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A Phase II Study of Antineoplastons A10 and AS2-1 in Adult Patients With Newly-Diagnosed Anaplastic Astrocytoma

Final Report (Protocol BT-08)

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Abstract

The paper reports the evaluation of efficacy and safety of Antineoplastons A10 and AS2-1 (ANP) in a phase II study of newly diagnosed patients with anaplastic astrocytoma (AA). The study was designed as a single-arm, two-stage, phase II trial of ANP as monotherapy. The primary endpoint, was to determine the overall response rate (confirmed complete response (CR) or partial response (PR)) to ANP, based on disappearance or more than 50% reduction of tumor size by contrast-enhanced magnetic resonance imaging (MRI). The goal of the study was a response rate to ANP of not less than 10% or 4 patients. 19 patients (12 men, 7 women) ages 22-60 years (median age, 44) were treated. The patients received ANP daily every four hours (median dose of A10 5.3 g/kg/d and AS2-1 0.3 g/kg/d). The duration of ANP ranged from 3 to 175 weeks. A complete response was documented in 21%, and stable disease in 26% of patients. The study was closed for enrollment after 19 of its subjects had been admitted, because the goal of trial had been accomplished. Progression-free survival and overall survival at 2 years was 16% and 37% and at 10 years was 5% and 14% respectively. Only 11% of patients reported grade 3 toxicities consisting of hypokalemia and fatigue, and 11% reported grade 4 toxicities consisting of hypernatremia and hypokalemia. There was no chronic toxicity and there was a high quality of life. ANP shows efficacy with very good toxicity profile in patients with newly diagnosed AA.

Keywords: anaplastic astrocytoma, antineoplastons, glioma, phase II clinical trial

1. Introduction

The CBTRUS reports the average annual incidence of anaplastic astrocytoma (AA) in the USA for the Years 2006 through 2010 as 5,621 patients and a 10-year survival rate from 4.4% to 17.6% (Ostrom et al., 2013). Classified as a WHO grade 3 tumor (Louis et al., 2007), AA is grouped together with glioblastoma into the high-grade glioma group. It is generally accepted that mutations in the PTEN, TP53 and RB pathways are main events in the pathogenesis of high-grade glioma (Chow et al., 2011; Kitange, Templeton, & Jenkins, 2003). Standard treatment for anaplastic astrocytoma and glioblastoma multiforme involves surgical resection followed by radiation therapy (RT), with six months of chemotherapy with temozolomide (TMZ) (Stupp et al., 2005). There are only two reports of phase II studies, published in peer-reviewed journals during the last eight years, on chemotherapy followed by RT in patients with newly diagnosed AA, but there are numerous reports on recurrent AA (Gilbert et al., 2002; Rao et al., 2005). Surprisingly, there were no phase II studies on single pharmacological agents in newly diagnosed AA without RT. Long-term survival remains low in AA and the quality of survival is poor in at least one-third of the patients (Greene-Schloesser & Robbins, 2012; Mu et al., 2012). A recent retrospective study in patients with AA treated with TMZ-based chemoradiotherapy reported a 5-year overall survival (OS) rate of 38% (Minniti et al., 2014). This study, however, was not prospective and correlated survival outcomes with the isocitrate dehydrogenase 1 (IDH1) mutation and O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation. On the other hand, the chemotherapy for postoperative patients has been criticized and suggested not to be used (Scoccianti et al., 2012). This creates the need for new treatment modalities.

Antineoplastons (ANP) A10 and AS2-1 are synthetic amino acid derivatives. A10 is a formulation consisting of a 4:1 ratio of phenylacetylglutaminate sodium (PG) and phenylacetylisoglutaminate sodium (isoPG). AS2-1 is a

formulation with a 4:1 ratio of phenylacetate sodium (PN) and PG (Burzynski, 2004). In preclinical studies on Human U87 glioblastoma (GBM) cell line PG and PN affected over 100 genomic targets and interrupted signal transmission in RAS/MAPK/ERK, and P13K/AKT/PTEN pathways (Burzynski & Patil, 2014). Phase I studies have shown responses in gliomas, and in the Phase II study of ANP A10 and AS2-1 for 20 subjects with astrocytomas conducted in 1988, complete response (CR) and partial response (PR) was documented in six subjects (30%), and two subjects diagnosed with diffuse intrinsic pontine glioma (DIPG) AA survived tumor-free for over 24 years (Burzynski & Kubove, 1986; S. Burzynski, B. Burzynski, & Mohabbat, 1986; S. Burzynski, Kubove, & B. Burzynski, 1992). The program of 14 phase II studies of ANP in primary brain tumors sanctioned by the FDA has been recently completed. (S. Burzynski, Janicki, G. Burzynski, & Marszalek, 2014; Burzynski & Patil, 2014; S. Burzynski, Janicki, G. Burzynski, & Marszalek, 2014; S. Burzynski, Janicki, & G. Burzynski, 2014; S. Burzynski, G. Burzynski, & Janicki, 2014; S. Burzynski, Janicki, G. Burzynski, Marszalek, & Brookman, 2014). This study assesses the results of ANP treatment in adult AA patients who have not undergone radiation or chemotherapy.

