Burzynski SR. Hypomethylation of nucleic acids: a key to the induction of terminal differentiation. *Internat J Exptl Clin Chemother.* 1989;2:187-199.
- Burzynski SR, Stolzmann Z, Szopa B, et al. Antineoplaston A in cancer therapy (I). *Physiol Chem Phys.* 1977;9:485-500.
- Gross S, Galicka N, Grabarczyk M, et al. Urinary peptides inhibit DNA synthesis in vitro in certain cultured neoplastic cells. *Clin Chem.* 1977;23:148-149.
- Beall P, Szopa B, Burzynski SR, et al. Polypeptides that inhibit human breast cancer division. *Cancer Biochem Biophys.* 1979;3:93-96.
- Lee SS, Mohabbat MO, Burzynski SR. Tissue culture and animal toxicity studies of antineoplaston A2. *Drugs Exptl Clin Res.* 1984;10:607-610.
- Burzynski SR. Antineoplaston A2. *Drugs of the Future.* 1986;11:549-550.
- Burzynski SR. Antineoplaston A3. *Drugs of the Future.* 1986;11:551-552.
- Burzynski SR. Antineoplaston A5. *Drugs of the Future.* 1986;11:824-825.
- Burzynski SR, Mohabbat MO. Chronic animal toxicity studies of antineoplaston A2. *Drugs Exptl Clin Res.* 1986;12(suppl 1):73-75.
- Lee SS, Mohabbat MO, Burzynski SR. In vitro cancer growth inhibition and animal toxicity studies of antineoplaston A3. *Drugs Exptl Clin Res.* 1987;13(suppl 1):57-60.
- Lee SS, Burzynski SR. Tissue culture and animal toxicity studies of antineoplaston A5. *Drugs Exptl Clin Res.* 1987;13(suppl 1):31-35.
- Burzynski SR, Kubove E. Initial clinical study with antineoplaston A2 injections in cancer patients with five years follow-up. *Drugs Exptl Clin Res.* 1987;13(suppl 1):1-12.
- Burzynski SR, Kubove E. Phase I clinical studies of antineoplaston A3 injections. *Drugs Exptl Clin Res.* 1987;13(suppl 1):17-29.
- Burzynski SR, Kubove E. Phase I clinical studies of antineoplaston A5 injections. *Drugs Exptl Clin Res.* 1987;13(suppl 1):37-43.
- Burzynski SR, Hendry LB, Mohabbat MO, et al. Purification structure determination, synthesis and animal toxicity studies of antineoplaston A10. In: *Proceedings of the 13th International Congress of Chemotherapy.* Vienna, Austria; 1983:17, PS. 12.4 11-4.
- Burzynski SR, Hai TT. Antineoplaston A10. *Drugs of the Future.* 1985;10:103-105.
- Burzynski SR. Synthetic antineoplastons and analogs. *Drugs of the Future.* 1986;11:679-688.
- Burzynski SR, Mohabbat MO, Lee SS. Preclinical studies of antineoplaston AS1-1 and antineoplaston AS2-5. *Drugs Exptl Clin Res.* 1986;12(suppl 1):11-16.
- Burzynski SR, Khalid M. Antineoplaston A10 injections. *Drugs of the Future.* 1986;11:364-365.
- Burzynski SR, Khalid M. Antineoplaston AS2-1. *Drugs of the Future.* 1986;11:361-363.
- Burzynski SR. Antineoplaston AS2-5. *Annual Drug Data Report.* 1986;8:319.
- Burzynski SR, Mohabbat MO, Burzynski B. Animal toxicology studies on oral formulation of antineoplaston A10. *Drugs Exptl Clin Res.* 1984;10:113-118.
- Burzynski SR. Phase I clinical studies of antineoplaston AS2-5 injections. In: Ishigami J, ed. *Recent Advances in Chemotherapy.* Tokyo, Japan: University of Tokyo Press; 1985.
- Burzynski SR, Burzynski B, Mohabbat MO. Toxicology studies of antineoplaston AS 2-1 injections in cancer patients. *Drugs Exptl Clin Res.* 1986;12(suppl 1):25-35.
