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Treatment of Recurrent Glioblastoma Multiforme (rGBM) with Antineoplaston AS2-1 in Combination with Targeted Therapy

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Abstract

Treatment of recurrent glioblastoma multiforme (rGBM) poses a difficult challenge. Therefore, the purpose of this report was to evaluate objective response (OR), progression-free survival (PFS), overall survival (OS), and the incidence of adverse events (AEs) in rGBM patients, age 19-70 years, who were treated with antineoplaston AS2-1 (Astugenal) plus targeted therapy. A retrospective analysis was performed. Tumor response was assessed by gadolinium-enhanced magnetic resonance imaging (MRI). Twenty-nine adult rGBM patients were treated between 9/11/2015 and 06/23/2018. Seven had no prior treatment with bevacizumab elsewhere, had radiologic evidence of rGBM, and had MRI assessment of tumor response. The median treatment time was 101 days (range: 55-208 days). OR was seen in six patients (85.7%) with complete disappearance of gadolinium enhancement in four patients (57.1%) and a 50% or greater reduction in gadolinium enhancement in two patients (28.6%). Progressive disease was seen in one patient (14.3%). The median time to first response was 29 days (range: 22-96 days) while the median duration of response was 141 days (range 55-739+ days). Six- and 12-month PFS was 57% and 19%, respectively while 6- and 12-month OS at was 86% and 54%, respectively. Treatment was well-tolerated with no patients experiencing grade 3 or 4 antineoplaston-related toxicity. Regarding response to treatment and toxicity, AS2-1 plus targeted therapy compares favorably with other reported rGBM therapies. Duration of response was shortened by the ill-advised decision of some patients to discontinue treatment after a tumor response was achieved.

Keywords: antineoplaston AS2-1, Astugenal, recurrent glioblastoma, survival in recurrent glioblastoma, targeted therapy

1. Introduction

Glioblastoma multiforme (GBM) is the most common and most aggressive primary malignant brain tumor (Ostrom et al., 2018). The prognosis for newly-diagnosed GBM patients is poor with a median survival of 14.6 months from diagnosis despite standard treatment with surgery, radiation therapy (RT), adjuvant temozolomide (TMZ) and bevacizumab (BVZ) (Stupp et al., 2005; Ohka, Natsume & Wakabayashi, 2012). After standard therapy, the 5-year overall survival (OS) rate is in the range of 1% - 2% (Olson et al., 2014; Stupp et al., 2005) while median progression-free survival (PFS) is 6 to 9 months (Stupp et al., 2009). The prognosis for patients with recurrent GBM (rGBM) is much worse, with most patients dying within six months of recurrence.

In phase II clinical trials (IND 43742), we have evaluated treatment of GBM and rGBM with antineoplastons, a group of peptides, amino acid derivatives, and carboxylic acids that inhibit the growth of neoplastic cells without inhibition of the growth of normal cells (Burzynski, 1976; Burzynski, 1986; Burzynski, 2004; Burzynski, 2006; SR Burzynski, Janicki, GS Burzynski & Marszalek, 2014, 2051-2061; SR Burzynski, Janicki, GS Burzynski & Marszalek, 2014, 565-577; SR Burzynski, Janicki & GS Burzynski, 2014; SR Burzynski, Janicki, GS Burzynski, Marszalek & Brookman, 2014; SR Burzynski, GS Burzynski & Janicki, 2014).

Phase II studies, utilizing antineoplastons AS2-1 (Astugenal) and A10 (Atengenal) in combination, evaluated their safety and efficacy in recurrent high-grade glioma, with a special emphasis on rGBM. Objective responses (ORs) were achieved in 17% of eligible patients while overall survival (OS) was 65.5% at six months, 56.7% at nine months, 39% at one year, and 4.4% at two, five, and ten years (SR Burzynski, Janicki & GS Burzynski, 2014).

AS2-1 is composed of phenylacetylglutaminate (PG) and phenylacetate (PN) and together with its metabolites affects approximately 100 abnormal genes in the GBM genome (Burzynski & Patil, 2014). Published data indicate that some GBM patients will respond to antineoplastons alone but for most GBM patients antineoplastons will not be effective in controlling approximately 650 abnormal genes in the GBM genome. Therefore, we elected to treat these terminal rGBM patients with a combination of AS2-1 plus targeted therapy that was based on genomic profiling. We report here on seven evaluable patients with rGBM treated with AS2-1 and targeted therapy. Three of these patients also received A10.

2. Patients and Methods

2.1 Patients

Between 9/11/2015 and 06/23/2018, 29 adult GBM patients age 19-70 years received treatment at the Burzynski Clinic (BC) under the state's "Right to Try" law. Of these 29 patients, 18 had not received standard of care treatment, had received prior treatment elsewhere with BVZ, and/or did not have radiologic evidence of recurrence. Of the remaining 11 patients, only seven had follow-up magnetic resonance imaging (MRI) for evaluation of tumor response. These seven patients received AS2-1 and, based on genomic testing, also received targeted therapies that were given sequentially over a period of days as determined by the characteristics of the individual targeted therapies. These seven patients are the focus of this report.