2. Patients

Recruited patients were over 18 years of age with subtotally resected or biopsied tumor prior to therapy. They had radiologic evidence of the tumor by a gadolinium-enhanced magnetic resonance imaging (MRI) performed within 14 days before ANP started, and no earlier than four weeks following tumor resection, confirmed by an outside radiologist. The patient's performance determined by the Karnofsky Performance Status (KPS) should be above 60. The patients should not have anemia, leukopenia, thrombocytopenia or significant elevations of serum bilirubin, creatinine and transaminases. The pathologic diagnosis of AA was confirmed by outside pathologists. Patients with brainstem location of the tumor and serious additional disease were excluded from admission.

The criteria of removal from the study included progressive disease (PD), unacceptable toxicity, interfering concurrent illness, the subject's request, non-compliance, and at least an additional 8 months of therapy after CR, PR or stable disease (SD).

The patients read, understood, and signed written informed consent prior to enrollment. The study was sponsored by the Burzynski Research Institute, Inc., (BRI) and conducted by the Burzynski Clinic (BC) in Houston, Texas. The patients did not pay for the investigational agents.

3. Methods

3.1 Study Design

The study was designed as a single-arm, two-stage, interventional Phase II trial of ANP. The study was listed by the National Cancer Institute (NCI). It was supervised by the independent Institutional Review Board (BRI-IRB, BC-BT-08).

The study was performed according to Protocol BT-08 which was submitted to the FDA under the IND 43,742. Subsequently, the protocol was amended by BRI several times; however, none of the amendments altered the aim or design of the original study objectives.

3.2 Statistical Considerations

The sample size was calculated based upon the method described by Chang et al. (1999). The distributions of survival and treatment failure were estimated by Kaplan-Meier analysis. All analyses were performed on an intent-to-treat (ITT) population.

3.3 Administration of the Medications, Evaluation and Follow-Up

ANP was the only anti-tumor treatment administered in this study. The formulations were delivered via a dual-channel infusion pump and single-lumen subclavian catheter (Broviac or Groshong) every 4 hours and according to the maintenance plan patients were taking up to 3.0 g six times a day of both A10 and AS2-1 as described before (S. Burzynski, T. Janicki, & G. Burzynski, 2014).

Medications that were considered necessary for the subjects' welfare and that did not interfere with the evaluation of treatment were given at the discretion of the investigator. The use of corticosteroids was carefully monitored. Subjects received full supportive care, including transfusions of blood products and antibiotics when appropriate.

The initial two weeks of therapy was administered by BC staff on an outpatient basis, in Houston, Texas. The treatment did not require hospitalization. The outpatient treatment was also used in another Phase II study for AA (Gilbert et al., 2002). The details of monitoring, testing and evaluation of response and toxicity were described previously (S. Burzynski et al., 2014).

4. Results

4.1 Patients Demographics

Subject enrollment started April 1995 and continued through August 2007. The results were analyzed prior to the target enrollment level of $N = 40$, due to sufficient evaluable subjects with an OR.

The 19 candidates were of a median age of 44 years and represented a final ITT population. Table 1 summarizes demographics for the patients.

Table 1. Study demographics – Protocol BT-08

ANTINEOPLASTONS		
Characteristic	*N*	Range or (%)
Total number of patients	19	
Median age years (range)	44	22-62
Sex, *N* (%)		
Male	12	(63)
Female	7	(37)
KPS, *N* (%)		
Median (range)	80	60-100
Median time from diagnosis (months)	14	
Surgery at initial diagnosis, *N* (%)		
Biopsy	12	(63)
Subtotal resection	7	(37)

Note. KPS-Karnofsky performance status; N-number.

Patient demographics were similar to the Gilbert et al study with the exception of lower median age (36 years vs. 44 years) and better KPS in the other study (Gilbert et al., 2002). The pathology diagnosis was confirmed at university hospitals in 12 cases and in regional hospitals in 7 cases. Baseline radiological confirmation was performed at universities in 5 cases, regional hospitals in 8 cases and regional radiology departments in 6 cases.