- Burzynski SR, Kubove E. Toxicology studies of antineoplaston A10 injections in cancer patients. *Drugs Exptl Clin Res.* 1986;12(suppl 1):47-55.
- Lehner AF, Burzynski SR, Hendry LB. 3-phenylacetylaminio-2,6-piperidinedione, a naturally-occurring peptide analog with apparent antineoplastic activity may bind to DNA. *Drugs Exptl Clin Res.* 1986;12(suppl 1):57-72.
- Ashraf AQ, Liau MC, Mohabbat MO, et al. Preclinical studies of antineoplaston A10 injections. *Drugs Exptl Clin Res.* 1986;12(suppl 1):37-45.
- Ashraf AQ, Liau MC, Kampalath BN, et al. Pharmacokinetic study of radioactive antineoplaston A10 following oral administration in rats. *Drugs Exptl Clin Res.* 1987;13(suppl 1):45-50.

41. Hendry LB, Muldoon TG, Burzynski SR et al. Stereochemical modeling studies of the interaction of Antineoplaston A10 with DNA. *Drugs Exptl Clin Res.* 1987;13(suppl 1):77-81.
42. Ashraf AQ, Burzynski SR. Comparative study of antineoplaston A10 levels in plasma of healthy people and cancer patients. *Adv Exptl Clin Chemother.* 1988;2:19-28.
43. Ashraf AQ, Kampalath BN, Burzynski SR. Pharmacokinetic analysis of antineoplaston A10 injections following intravenous administration in rats. *Adv Exptl Clin Chemother.* 1988;6:33-39.
44. Burzynski SR, Kubove E, Burzynski B. Phase I clinical studies of oral formulation of antineoplaston AS2-1. *Adv Exptl Clin Chemother.* 1988;2:29-36.
45. Burzynski SR, Kubove E, Burzynski B. Phase II clinical trials of antineoplastons A10 and AS2-1 infusions in astrocytoma. In: Adam D, ed. *Recent Advances in Chemotherapy.* Munich, Germany: Futuramed; 1992.
46. Burzynski SR, Kubove E, Szymkowski B. Phase II clinical trials of antineoplaston A10 and AS2-1 infusions in high-grade glioma. Paper presented at: 18th International Congress of Chemotherapy; June 1993; Stockholm, Sweden.
47. Hawkins MG, Friedman MA. National Cancer Institute's evaluation of unconventional cancer treatments. *J Natl Cancer Inst.* 1992;84:1699-1702.
48. Buckner JD, Malkin MG, Reed E, et al. Phase II study of antineoplaston A10 (NSC 648539) and AS2-1 (NSC 6200261) in patients with recurrent glioma. *Mayo Clin Proc.* 1999;74:137-145.
49. Burzynski SR. Efficacy of antineoplastons A10 and AS2-1. *Mayo Clin Proc.* 1999;74:641-642.
50. Burzynski SR, Conde AB, Peters A, et al. A retrospective study of antineoplastons A10 and AS2-1 in primary brain tumours. *Clin Drug Invest.* 1999;18:1-10.
51. Burzynski SR. Antineoplastons. In: Novey DW, ed. *Clinician's Complete Reference to Complementary/Alternative Medicine.* St. Louis (MO): Mosby; 2000:496-507.
52. Burzynski SR, Lewy RI, Weaver RA, et al. Phase II study of antineoplastons A10 and AS2-1 in patients with recurrent diffuse intrinsic brain stem glioma (preliminary report). *Drugs in R&D.* 2003;4:91-101.
53. Shack S, Chen LC, Miller AC, et al. Increased susceptibility of ras transformed cells to phenylacetate is associated with inhibition of p21^{ras} isoprenylation and phenotypic reversion. *Int J Cancer.* 1995;63:124-129.
54. Adam L, Crepin M, Savin C et al. Sodium phenylbutyrate induces growth inhibition and Bcl-2 down-regulation and apoptosis in MCF7 ras cells *in vitro* and in nude mice. *Cancer Res.* 1995;55:5156-5160.