Data were collected by retrospective medical record review. Table 1 shows the patients' pathology, treatment, and recurrence data (as reported from outside institutions) prior to treatment with AS2-1 plus targeted therapy at BC. Cases #2, #6, and #7 received sodium phenylbutyrate (PB) plus targeted therapy (including BVZ) at BC prior to receiving AS2-1 plus targeted therapy. However, their disease did not progress during prior BVZ therapy at BC.

Standard blood and urine analyses were performed at BC and at other institutions. Molecular profiling of tissue samples was performed by Foundation Medicine (FMI) of Cambridge, MA and Caris Life Science (CLS) of Phoenix, AZ, neither of which have any affiliation with BC. See Table 2.

All patients read, indicated their understanding of, and signed an informed consent document, which explained in detail the treatment and possible associated adverse events (AEs).

As described above, the treatment plan for any patient was based on genomic profiling and consisted of antineoplaston AS2-1 for broad-spectrum coverage and selected targeted agents for specific genomic abnormalities (SR Burzynski, GS Burzynski & Janicki, 2014).

Table 1. Pathology and prior treatment and recurrence data

Case #	Pathology diagnosis and date	Treatment prior to AS2-1 plus targeted therapy	Recurrence date	Recurrence assessment
1	GBM - 6/9/2015	SU 6/1/2015 – Subtotal resection		
		RT 6/24/2015-7/30/2015 60Gy	9/1/2015	Recurrence
		TMZ 7/30/2015 - 7/30/2015		+ new lesion
2	GBM - 11/14/2011	SU 11/1/2011 - Total resection		
		RT 12/12/2011-1/24/2012		
		TMZ 12/12/2011-1/24/2012		
		SU 2/25/2012		
		BC: 4/18/2012-10/21/2015		
		PB, erlotinib, BVZ, TMZ, sirolimus	8/14/2015	New lesion
	GBM - 9/17/2015	SU 09/17/2015 - Total resection	10/15/2015	Progression
3	GBM - 4/8/2014	SU 4/8/2014		
		RT 4/30/2014-6/12/2014 60Gy		
		TMZ 4/30/2014-7/8/2014	8/2014	Recurrence
	GBM - 8/14/2014	SU 8/14/2014		
		Aug 2014 Vaccination trial (UCLA)		
		TMZ 10/1/2014 - April 2015	9/2015	Progression
		September 2015		
		pembrolizumab + nivolumab		
		SU 1/18/2016, 6/12/2016; 10/2/2016	12/9/2016	Progression
4	Astrocytoma Grade IV	SU 1/5/2009		
	(GBM) - 1/5/2009	RT 2/16/2009-4/1/2009 59Gy		
	GBM - 1/4/2016	TMZ 2/16/2009-4/4/2009	12/24/2015	New lesions
		SURG 1/4/2016		
		RT 2/2/2016-3/7/2016 59 Gy		
		TMZ 2/2/2016-9/16/2016	12/12/2016	Recurrence
		RT 12/20/2016 (Gamma knife)	1/2017	Progression
5	GBM - 10/3/2016	SU 9/20/2016 - Total resection	3/1/2017	Progression
		RT 10/2016 -11/30/2016		
		TMZ 10/2016 -11/30/2016		
		TMZ 01/2017 - 03/7/2017		
6	GBM - 6/8/2015	SU 5/11/2015 - Total resection		
		RT 6/1/2015-7/22/2015 60 Gy		
		TMZ 6/1/2015-7/07/2016	2/8/2017	Progression
		BC: 2/22/2017-7/10/2017		
		PB, dasatinib, pazopanib,		
		everolimus, BVZ (5/26/2017)	6/28/2017	Progression
7	Diffuse glioma with possible	SU 8/12/2014 - Subtotal resection		
		SU 10/2/2015		
	oligodendro-glioma	RT 11/12/2015-12/24/2015	8/5/2015	Progression
	component - 8/18/2014	BC: 1/8/2016-5/20/2016		
	GBM – 10/16/15	PB, everolimus, dasatinib,		
		sorafenib, BVZ	5/24/2016	Progression

Abbreviations: BC, Burzynski Clinic; BVZ, bevacizumab; GBM, glioblastoma multiforme; PB, sodium phenylbutyrate; RT, radiation therapy; SU, surgery; TMZ, temozolomide

Therapy was performed on an out-patient basis. Following an initial two to four week treatment period at BC, patients continued their treatment at home under our care and in cooperation with local oncologists. Prior to the start of treatment, an MRI with and without gadolinium-enhancement, was performed. For each measurable lesion (gadolinium enhancing and at least 5 mm in diameter) the product of the two largest perpendicular diameters was calculated and then the products of all measureable lesions were totaled (SUM), providing a baseline evaluation for each study subject and a reference for determining response to the treatment, which was assessed by MRIs performed every four to six weeks during initial treatment and every three months thereafter.