Four (21.0%) subjects presented with multicentric disease and two (10.5%) had multifocal enhancements. The median size of enhancement at the baseline was 4.3 cm^2.

The pre-treatment tumor evaluation and treatment prior to enrolment is summarized in Table 2.

Table 2. Pre-treatment tumor evaluation and prior treatment in AA patients

Case no.	Prior Surgery	Location	Tumor type	Product of LPD (cm^2)
1	Biopsy	Lt parietal	Solitary	3.74
2	STR	Lt temporal	Multicentric	1.95
3	STR	Lt frontal	Solitary	18.2
4	STR	Lt frontal	Solitary	18
5	Biopsy	Lt insular cortex	Solitary	8
6	Biopsy	Lt temporal	Solitary	1.8
7	Biopsy	thalamus	Solitary	21.6
8	Biopsy	Rt parietal Rt frontal Rt Sylvian fissure	Multicentric	2.32
9	STR	Rt periventricular	Solitary	9
10	Biopsy	Rt anterior parietal	Solitary	7.56
11	Biopsy	Lt frontoparietal	Multifocal	0.17
12	Biopsy	Lt temporal Lt frontal convexity	Multicentric	31.2
13	PRR	Rt uncal Rt temporal horn	Multicentric	4.32
14	Biopsy	Rt thalamus	Solitary	2.2
15	GTR	Lt frontal	Solitary	3
16	Biopsy	Lt frontal	Solitary	10.88
17	Biopsy	Lt temporal	Multifocal	4.63
18	Biopsy	Rt parietal	Solitary	0.56
19	GTR	Rt frontal	Solitary	1.54

Note. GTR–gross-total resection; LPD–greatest perpendicular diameters; Lt–left; PRR–partial resection; Rt–right; STR–subtotal resection.

4.2 Treatment

The median daily dose of ANP A10 ranged from 1.4 to 11.56 g/kg/d with a median of 5.33 g/kg/d. For AS2-1, the median daily dose was 0.29 g/kg/d, with a range of 0.18 to 0.38 g/kg/d.

The median daily dose of A10 in subjects with CR observed was 3.51 g/kg/d; (range 1.0 to 8.61 g/kg/d) and the median daily dose of AS2-1 was 0.29 g/kg/d; (range 0.19 to 0.40). The median time to first OR was 13.6 weeks (range 4 to 78 weeks). In the group of subjects with an SD response, the median daily dose of A10 was 7.94 g/kg/d (range from 4.19 to 11.34 g/kg/d) and the median daily dose of AS2-1 was 0.30 g/kg/d (range 0.22 to 0.38 g/kg/d). It was speculated that by increasing the A10 dose in patients who obtained SD, it will be possible to accomplish an OR. The duration of IV ANP therapy in the ITT group ranged from 2.6 to 174.7 weeks with a median of 16 weeks. Three CR patients were eligible for a maintenance oral treatment of A10 and AS2-1 capsules. One additional SD patient was given maintenance oral treatment.

4.3 Responses and Survival

In accordance with other phase II studies conducted at the initiation of this trial, the possible responses to the treatment were CR, PR, SD and PD as described previously (S Burzynski, T Janicki & G Burzynski, 2014).

The results were analyzed prior to the target enrollment level of $N = 40$, due to sufficient enrolled subjects with CR (4 out of 19). Out of the 19 enrollees, 4 (21%) had a CR, 5 (26%) had SD, 9 (47%) had PD, and 1 patient was not evaluable because he did not have a follow-up evaluation including MRI. It took a median of 14 weeks for a CR to be reached (range 4-78 weeks). The response to antineoplaston treatment compared to TMZ followed

by RT is shown in Table 3. It should be noted that the TMZ study was not designed as a "head-to-head" comparison with the ANP study.

Table 3. Response to antineoplastons treatment compared to temozolomide followed by radiation therapy.

Antineoplastons		Temozolomide (Gilbert et al., 2002) followed by radiation therapy		
Response N%, N%				
CR*	4	21	2	10
PR	0	0	5	24
SD	5	26	8	38
Overall Survival KM				
1 year		53		70
2 years		37		50
3 years		26		
5 years		21		
10 years		14		
Progression-Free Survival KM				
1 year		26		
2 years		16		
3 years		11		
5 years		5		
10 years		5		

Note. CR-complete response; PR-partial response; SD-stable disease.

*3 out of 4 OR patients obtained CR (patients 1, 8, 11 and 19) without prior tumor resection (biopsy only).

The survival data are illustrated by the Kaplan-Meier curve (Figure 1).

