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Preliminary Findings on the Use of Targeted Therapy with Pazopanib and Other Agents in Combination with Sodium Phenylbutyrate in the Treatment of Glioblastoma Multiforme

Stanislaw R. Burzynski, Tomasz J. Janicki, Gregory S. Burzynski, Sheldon Brookman

Burzynski Clinic, Houston, USA
Email: srb@burzynskiclinic.com

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Abstract

The most common and aggressive type of brain tumor is glioblastoma multiforme (GBM). The prognosis for GBM remains poor with a five-year survival rate between 1% and 2%. The prospects for patients with recurrent GBM (RGBM) are much worse, with the majority dying within 6 months. This publication provides a brief description of the treatment of 11 GBM patients treated with sodium phenylbutyrate (PB) in combination with pazopanib, m-TOR inhibitors, and other agents. The treatment was associated with tolerable side effects and resulted in objective responses in 54.5% of cases (complete response 18.2%, partial response 36.3%) and 27.3% cases of stable disease. The preferable treatment regimen consisted of PB, pazopanib, dasatinib, everolimus, and bevacizumab (BVZ). For various reasons not all patients were compliant with the treatment regimen. In patients who strictly complied with the treatment plan, all responded as CR or PR. Based on preliminary findings, the authors propose further phase I/II clinical trials with PB in combination with pazopanib, dasatinib, everolimus, and BVZ in patients with RGBM who failed standard surgery, radiation therapy and chemotherapy. With proper dose reductions, the treatment appears to be well-tolerated. Molecular profiling of patient subgroups with favorable genomic signatures may help to select patients for future studies.

Keywords

Gliobastoma Multiforme, Personalized Targeted Agents, Sodium Phenylbutyrate, Treatment of Glioblastoma Multiforme

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1. Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive type of primary malignant brain tumor [1]. Prognosis has been extremely poor, with a median survival of 14.6 months from diagnosis despite surgery, radiation therapy (RT), adjuvant temozolomide (TMZ) and bevacizumab (BVZ) [2]. The 5-year survival rates are between 1% and 2% for newly-diagnosed GBM patients [3] [4]. Median progression-free survival (PFS) after standard therapy is 6 to 9 months [5] and the prognosis for patients with recurrent GBM (RGBM) appears much worse, with the majority of patients dying within 6 months.

Our team evaluated treatment of GBM and other primary brain tumors with sodium phenylbutyrate (PB), a histone deacetylase (HDAC) inhibitor as well as with antineoplastons (ANP), 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 [6]-[14]. A recently published phase II study evaluated the safety and efficacy of ANP A10 and AS2-1 in recurrent high-grade glioma, with special emphasis to RGBM. Objective responses (OR) were determined in 17% of eligible patients, overall survival (OS) was 65.5% at 6 months; 56.7% at 9 months, 39% at 1 year and 4.4% at 2, 5, and 10 years [12].

Parallel to the ANP studies, PB was also used in the treatment of patients with primary brain tumors. PB, approved for urea cycle disorders has also been used for the treatment of glioma and acute promyelocytic leukemia [15] [16]. PB shares its metabolites phenylacetylglutaminate (PG) and phenylacetate (PN) with some ingredients of ANP. The results of testing of PG and PN on the GBM genome indicate that PG and PN affect over 100 abnormal genes [17]. In 2012, a GBM classification into six biological subgroups had been proposed [18]. Each of the subgroups had distinct molecular and biological characteristics. PB and ANP have similar spectrums of activity on the GBM genome. Published data indicate that only some GBM patients will respond to monotherapy of ANP and PB [12] [14] [19] [20]. However, the activity of PB may not be as potent, since it is administered orally. The effect on over 100 abnormal genes in laboratory models of GBM suggests that it may not be sufficient to control the GBM genome that has an average of 650 abnormal genes. It was thus decided to treat advanced GBM patients who were not candidates for phase II studies with a combination of PB and additional targeted agents. The treatment plans of these patients were based on genomic profiling and consisted of PB for broad-spectrum coverage and selected targeted agents indicated by genomic abnormalities [14]. A number of ORs were determined within the course of treatment as was long-term OS that was considered highly unusual in RGBM. This publication provides a brief description of 11 evaluable cases of GBM treated with PB in combination with pazopanib, mammalian target of rapamycin (mTOR) inhibitors and other agents.