Additional assessments included demographics, medical history, current medications, physical examination, vital signs, clinical disease status, and the Karnofsky Performance Status (KPS).

Table 2. Genomic testing, alterations identified, and GBM subtype

Case #	Institution	Genomic alterations identified (up to five alterations listed)	GBM subtype
1	Foundation Medicine International Cambridge, MA	CDK4 amplification KDR amplification KIT amplification PDGFRA amplification PIK3CA amplification	Proneural
2	Caris Life Science Phoenix, AZ	PTEN amplification EGFR low MGMT negative RRM1 negative TS negative	Classical
3	Caris Life Science Phoenix, AZ	TOP2A positive TOPO1 positive TS positive	Classical
4	Not performed		IDH wild type
5	Foundation Medicine International Cambridge, MA	AKT3 amplification EGFR amplification CDKN2A/B loss SMARCA4 rearrangement TERT promoter -124C>T	Classical
6	Foundation Medicine International Cambridge, MA	ATM MAP2K1 PDGFRA PTEN IRF2	Proneural
7	Foundation Medicine International Cambridge, MA	BRAF V600E- alteration	Atypical

Possible responses to treatment included an OR, specifically, complete disappearance of gadolinium enhancement (CR) or ≥ 50% reduction in gadolinium enhancement (PR), i.e., SUM ≤ 50% of baseline SUM, and progressive disease (PD), which was evident when there was a ≥25% increase in gadolinium enhancement (i.e., SUM ≥ 25% of the baseline SUM or ≥ 25% of the smallest SUM achieved during treatment) or the appearance of new measureable lesions. The duration of each response was measured from the date that the response was first observed until the date that PD became evident.

Common Terminology Criteria for Adverse Events was used to document toxicities related to therapy.

2.2 Treatment

Based on genetic testing, five patients received a combination of, AS2-1, A10, BVZ, dasatinib, everolimus and pazopanib while one patient received AS2-1, ipilimumab and nivolumab, and one patient received AS2-1, BVZ, and PB. See Table 3.

Table 3. Treatment received at BC

Case #	Age (yrs)	KPS at treatment start	Onset of targeted therapy	Days on treatment
1	47	90	9/9/15 - pazopanib 9/11/15 – ANP 9/11/15 - dasatinib 9/15/15 - BVZ 9/17/15 - everolimus	55
2	57	80	10/22/15 – ANP 10/23/15 - everolimus 10/23/15 - dasatinib 10/27/15 - BVZ 11/02/15 - pazopanib	65
3	39	50	12/15/16 - AS2-1 12/20/16 - BVZ 12/20/16 - everolimus 12/27/16 - pazopanib 12/27/16 - dasatinib 2/15/2017 - A10	119
4	27	90	2/15/17 - AS2-1 2/16/17 - dasatinib 2/20/17 - everolimus 2/22/17 - pazopanib 2/27/17 - BVZ	181
5	55	70	3/27/2017 - AS2-1 3/28/17 - BVZ 3/29/17 - pazopanib 3/29/17 - everolimus 4/4/17 - dasatinib	101
6	50	60	7/13/17 AS2-1 7/18/17 - ipilimumab 7/18/17 - nivolumab	79
7	23	90	5/26/2016 - AS2-1 6/17/16-7/14/16 - BVZ 6/2/16 - 11/25/16 PB	208

Abbreviations: ANP, antineoplaston therapy (AS2-1 + A10); BC, Burzynski Clinic; BVZ, bevacizumab; KPS, Karnofsky Performance Status; PB, sodium phenylbutyrate

Antineoplastons AS2-1 and A10 were administered intravenously (IV) via a subclavian vein catheter (Hickman, Groshong, Broviac) and portable infusion pump as previously described (SR Burzynski, Janicki & GS Burzynski, 2014). A single channel infusion pump was used for the administration of AS2-1 while a double channel infusion pump was used for the administration of AS2-1 and A10. BVZ (10.0 mg/kg) was infused IV every two weeks while everolimus (Afinitor), 2.5-5.0 mg, dasatinib (Sprycel), 20.0-50.0 mg, and pazopanib (Votrient), 200.0 mg, were administered orally, on a daily basis, at a 50% to 75% dose reduction. Ipilimumab (Yervoy), 1.0 mg/kg, and nivolumab (Opdivo), 3 mg/kg, were infused IV every three weeks.

3. Results

3.1 Demographics

Seven evaluable patients are the focus of this report. The demographics of these seven patients are shown in Table 4.