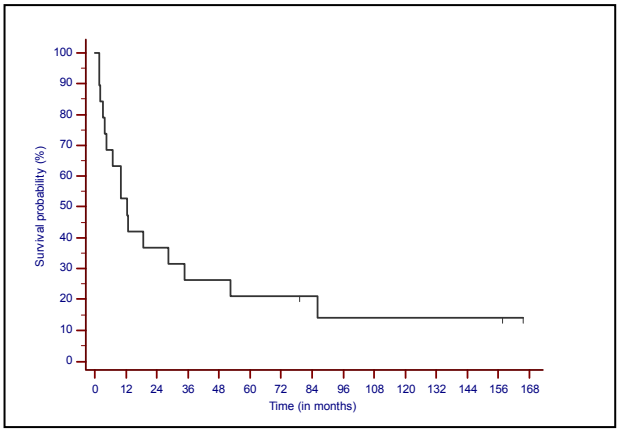

Figure 1. The Kaplan-Meier survival curve

Survival analysis revealed a progression-free survival (PFS) rate at 1 year of 26%, 2 years 16%, 3 years 11%, 5 years 5%, and 10 years 5%. OS is 53% at 1 year, 37% at 2 years, 26% at 3 years, 21% at 5 years, and 14% at 10 years. The quality of survival is very good, and KPS increased or normalized in 36% of patients. The KPS at the

baseline in four cases of CR was 90, 90, 60 and 80 and the median size of enhancement in this group was 1.9 cm^2.

In one CR case the patient accomplished 58% decrease of LPD on repeat MRI which would be classified as PR. However, two repeat positron emission tomography (PET) scans demonstrated a decrease and resolution of metabolic activity permitting classification as CR. This patient underwent stereotactic radiotherapy four years after the treatment start, and approximately a year and 8 months after determination of CR. She passed away four years and four months after treatment start and two years and one month after the completion of radiotherapy. The cause of death was not reported to us. Patient maintained CR for 34 months.

Another case of CR died from pneumonia approximately three years after treatment start, and 3 months after the last dose of ANP and one month after starting thalidomide. Patient developed PD after 21 months of CR and 25 months since first PR.

Two remaining cases of CR are alive and well at present with KPS of 100. One of these patients is approaching 14 years of tumor-free survival, lives a normal life and delivered two healthy children. She did not receive any treatment after the discontinuation of ANP.

The second surviving CR case discontinued ANP after a year and two months from treatment start, due to allergic skin rash on his lower extremities. He decided to undergo craniotomy with subtotal resection of residual tumor. The pathology examination indicated recurrence of AA after prior CR, which was maintained for 10 months. He was followed with RT with TMZ, bevacizumab and irinotecan, which contributed to his survival. These treatments were discontinued on March 15, 2009. At the time of this report his follow-up MRIs do not show any signs of tumor recurrence. He lives a normal productive life with a KPS of 100, and does not take any treatment. The majority of patients treated in this study were admitted before molecular cytogenetics techniques were available to define the "genetic signature" of patients' tumors; however, this CR case is an exception. His studies demonstrated that he was negative for *1p19q* deletion of and amplification of *EGFR*. All of his cells were positive for *TP53* mutation, and there was loss of *PTEN*. According to current classification of high-grade glioma, he would be assigned to a "mixed gene expression subgroup" which carries an inferior survival prognosis with OS not higher than 60 months (Sturm et al., 2012).There is an additional case of the patient who is currently surviving over 12 years since the treatment start. This patient started the treatment on February 7, 2001, but discontinued after six weeks when he developed pneumonia and infection of his subclavian vein catheter. Due to the lack of follow-up MRI evaluations, this case was defined as nonevaluable. He decided to take standard RT in October 2002 and TMZ for one year and discontinued in November 2003 which contributed to his survival. On August 1, 2012, eleven-and-a-half years from treatment start, he underwent craniotomy with resection of brain cyst, which represented residual lesion. There was no tumor recurrence, which was also confirmed by his MRI of November 9, 2012. He lives a normal productive life, and his KPS is 100.

The duration of IV ANP therapy ranged from 3 to 175 weeks, with a median of 16 weeks. Four patients continued therapy on a maintenance schedule in a capsule form for a median of 48 weeks (range 30-80 weeks). Among CR cases, there was a single discontinuation due to CR, two due to patient request and one due to worsening.

In summary, three patients (16%) are currently alive in excess of 14 to 6 years from treatment start and are in excellent health and live productive lives; however, quality of life was not formally assessed as an endpoint. The KPS normalized in 26% of patients, and increased in 42% compared to pretreatment as the result of ANP.