2. Patients and Methods

Patients were diagnosed with inoperable, recurrent, persistent or progressed GBM and received the treatment at Burzynski Clinic (BC) in Houston, Texas. Pathology and radiologic evaluations were performed by institutions not associated with BC. Tests performed by both outside laboratories and by the laboratory at BC included standard blood and urine analyses as well as a determination of genomic markers. Molecular profiling of tissue samples was performed by Foundation Medicine of Cambridge, Massachusetts. All patients read, understood and signed informed consent documents, which explained in detail the treatment prior to admission. The treatment plan, based on molecular profiling, included PB given in combination with targeted and chemotherapeutic agents. Therapy was performed on an out-patient basis, and after the initial two to four weeks of treatment at BC, patients continued their management under the care of local oncologists. Prior to the start of treatment, a magnetic resonance imaging (MRI) with and without contrast and in some instances a positron emission tomography (PET) scan was undertaken. The products of the two largest perpendicular diameters (LPD) of measurable lesions were calculated and totaled, providing a baseline evaluation for each study subject and a reference for determining response outcome to the treatment. Additional pretreatment measurements included Karnofsky Performance Status (KPS), vital signs, clinical disease status, demographics, medical history and current medications, physical examination, and electrocardiogram (EKG). Toxicity was evaluated according to Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0). Possible response to treatment included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR required the disappearance of all lesions confirmed at the end of four weeks, PR required 50% or higher decrease of the LPD of measurable lesions. PD was determined when there was over 25% increase of the lesions or new lesions, and SD was the status between PR and PD. The duration of each response was measured from the date that the criteria

of the outcome were first met and until the date that PD was documented. In the case of SD, the duration was measured from the time that therapy had commenced.

3. Results

3.1. Patient's Demographics

Patients included all consecutively admitted, evaluable subjects between October 6, 2010 and August 15, 2014. The majority of patients failed the available standard treatments with 45% having unsuccessful resection, radiation, and chemotherapy with TMZ, 9% having resection, RT, TMZ, and BVZ, and an additional 18% who were inoperable and received RT and TMZ. One patient refused radiation and chemotherapy and underwent two tumor resections and another patient who was inoperable refused standard radiation and chemotherapy and underwent a craniotomy and tumor biopsy only. Another patient was operated on three times and had been treated with radiation and two types of chemotherapy. Patient's demographics are described in **Table 1**. Data on confirmation of diagnosis, recurrence, and response to treatment received are shown in **Table 2**.

Table 1. Study population demographics.

Studied subjects		
Characteristic	N = 11	%
Age (year)	N	%
Median	52	
Range	26 - 67	
Sex		
Male	6	55
Female	5	45
KPS (Karnofsky performance status score)		
Median	80	
Range	50 - 90	
Diagnosis		
GBM	11	100
Clinical characteristics		
Recurrent tumors	6	55
Persistent tumors	4	36
Progressing tumors	1	9
Inoperable	2	18
Biopsy only (refused standard treatment)	1	9
Resection only (2×)	1	9
Resection, RT and TMZ	5	45
Resection 3×, RT, TMZ and PCV	1	9
Resection, RT, TMZ and BVZ	1	9
RT and TMZ	2	18

Abbreviations: BVZ: bevacizumab, GBM: glioblastoma multiforme, PCV: procarbazine, lomustine, and vincristine, RT: radiation therapy, TMZ: temozolomide.

Table 2. GBM patients treated with PB, pazopanib, mTOR inhibitors and targeted drugs.