Table 4. Demographics

Age (yrs)		Gender		KPS	
Median	Range	Male	Female	Median	Range
47	23.9-57.2	5	2	80	50 - 90

Abbreviations: KPS, Karnofsky Performance Status

3.2 Response and Survival

An OR was achieved in six patients (85.7%) with a CR (complete disappearance of gadolinium enhancement) being achieved in four patients (57.1%) and a PR, i.e., ≥ 50% reduction in gadolinium enhancement (SUM ≤ to 50% of baseline SUM) was achieved in two patients (28.6%). PD, a ≥ 25% increase in gadolinium enhancement (i.e., SUM ≥ 25% of the baseline SUM or ≥ 25% of the smallest SUM achieved during treatment) or the appearance of new measureable lesions, was seen in one patient (14.3%). The median time to first response was 29 days (range: 22-96 days) while the median duration of response was 141 days (range 55-739+ days). See Table 4.

Figure 1 shows Kaplan Meier survival curves for this group of patients. Six- and 12-months PFS was 57% and 19%, respectively, while 6- and 12-month OS was 86% and 54%, respectively.

3.3 Safety and Adverse Events

There were no grade 4 AEs while there were five grade 3 AEs, none of which were related to antineoplastons. Grade 1 or 2 AEs related to antineoplastons were fatigue (2 patients), polydipsia (1 patient), and paresthesia (1 patient).

3.4 Case Studies

3.4.1 Case #2 as listed in Tables 1-5

In October 2011, this patient, a 57-year-old Caucasian male, experienced seizures. MRI showed a mass in the right parietal area and biopsy suggested a grade 3 oligoastrocytoma and gliomatosis. In November 2011, he underwent subtotal resection of an 8 x 5.5 cm tumor that involved the right temporal, occipital and parietal lobes and the corpus callosum. Pathologic examination demonstrated a GBM. RT and TMZ were administered, but two weeks after completion of RT, the patient developed recurrent disease and subsequently underwent a second resection. Six weeks later he began treatment at BC (Table 1) where baseline MRI revealed a 5.2 x 4.3 cm tumor. Following six weeks of treatment with PB, BVZ, erlotinib, sirolimus, and TMZ, the patient achieved a CR, which persisted for three and one-half years. In August 2015, MRI showed recurrent tumor in the right temporal and parietal lobes and the patient underwent another tumor resection with pathologic examination demonstrating rGBM. The patient returned to BC (Table 5) and received pazopanib, AS2-1, A10, dasatinib, BVZ, and everolimus. On this treatment, he achieved a CR (see Figure 2) with PFS of 9.4 months and OS (from the start of his second course of treatment at BC) of 12.0 months. The patient's treatment was prematurely discontinued after eight weeks.

Table 5. Results of AS2-1 plus targeted therapy received at BC

Case #	Best response	MRIs: Baseline and follow-up with response status	PFS (days)	OS (days) and patient status
1	CR	9/1/15 BL 10/5/15 CR 11/2/15 CR 2/1/16 CR 5/10/16 CR 7/5/16 PD	298	732+ alive
2	CR	10/15/15 BL 11/30/15 1/4/16 CR 4/15/16 CR 8/4/16 PD	287	376 deceased
3	PR	12/9/16 BL 1/10/17 PR 2/4/17 PR 3/30/17 PR	---	224+ alive
4	CR	2/14/17 BL 3/19/17 CR***** 5/4/17 PD 6/15/17 PD 7/19/17 PD	78	211 deceased
5	PR	3/1/17 BL 3/28/17 4/27/17 PR***** 6/1/17 PD	66	314 deceased
6	PD	6/28/17 BL 7/26/17 PD	13	120 deceased
7	CR	5/24/16 BL 8/30/16 CR 12/9/16 CR 2/22/17 CR 6/1/17 CR 6/30/17 CR 8/11/17 CR 12/20/17 CR 5/29/18 CR	---	739 + alive

* A second MRI was not performed for confirmation of the CR/PR.

Abbreviations: BC, Burzynski Clinic; BL, baseline; CR, complete response; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response