55. Castillo M, Iglesias J, Zafra MF, et al. Inhibition of chick brain cholesterogenic enzymes by phenyl and phenolic derivatives of phenylalanine. *Neurochem.* 1991;18:171-174.
56. DiCroce L, Raker VA, Corsaro M, et al. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science.* 2002;295:1079-1082.
57. Gorospe M, Shack S, Guyton KZ, et al. Up-regulation and functional role of p21^{WAF1/Cip1} during growth arrest of human breast carcinoma MCF-7 cells by phenylacetate. *Cell Growth Differ.* 1996;7:1609-1615.
58. Liu L, Samid D. Mutant p53 as a target of phenylacetate in human glioblastoma [abstract 2580]. *Proceedings of the 86th Annual Meeting of the American Association for Cancer Research.* Toronto, Canada; 1995:433.
59. Kamitani H, Tanjura S, Watanbe K, et al. Histone acetylation may suppress human glioma cell proliferation with p21^{WAF1/Cip1} and gelsolin are induced. *Neuro-Oncology.* 2002;4:95-101.
60. Burzynski SR. Gene therapy with antineoplastons. Paper presented at: Comprehensive Cancer Care; 1998; Washington, DC.
61. Waldbillig RJ, Burzynski SR. Mechanism of action, uptake and gene array studies on the antineoplastic agent phenylacetylglutamine (PG) in human glioma cells U-87. Paper presented at: 8th Annual Meeting of the Society for Neuro-Oncology; November 13-16, 2003; Keystone, Colorado.
62. Mahoney DH, Cohen ME, Friedman HS, et al. Carboplatin is effective therapy for young children with progressive optic pathway tumors: a Pediatric Oncology Group phase II study. *Neuro-Oncology.* 2000;2:213-220.
63. Allen JC, Hancock, Walker R, et al. PCNU and recurrent childhood brain tumors. *J Neurooncol.* 1987;5:241-244.
64. Heideman RL, Packer RJ, Reaman GH, et al. A phase II evaluation of thiotepa in pediatric central nervous system malignancies. *Cancer.* 1993;72:271-275.
65. Mandell LR, Kadota R, Freeman C, et al. There is no role for hyperfractionated radiotherapy in the management of children with newly diagnosed diffuse intrinsic brain stem tumors: results of pediatric oncology group phase III trial comparing conventional vs. hyperfractionated radiotherapy. *Int J Radiat Oncol Biol Phys.* 1999;43:959-964.
66. Phuphanich S, Selph J, Snodgrass S, et al. Low dose thalidomide and temodar as salvage therapy for recurrent malignant glioma. *Neuro-Oncology.* 2002;4:374.
67. Prados M, Schold SC Jr, Fine HA, et al. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro-Oncology.* 2003;5:96-103.
68. Ogata Y, Tsuda H, Matono K, et al. Long-term survival following treatment with antineoplastons for colon cancer with unresectable multiple liver metastases: report of a case. *Surg Today.* 2003;33:448-453.
69. Tsuda H, Sata M, Saitsu H, et al. Antineoplaston AS2-1 for maintenance therapy in liver cancer. *Oncol Rep.* 1997;4:1213-1216.
70. Kumabe T, Tsuda H, Uchida M, et al. Antineoplaston treatment for advanced hepatocellular carcinoma. *Oncol Rep.* 1998;5:1363-1367.
71. Tsuda H, Sata M, Ijuuin H. A novel strategy for remission induction and maintenance in cancer therapy. *Oncol Rep.* 2002;9:65-68.
72. Tsuda H, Sata M, Kumabe T, Uchida M, Hara H. The preventive effect of antineoplaston AS2-1 on HCC recurrence. *Oncol Rep.* 2003;10:391-397.
73. Richardson MA, Russell NC, Sanders T, et al. Assessment of outcomes at alternative medicine cancer clinics: a feasibility study. *J Altern Complement Med.* 2001;7:19-32.