Confirmation of diagnosis					Treatment	Confirmation of recurrence		Confirmation of response to PBT		Molecular profiling
Pathology			Radiology							
Patient	Place and date	Diagnosis	Place and date	Diagnosis		Place and date	Assessment	Place and date	Assessment	
1	University hospital April 28, 2010	GBM	Regional radiology MRI April 26, 2010	Two enhancing masses in left parietal and temporal lobes	Subtotal tumor resection April 27, 2010					
					RT + TMZ May 20, 2010 to July 1, 2010					
					TMZ ×2 August 21, 2010 to September 21, 2010	Regional radiology MRI September 21, 2010	Recurrence			
					BC. October 6, 2010 PB, vorinostat, erlotinib, pazopanib, everolimus, BVZ. Discontinued vorinostat on December 15, 2010 and had last BVZ infusion on December 12, 2010. Discontinued erlotinib, everolimus, and pazopanib January 18, 2011. Recurrence of rectal abscess. Discontinued PB on March 1, 2011.Died March 1, 2011 due to septicemia.			Regional radiology MRI November 30, 2010	PR	Elevated VEGF (Blood) c-Kit-wild type genotype (tissue-Caris)
2	Regional hospital December 2010	GBM	Regional radiology CT November 25, 2010	A large 5.5 × 4 cm lesion of the left temporal parietal lobe	Tumor resection December 2, 2010					
					RT and TMZ					
					Craniotomy for epidural abscess	Regional radiology MRI November 10, 2011 (Baseline)	Persistent tumor			
					TMZ ×4					
					BC. November 30, 2011 PB, pazopanib, dasatinib, sirolimus, erlotinib, BVZ			Regional radiology MRI May 17, 2012	SD	Blood and tissue profiling not done
3	Regional hospital September 1, 2011	GBM	Regional hospital MRI August 25, 2011	Left parietal lobe enhancing tumor	Tumor resection August 31, 2011					
					RT 60 Gy and TMZ October 10, 2011-November 18, 2011					
					TMZ	Regional radiology MRI March 14, 2012	Recurrence			
					BVZ March 2012 to December 28, 2012	Regional hospital MRI December 12, 2012.	Recurrence			
						Regional radiology MRI January 9, 2013.	Recurrence			
					BC. January 15, 2013 PB, pazopanib, everolimus, dasatinib, BVZ. Discontinued everolimus February 13, 2013 (lack of insurance). Added erlotinib April 16, 2013. Discontinued erlotinib May 21, 2013. Discontinued PB March 3, 2014. Died June 23, 2014 due to disease progression.			Regional radiology MRI March 20, 2013	PD	Normal (blood) FGFR3-amplification, PTEN-mutation U1331, CDK4-amplification, TP53-loss, mutation D281H (tissue)

Continued

4	Regional hospital September 2011	AA	Regional hospital CT July 20, 2011	Frontotemporal mass (4 × 3.7 cm)	Regional hospital September 15, 2011 Total tumor resection	Regional radiology MRI August 29, 2012	Recurrence			
	Regional hospital October 26, 2012	GBM			Regional hospital October 25, 2012 Total tumor resection	Regional radiology MRI February 21, 2013 Mass 3.8 × 3.6 cm	Recurrence			
					BC. February 21, 2013 PB, erlotinib, everolimus, BVZ, pazopanib, TMZ					
					Discontinued erlotinib after 6 weeks and replaced by dasatinib. Discontinued dasatinib after 9 months, and everolimus, BVZ and TMZ after 1 year. Discontinued pazopanib in August 2013 and PB in April 2014.			Regional radiology MRI April 8, 2013	CR	IDH1-R132C mutation, TP53 loss-P190 del, Splice site 672 + 2 T7G, ATRX loss-F395fs19, FBXW7-K189fs66 (tissue)
5	Regional hospital May 20, 2013	GBM	Regional hospital MRI May 2, 2013	Enhancing lesion in corpus callosum and corona radiata	None	Regional radiology MRI May 2, 2013	Left frontal mass (Baseline)			
					BC. May 29, 2013 PB, pazopanib, everolimus, lapatinib, BVZ			Regional radiology MRI July 11, 2013	PR	PIK3R1-L380-del, CDKN2A and CDKN2B-Loss, HF1-E318fs*58 mutation (tissue)
6	Regional hospital August 5, 2013	GBM	Regional radiology MRI July 24, 2013	Left temporal-parietal-occipital region tumor	Tumor resection August 5, 2013					
					RT and TMZ August 28, 2013-September 30, 2013					
					TMZ 290 mg for 5 days of a 28 day cycle ×6	Regional radiology MRI October 12, 2013	Enhancing lesion 1.2 × 0.9 cm			
					BC. November 1, 2013 PB, pazopanib, everolimus, dasatinib, erlotinib			Regional radiology MRIs February 19, 2014	SD (post-operative changes)	PTEN-A79T mutation, EGFR-A289D subclonal amplification, EGFRVIII mutation, G598V mutation (subclonal), L62R mutation, CDKN2A/B/C loss (tissue)
7	Regional hospital September 6, 2013	GBM	Regional radiology MRI September 2, 2013	Enhancing left parietal temporal tumor	RT and TMZ September 2013-November 26, 2013					
					TMZ-December 4, 2013	Regional radiology MRI January 22, 2014	Large enhancing tumor in the left cerebral hemisphere			
					BC. January 22, 2014 PB, pazopanib, everolimus, dasatinib, BVZ			Regional radiology CT/PET April 24, 2014	PD	PTEN-R130e mutation, CDKN2A/B-loss, VHL-N67M mutation (tissue)
8	Regional medical center January 27, 2014	GBM	Regional hospital MRI January 25, 2014	Butterfly shaped glioma surrounding the corpus callosum	RT, 30 treatments with TMZ 160 mg daily February 24, 2014-April 4, 2014					
					TMZ 320 mg, 5 days on and 23 days off May 12, 2014-June 4, 2014					
					BC. June 6, 2014 PB, dasatinib, everolimus, pazopanib, BVZ. Discontinued everolimus July 10, 2014. Discontinued treatment July 31, 2014. BVZ August 25, 2014-September 8, 2014 (2×). PB August 25-September 8, 2014. Erlotinib September 10-September 15, 2014			Regional hospital MRI September 3, 2014	SD	VEGF-elevated (blood) EGFR-amplification CDKN2A/B-loss (tissue)