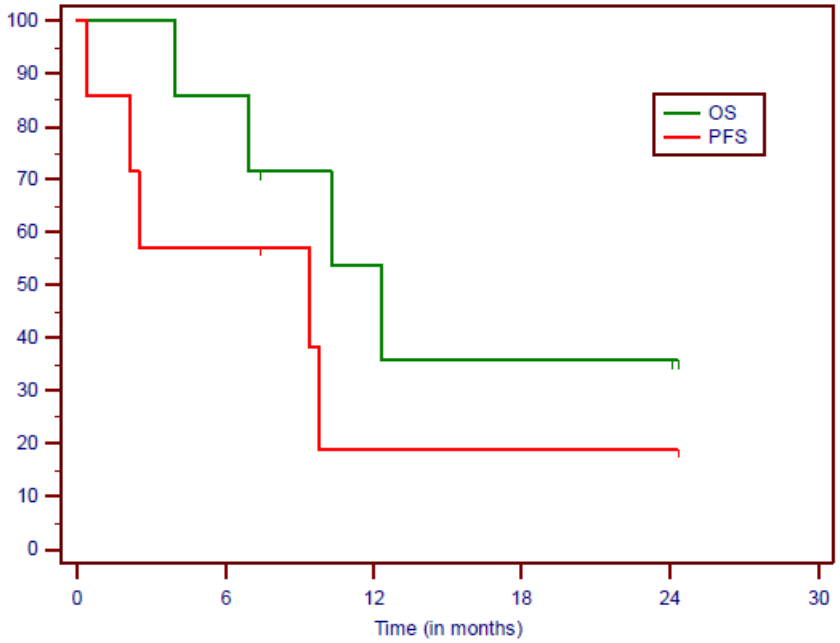

Figure 1. Kaplan Meier survival curves for study population

Abbreviations: OS, overall survival from treatment start; PFS, pro-gression-free survival from treatment start

3.4.2 Case #3 as listed in Tables 1-5

This patient, a 39-year-old Caucasian male, underwent resection of a brain tumor in April 2014. Pathologic examination demonstrated a GBM. RT and TMZ were administered (Table 1), but he developed recurrent disease in August 2014 and underwent a second resection with pathologic examination demonstrating rGBM. The patient then received an experimental vaccine and additional TMZ. In September 2015, he developed PD and was treated with pembrolizumab and then nivolumab. Subsequently, he underwent a third, fourth, and fifth tumor resection. In December 2016, the patient began treatment at BC (Table 5) where he received AS2-1, BVZ, everolimus, pazopanib, dasatinib, and A10. On this treatment, he achieved a PR approximately one month after starting treatment (see Figure 3). From the start of his treatment at BC, OS was 7.3 months, but the patient prematurely discontinued his treatment three months after achieving a PR.

4. Discussion

No contemporary treatments for newly-diagnosed GBM or rGBM are curative. Photodynamic therapy following aggressive tumor resection (Kaneko et al., 2018), High-intensity focused ultrasound ablation (Alkins, et al., 2018), and nanotechnology-driven neuro-oncological interventions (Yoon, et al., 2018) may have impact on high grade gliomas.

However, an understanding of the molecular mechanisms and gene mutations involved in the development and growth of GBM are leading to more promising and tailored therapeutic approaches. Multiple challenges remain including tumor heterogeneity and rapid, aggressive tumor relapse. Therefore, the treatment of patients with newly-diagnosed GBM remains palliative and consists of maximal surgical resection, RT, and concomitant and adjuvant chemotherapy with TMZ (Stupp et al., 2005; Stupp et al., 2009). PFS and OS with TMZ is enhanced in patients with MGMT (O^6-methylguanine–DNA methyltransferase) promoter methylation (Hegi et al., 2004).