4.4 Safety and Adverse Events

Safety assessments were analyzed based upon the total number of enrolled patients in the study (*N = 19*) (Table 4).

The treatment was tolerated very well. There were Grade 3 toxicities consisting of hypokalemia and fatigue in 11% of patients, and another 11% reported Grade 4 hypernatremia and hypokalemia. Adverse drug events (ADEs) were completely reversible.

Grade 2 toxicities included: allergic reaction (21%), anemia (5%), confusion (5%), fatigue (21%), headache (5%), hypocalcemia (5%), joint pain (11%), SGOT (5%), somnolence (16%), urinary frequency (16%), vomiting (5%), xerostomia (11%).

Grade 1 toxicities included: allergic reaction (16%), dizziness (5%), fatigue (5%), fever (5%), hypernatremia (16%), hyponatremia (5%), nausea (5%), joint pain (11%), rigors/chills (16%), sweating (5%), taste alteration (5%), urinary frequency (11%), xerostomia (5%).

There were no chronic toxicities.

Table 4. Adverse drug experiences, Grades 3 or 4, to antineoplastons treatment compared with temozolomide (Gilbert et al., 2002)

ANTINEOPLASTONS			TEMOZOLOMIDE (Gilbert et al., 2002)	
ADE	Grade 3 – N *(%)*	Grade 4 – N *(%)*	Grade 3 – N *(%)*	Grade 4 – N *(%)*
Total	2 (11)	2 (11)	16 (28)	7 (12)
Hypernatremia		1 (5)		
Hypokalemia	1 (5)	1 (5)		
Somnolence (fatigue)	1 (5)		2 (4)	
Constipation			3 (5)	
Nausea			2 (4)	
Thrombocytopenia			2 (4)	3 (5)
Anemia				1 (2)
Neutropenia				1 (2)
Ataxia			1 (2)	
Impaired cognition			1 (2)	
Convulsions			1 (2)	1 (2)
Speech disorder			1 (2)	
Behavior disorder			1 (2)	
Altered mental status			1 (2)	
Suicide attempt				1 (2)
Hypotension			1 (2)	
Asthenia			1 (2)	
Back pain			1 (2)	
Headache			4 (7)	
Weight decrease			1 (2)	
Syncope			2 (4)	
Bradycardia				1 (2)
Bilirubinemia			1 (2)	
Hyperglycemia			1 (2)	
Apnea			1 (2)	
Basal cell carcinoma			1 (2)	
Urinary tract infection			1 (2)	
Intracranial hemorrhage			1 (2)	

Note. ADE-adverse drug event; N-number.

5. Discussion

Randomized, controlled clinical studies and meta-analyses have demonstrated that chemotherapy is of value in the management of patients with malignant glioma (Stupp et al., 2005; Stupp, Reni, Gatta, Mazza, &Vecht, 2007; Fine, Dear, Loeffler, Black, & Canellos, 1993; Stewart, 2002). However, evidence of a beneficial effect in the subgroups of AA patients is weak. Radiation necrosis, cranial neuropathy, and endocrine dysfunction, including growth hormone deficiency, thyroid dysregulation and gonadotropin deficiency, are delayed toxicities that can develop in patients following RT. It is assumed that RT is less effective in prolonging survival in the older patient

group due to increased toxicity on the aging brain and lower effectiveness (Glantz, Chamberlain, Liu, Litofsky, & Recht, 2003). Unfortunately, phase II clinical trials of pharmacological agent as a single treatment (without RT) for newly diagnosed AA have not been presented in peer-reviewed literature during the last eight years. Contrary to that there were numerous studies on concomitant treatment with TMZ and other agents and RT and TMZ followed by RT for AA and GBM (Stupp et al., 2005; Stupp et al., 1993; Stewart, 2002). The first report published by Gilbert et al, for newly diagnosed AA, was a multicentric phase II study of TMZ followed by RT in patients with newly diagnosed supratentorial malignant glioma (Gilbert et al., 2002). The trial accrued 57 patients, and among them 18 adult patients diagnosed with AA. The study accomplished CR in 10%, PR in 24% and SD in 38% of patients. The overall survival at 2 years was 50%. It was concluded that TMZ was a safe and effective in treating newly diagnosed AA before RT.

The second study was conducted by Rao et al to evaluate the efficacy and safety of treatment consisting of carmustine, etoposide, and cisplatin IV followed by RT (Rao et al., 2005). Out of 29 patients who were accrued for the trial, only 14 (48%) completed the chemotherapy regimen, and 76% completed the RT. It was concluded that the pre-radiation chemotherapy was not effective in this group of patients. Another trial investigating primary therapy for anaplastic glioma is a phase III randomized study of RT and chemotherapy with procarbazine, lomustine, vincristine and temozolomide, but it cannot be compared to the current study with ANP (Wick et al., 2009).