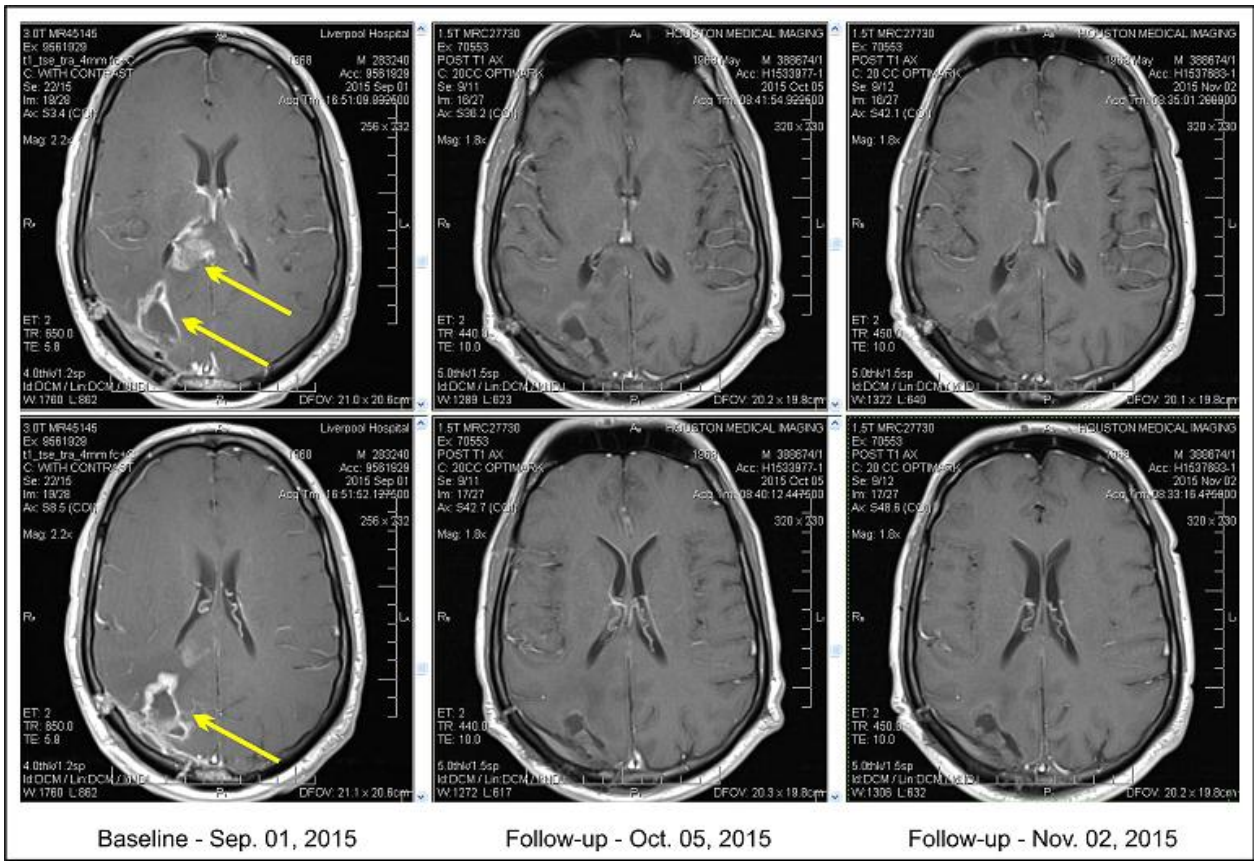

Figure 2. MRI of the head in Case #2. Post-gadolinium contrast T1 studies. Baseline - Sep. 01, 2015; Follow-up - Oct. 05, 2015 (first scan to show a CR); Follow-up – Nov. 02, 2015 (confirms the CR)

At BC, 29 rGBM patients were treated between 9/11/2015 and 06/23/2018. Seven had no prior treatment with bevacizumab elsewhere, had radiologic evidence of rGBM, and had MRI assessment of tumor response. These seven patients received AS2-1 and, based on genomic testing, also received targeted therapies that were given sequentially over a period of days as determined by the characteristics of the individual targeted therapies. Four of these seven patients achieved a CR while two patients achieved a PR.

E. Lee and colleagues reported on a multi-institutional study in which patients with recurrent high-grade glioma (HHG) were treated with oral panobinostat 30 mg three times per week, every other week, in combination with BVZ 10 mg/kg every other week (Lee et al., 2015). The primary endpoint was 6-month PFS for patients with rGBM while patients with recurrent anaplastic glioma (AG) were evaluated as an exploratory arm of the study. Panobinostat is a histone deacetylase inhibitor with antineoplastic and antiangiogenic effects in glioma that may work synergistically with BVZ.

At interim analysis, the rGBM arm did not meet criteria for accrual (see Table 6). A total of 24 rGBM patients were accrued prior to study closure. The 6-month PFS was 30.4%, median PFS was 5 months, and median OS was 9 months. The addition of panobinostat to BVZ in patients with rGBM did not improve 6-month PFS compared with historical controls of BVZ monotherapy. Our results show a better 6-month PFS (57% vs 30.4%), median PFS (9.4 vs 5 months), median OS (12.4 months vs 9 months) and CR (57% vs 0). See Table 6.

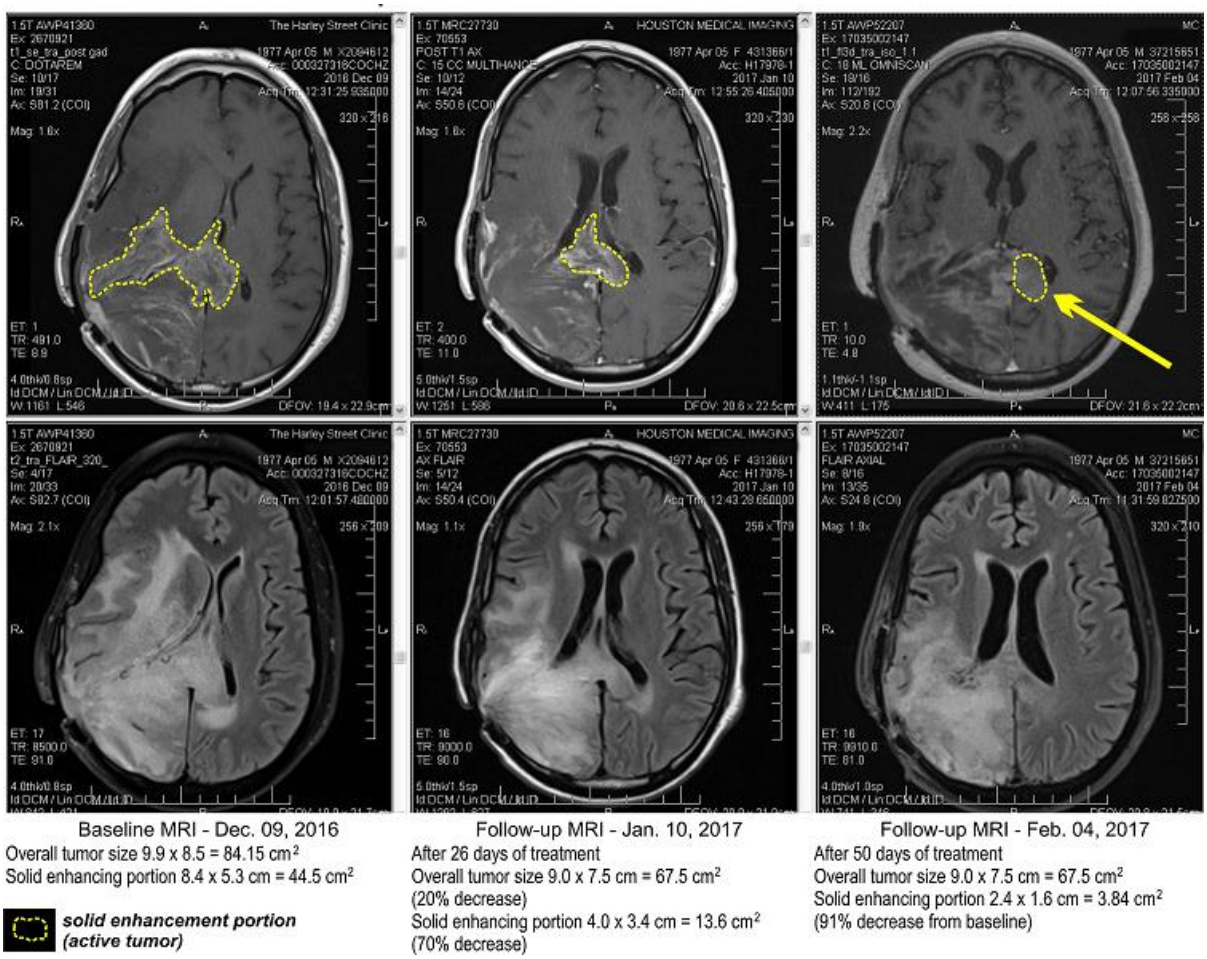

Figure 3. MRI of the head in Case #3. Upper row shows post-gadolinium contrast T1 studies while lower row shows T2/FLAIR studies; Baseline - Dec. 09, 2016; Follow-up - Jan. 10, 2015 (first scan to show a PR); Follow-up – Feb. 04, 2017 (confirms a PR)

J. Vredenburgh and colleagues reported on a phase II study of adults with recurrent grade II-IV glioma in which intravenous BVZ and irinotecan were given every two weeks of a six-week cycle (Vredenburgh et al., 2/2007). BVZ was administered at 10 mg/kg while the dose of irinotecan was determined based on the use of antiepileptics. Patients taking enzyme-inducing antiepileptic drugs received 340 mg/m^2 while patients not taking enzyme-inducing antiepileptic drugs received 125 mg/m^2.

Twenty-three patients with recurrent high-grade glioma in the phase II study were supplemented by a second cohort of 12 rGBM patients (Vredenburgh et al., 10/2007). This second cohort of patients received intravenous BVZ at 15 mg/kg every 21 days while irinotecan was administered on days 1, 8, 22, and 29 of a 42-day cycle at doses similar to those given to the first 23 patients.

Table 6. Selected clinical studies in rGBM compared our study

Study	Lee et al., 2015	Vredenburgh et al., 10/2007	Burzynski et al.
	Phase II panobinostat with BVZ in rGBM and AA (only rGBM data are presented) January 2015	BVZ and irinotecan in rGBM October 2007	Retrospective analysis of AS2-1 + targeted therapy in rGBM November 2018
Treatment	Oral panobinostat 30 mg 3x per week, every other week, in combination with BVZ 10 mg/kg every other week	Two cohorts 1. BVZ 10 mg/kg + irinotecan every 2 weeks (n=23) 2. BVZ 15 mg/kg every 3 weeks + irinotecan on days 1, 8, 22, 29 (n=12)	Three combinations 1. ANP + pazopanib, dasatinib, everolimus and BVZ (n=5) 2. AS2-1 + ipilimumab and nivolumab (n=1) 3. AS2-1 + BVZ + PB (n=1)
Primary end point	Six-month PFS	Six-month PFS	Retrospective analysis
Exclusion	Prior VEGF-targeted therapies and/or HDAC inhibitors, use of anticonvulsants, use of QT-prolonging drugs	Previous BVZ	BVZ given elsewhere.
Age	≥ 18	≥ 18	≥ 18
KPS	≥ 60	≥ 60	≥ 50
# of rGBM patients	24	35	7
Six-month PFS	30.4%	46%	57%
Median PFS (months)	5	5.5	9.4
Six-month OS	NA	77%	86%
Median OS (months)	9	15.5	12.4
Evaluation criteria	RANO	MacDonald	RANO
MRI	Every 8 weeks	Every 6 weeks	Every 4-12 weeks
CR	0	6 (17%)	4 (57%)
PR	7 (29%)	14 (40%)	2 (29%)
SD	14 (58%)	15 (43%) (SD+PD)	---
PD	3 (13%)		1 (14%)