The Phase II study with ANP described in this report includes a patient group similar to the Gilbert et al trial, but the median age of patients was higher (44 vs. 36 years) and the KPS was lower in the ANP study. It can be assumed that the patient population in the ANP study was both older and sicker which could compromise the results. The percentage of CR in the ANP study was more than twice that of the TMZ study, but there were no PR in ANP group whereas 24% of patients were classified as PR in the TMZ trial. At this time we do not have a clear explanation of this fact except that in clinical trials involving agents that work through biological mechanisms, there seems to be a higher CR rate compared to PR than in studies with the other agents. Correlative studies including *1p19q* deletions and *MGMT* silencing were not performed except for the most recent admission, because they were not yet introduced when the study began and the majority of patients were accrued. The only CR case which had a cytogenetics study belongs to an unfavorable survival subgroup. Based on prior studies ANP work on *PTEN*, *TP53* and *RB* pathways and most likely will show effectiveness only in patients who have the proper "genomic signature" for their tumors (Sturm, et al., 2012; Burzynski et al., 2005; S. Burzynski, Janicki, Weaver, & B. Burzynski, 2006; Burzynski & Patil, 2014).

The overall survival at 2 years in our study is 37%, which is lower than that of the TMZ study (50%), but the survival in TMZ was accomplished in combination with RT which started no later than after four months of TMZ treatment, and not through the use of TMZ as a single agent. It can be speculated that if ANP would be followed by RT that the OS would be higher. The Gilbert et al study does not provide data for 5 and 10 years of OS, but in the ANP trials there is a substantial percentage of OS at 5 years (21%), and 10 years (14%). It can be concluded that ANP is efficacious in a subgroup of patients who will obtain CR and these patients will have long-term OS and excellent quality of survival. The currently conducted study on genomic changes in successfully treated patients should provide further information about possible markers for prediction of response.

While less disabling than RT, long-term toxicity is also seen with chemotherapy. Chronic myelosuppression, gonadal dysfunction, renal insufficiency, pulmonary toxicity, hepatotoxicity, and neurotoxicity including hearing loss can develop (Stewart, 2002; Armstrong et al., 2009; Sarganas et al., 2012; Grossman et al., 2011). Therefore, the search for safer therapies with strong efficacy needs to continue. Whereas there is evidence for the benefit of adding TMZ to RT in the therapy of GBM, there is no evidence to justify the addition of TMZ to postoperative RT for patients with newly diagnosed AA outside of clinical trials (Mu et al., 2012).

Comparison of ADEs reported in TMZ to those reported in ANP trials revealed markedly higher incidences of grades 3 and 4 toxicities for TMZ. Only 11% of patients reported grade 4 toxicity in ANP trials, and another 11% of patients had reversible grade 3 toxicity, whereas in the TMZ trial 40% of patients suffered grades 3 and 4 toxicity. The ADE in ANP trial consisted of reversible hypernatremia and somnolence (fatigue) whereas the TMZ trial patients reported severe myelosuppression, neurotoxicity and nausea in addition to less frequent ADE (Stupp et al., 2005). There were no chronic ADE in the ANP study, and the quality of survival was very high.

It is recognized that the impact of this Phase II trial is limited by a population without a "control" cohort. However, the patient population was representative of the general population at risk, and the efficacy and safety of the treatments was able to be evaluated. Future prospective, randomized studies will be able to directly assess

the comparative effectiveness of the antineoplastons regimen versus the standard radiation therapy or chemotherapy.

The study reached the goal with 4 cases of CR in ITT population. It provides evidence-based research supporting the safety and efficacy of antineoplastons for the treatment of anaplastic astrocytoma which should be confirmed by further studies. More importantly, this treatment was found to be reasonably well tolerated.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

Armstrong T. S., Cao, Y., Scheurer, M. E., Vera-Bolaños, E., Manning, R., Okcu, M. F., Bondy M., … Gilbert, M. R. (2009). Risk analysis of severe myelotoxicity with temozolomide: the effects of clinical and genetic factors. *Neuro-Oncology, 11*, 825-832. http://dx.doi.org/10.1215/15228517-2008-120

Burzynski, S. R., & Kubove, E. (1986). Toxicology studies on antineoplaston A10 injections in cancer patients. *Drugs under Experimental and Clinical Research, suppl 12*, 47-55.