Abbreviations: AA, anaplastic astrocytoma; ANP, antineoplaston therapy (AS2-1 + A10); BC, Burzynski Clinic; BVZ, bevacizumab; CR, complete response; HDAC, histone deacetylase; KPS, Karnofsky Performance Status; NA, not applicable; OS, overall survival; PB, sodium phenylbutyrate; PD, progressive disease; PFS, progression-free survival; PR, partial response; RANO, response assessment in neuro-oncology; rGBM, recurrent glioblastoma multiforme; SD, stable disease; VEGF, vascular endothelial growth factor

The 6-month PFS for the combined patient cohorts (n=35) was 46% while the 6-month OS was 77%. Twenty of the 35 patients (57%) had an objective tumor response. Based on these findings, there was no apparent difference in the efficacy of the therapeutic regimes utilized for the first and second cohorts of patients. Our results show a higher 6-month PFS (57% vs 46%), median PFS (9.4 vs 5.5 months), 6-month OS (86% vs 77%), CR (57% vs 17%), but a lower PR (29% vs 40%) and median OS (12.4 vs 15.5 months). See Table 6.

For the seven rGBM patients reported here, an OR was achieved in 85.7%. Six-month PFS was 57% and 6-month OS was 86%. While only a general comparison can be made between results reported for the Lee and Vredenburg studies and our results, AS2-1 plus targeted therapy appears to provide for a better outcome in rGBM.

The duration of response at BC was negatively influenced by premature discontinuation of treatment based on patients' ill-advised decisions to stop treatment after achieving an OR. (Patients were responsible for the cost of targeted therapy.) It is known that GBM growth accelerates after discontinuation of BVZ.

We believe a phase II study is warranted. In keeping with the evidence presented in this report, patients will receive AS2-1 and targeted therapies given sequentially over a period of days. The mechanisms of action of AS2-1 and targeted therapy in GBM and rGBM have been described (Wick et al., 2011; Goel & Mercurio, 2013;

Prud'homme & Glinka, 2012; Soker, Takashima, Miao, Neufeld & Klagsbrun, 1998; Zachary, Frankel, Evans & Pellet-Many, 2009; Kolodkin et al., 1997; Neufeld, Kessler & Herzog, 2002; Nakayama & Berger, 2013; Goel et al., 2012; Lu et al., 2012; Rizzolio et al., 2012; Grandclement et al., 2011; Ginka, Stoilova, Mohammed & Prud'homme, 2011; West et al., 2005; Banerjee et al., 2006; Byzova et al., 2000; Wey et al., 2005; Staton et al., 2013; Beck et al., 2011; Snuderl et al., 2013; Ellis & Hicklin, 2008; De Bacco et al., 2012; Bottsford-Miller, Coleman & Sood, 2012).

In a phase II study of AS2-1 and targeted therapy, the number of patients enrolled will be based on the OR rate (see below). However, 6- and 12-month PFS and OS will be emphasized as endpoints because of the pseudoresponses that can be induced by BVZ. In addition, such a phase II study will be designed to allow a comparison of 1) AS2-1 plus targeted therapy for rGBM and 2) BVZ plus irinotecan for rGBM, especially the second Vredenburg study cited (Vredenburgh et al., 10/2007). Studies of BVZ and irinotecan have not proven their usefulness in the practice of neuro-oncology. It has been stated that the addition of irinotecan to BVZ has "failed to show any additional activity" (Lee, McFaline-Figueroa, Cloughesy & Wen, 2018).

The phase II study envisioned would incorporate a strategy for early assessment of OR rates with procedures for termination of enrollment for lack of efficacy. An optimal two-stage design would be utilized (Simon, 1989). If the OR rate is $\geq$ 19%, then this design allows for enrollment of sufficient patients to compare the OS and PFS in the phase II study of AS2-1 and targeted therapy to the OS and PFS in the second Vredenburg study sited (Vredenburgh et al., 10/2007).

5. Conclusion

There is no standard of care for rGBM. Clinical studies with single chemotherapy and targeted agents or their combination have shown some promise, but progress has been modest at best. Our report provides evidence that a high response rate in rGBM utilizing AS2-1 and targeted agents can be achieved. With appropriate dose reductions, we have shown that such treatment is well tolerated. These findings suggest that AS2-1 in combination with targeted agent carries significant promise for a rapid and durable response in rGBM with acceptable toxicity. Therefore, as described above, we propose a phase II study of AS2-1 plus targeted therapy in patients with rGBM following standard of care therapy.

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