Burzynski, S. R., Burzynski, B., & Mohabbat, M. O. (1986). Toxicology studies on antineoplastons AS2-1 injections in cancer patients. *Drugs under Experimental and Clinical Research, suppl 12*, 25-35.

Burzynski, S. R., Kubove, E. & Burzynski, B. (1992). Phase II clinical trials of antineoplastons A10 and AS2-1 infusions in astrocytoma. In: D. Adam (Ed.), *17th International Congress of Chemotherapy*. Munich, Germany: Futuramed.

Burzynski, S. R. (2004). The present state of antineoplaston research (1). *Integrative Cancer Therapies, 3*, 47-58. http://dx.doi.org/10.1177/1534735403261964

Burzynski, S. R., Weaver, R.,A., Janicki, T., Szymkowski, B., Jurida, G., Khan, M.,& Dolgopolov, V. (2005). Long-term survival of high-risk pediatric patients with primitive neuroectodermal tumors treated with Antineoplastons A10 AS2-1. *Integrative Cancer Therapies, 4*, 168-77. http://dx.doi.org/10.1177/1534735405276835

Burzynski, S. R., Janicki, T. J., Weaver, R. A. & Burzynski, B. (2006). Targeted therapy with Antineoplastons A10 and AS2-1 of high grade, recurrent and progressive brainstem glioma. *Integrative Cancer Therapies, 5*, 40-47. http://dx.doi.org/10.1177/1534735405285380

Burzynski, S. R., Janicki, T. J., Burzynski, G. S., & Marszalek, A. (2014). The response and survival of children with recurrent diffuse intrinsic pontine glioma based on phase II study of Antineoplastons A10 and AS2-1 in patients with brainstem glioma [Online]. *Child's Nervous System*. http://DOI 10.1007/s00381-014-2401-z

Burzynski, S. R., & Patil, S. (2014). The effect of antineoplastons A10 and AS2-1 and metabolites of sodium phenylbutyrate on gene expression in glioblastoma multiforme. *Journal of Cancer Therapy, 5*, 929-945. http://dx.doi.org/10.4236/jct.2014.510099

Burzynski, S. R., Janicki, T., Burzynski, G., & Marszalek, A. (2014). A phase II study of antineoplastons A10 and AS2-1 in children with high-grade glioma. Final report (Protocol BT-06), and review of recent trials. *Journal of Cancer Therapy, 5*, 565-577. http://dx.doi.org/10.4236/jct.2014.56065

Burzynski, S. R., Janicki, T. J., & Burzynski, G. S. (2014). A phase II study of antineoplastons A10 and AS2-1 in adult patients with recurrent glioblastoma multiforme: Final report (Protocol BT-21). *Journal of Cancer Therapy, 5*, 946-956. http://dx.doi.org/10.4236/jct.2014.510100

Burzynski, S. R., Burzynski, G. S., & Janicki, T. J. (2014). Recurrent glioblastoma multiforme – A strategy for long-term survival. *Journal of Cancer Therapy, 5*, 957-976. http://dx.doi.org/10.4236/jct.2014.510101

Burzynski, S. R., Janicki, T. J., Burzynski, G. S., Marszalek, A., & Brookman, S. (2014). A phase II study of antineoplastons A10 and AS2-1 in children with recurrent, refractory or progressive primary brain tumors – Final report (Protocol BT-22). *Journal of Cancer Therapy, 5*, 977-988. http://dx.doi.org/10.4236/jct.2014.510102

Chang, S. M., Kuhn, J. G., Robins, H. I., Schold, S. C., Spence, A. M., Berger, M. S., Mehta, M. P., … Prados, M. D. (1999). Phase II study of phenylacetate in patients with recurrent malignant glioma: a North American Brain Tumor Consortium report. *Journal of Clinical Oncology, 17*, 984-90.

Chow, L. M., Endersby, R., Zhu, X., Rankin, S., Qu, C., Zhang, J., Broniscer A., … Baker, S. Jr. (2011). Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain. *Cancer Cell, 19*, 305-16. http://dx.doi.org/10.1016/j.ccr.2011.01.039

Fine, H. A., Dear, K. B., Loeffler, J. S., Black, P. M., & Canellos, G. P. (1993). Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. *Cancer, 71*, 2585-97.

Gilbert, M. R., Friedman, H. S., Kuttesch, J. F., Prados, M. D., Olson, J. J., Reaman, G. H. & Zaknoen, S. L. (2002). A phase II study of temozolomide in patients with newly diagnosed supratentorial malignant glioma before radiation therapy. *Neuro-Oncology, 4*, 261-7.

Glantz, M., Chamberlain, M., Liu, Q., Litofsky, N. S. & Recht, L. D. (2003). Temozolomide as an alternative to irradiation for elderly patients with newly diagnosed malignant gliomas. *Cancer, 97*, 2262-2266.

Greene-Schloesser, D. & Robbins, M. E. (2012). Radiation-induced cognitive impairment-from bench to bedside. *Neuro-Oncology, 14*, iv37-iv44. http://dx.doi.org/10.1093/neuonc/nos196

Grossman, S. A., Ye, X., Lesser, G., Sloan, A., Carraway, H., Desideri, S., & Piantadosi, S. (2011). Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. *Clinical Cancer Research, 17*, 5473-80. http://dx.doi.org/10.1158/1078-0432.CCR-11-0774

Kitange, G. J., Templeton, K. L. & Jenkins, R. B. (2003). Recent advances in the molecular genetics of primary gliomas. *Current Opinion in Oncology, 15*, 197-203.

Louis, D. N., Ohgaki, H., Wiestler, O. D., Cavenee, W. K., Burger, P. C., Jouvet, A., Scheithauer, B. W. & Kleihues, P. (2007). The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathologica, 114*, 97-109. http://dx.doi.org/10.1007/s00401-007-0243-4

Minniti, G., Searingi, C., Arcella, A., Lanzetta, G., Di Stefano, D., Scarpino, S., Bozzao, A., … Enrici, R. M. (2014). IDH1 mutation and MGMT methylation status predict survival in patients with anaplastic astrocytoma treated with temozolomide-based chemoradiotherapy. *Journal of Neuro-Oncology, 118*, 377-383. http://dx.doi.org/10.1007/s11060-014-1443-0

Mu, Y. G., Huang, L. J., Li, S. Y., Ke, C., Chen, Y., Jin, Y., & Chen, Z. P. (2012). Working memory and the identification of facial expression in patients with left frontal glioma. *Neuro-Oncology, 14*, iv81-iv89. http://dx.doi.org/10.1093/neuonc/nos215

Ostrom, Q. T., Gittleman, H., Farah, P., Ondracek, A., Chen, Y., Wolinsky, Y., Stroup, N., … Barnholtz-Sloan, J. S. (2013). CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro-Oncology, 15*, ii1-ii56. http://dx.doi.org/10.1093/neuonc/not151

Rao R.D., Krishnan S., Fitch T.R., Schomberg P. J., Dinapoli R. P., Nordstrom K., Scheithauer B., … Buckner J. C. (2005). Phase II trial of carmustine, cisplatin, and oral etoposide chemotherapy before radiotherapy for grade 3 astrocytoma (anaplastic astrocytoma): results of North Central Cancer Treatment Group trial 98-72-51. *International Journal of Radiation Oncology, Biology, Physics, 61*, 380-86.

Sarganas, G., Orzechowski, H. D., Klimpel, A., Thomae, M., Kauffmann, W., Herbst, H., Bronder, E., & Garbe, E. (2012). Severe sustained cholestatic hepatitis following temozolomide in a patient with glioblastoma multiforme: case study and review of data from the FDA adverse event reporting system. *Neuro-Oncology, 14*, 541-6. http://dx.doi.org/10.1093/neuonc/nos056

Scoccianti, S., Magrini, S. M., Ricardi, U., Detti, B., Krengli, M., Parisi, S., Bertoni, F., … Biti, G. (2012). Radiotherapy and temozolomide in anaplastic astrocytoma: a retrospective multicenter study by the Central Nervous System Study Group of AIRO (Italian Association of Radiation Oncology). *Neuro-Oncology, 14*, 798-807. http://dx.doi.org/10.1093/neuonc/nos081

Stewart, L. A. (2002). Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet, 359*, 1011-18.

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., Belanger, K., …Mirimanoff R. O. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New England Journal of Medicine, 352*, 987-96.

Stupp R., Reni M., Gatta G., Mazza E. & Vecht C. (2007). Anaplastic astrocytoma in adults. *Critical Reviews in*

Oncology/Hematology, 63, 72-80.

Sturm D., Witt H., Hovestadt V., Khuong-Quang D. A., Jones D. T., Konermann C., Pfaff E., …Pfister S. M. (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*, *22*, 425-37.

Wick, W., Hartmann, C., Engel, C., Stoffels, M., Felsberg, J., Stockhammer, F., Sabel, M. C., … Weller M. (2009). NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *Journal of Clinical Oncology, 27*, 5874-5880. http://dx.doi.org/10.1200/JCO.2009.23.6497