Continued

9	Cancer institute August 25, 2011	Fibrillary astrocytoma, Grade 2	Regional hospital MRI June 27, 2011	Contrast-enhancing lesion in right temporo-parieto-occipital area	Partial tumor resection August 19, 2011					
	Cancer institute November 22, 2011	Astrocytoma, Grade 2/3			Repeat partial tumor resection November 7, 2011					
	Institute of neurology May 23, 2013	GBM			RT 5400 cGy January 16, 2012 to February 20, 2012	Cancer institute MRI February 2, 2013	Recurrence			
					Repeat partial tumor resection May 15, 2013					
					TMZ 5 days (28 day cycle x9) May 2013 to February 2014	Cancer institute MRI January 27, 2014	Recurrence			
					PCV chemotherapy ×2 March 2014 to May 14, 2014	Cancer institute MRI May 14, 2014	Recurrence			
					BC. June 19, 2014 PB, sorafenib, pazopanib, everolimus, dasatinib, BVZ			Regional radiology MRI August 28, 2014	PR	IDH1-R132H mutation, CDK4-amplification, TP53 loss (G245S), ATRX-R808 mutation EPHA3 loss (V4121) (tissue)
10	University hospital September 4, 2013	GBM	University hospital MRI August 20, 2013	Enhancing mass in the right occipital lobe	Gross tumor resection August 23, 2013. Standard RT and TMZ September 20, 2013 to November 20, 2013. TMZ, 28 day cycle December 2013 to June 30, 2014.	Regional radiology MRI July 18, 2014	Recurrence			
					BC. July 28, 2014 PB, pazopanib, everolimus, dasatinib, BVZ			Regional radiology MRI September 4, 2014	CR*	HER-2-elevated, EGFR and VEGF-normal (blood), NF1-loss of function rearrangement exxon 31, PIK3CA-H1047L mutation, CDKN2A-loss equivocal (tissue)
11	University hospital January 27, 2014	GBM	University hospital MRI January 7, 2014	Left frontal lobe tumor	Partial tumor resection January 9, 2014. Standard RT with TMZ February 28, 2014 to April 8, 2014. TMZ every 28 days May 5, 2014 to August 1, 2014.	Regional radiology August 19, 2014	Recurrence			
					BC. August 18, 2014 PB, pazopanib, everolimus, dasatinib, BVZ			Regional radiology MRI September 16, 2014	PR**	HER-2, VEGF-elevated, EGFR-normal (blood), NF1 loss-Y2885 fs*5 mutation, NF2 loss-5518 fs*4 mutation, FANCG, GATA2, HSD3B1, IKBKE, NOTCH2 mutations (tissue)

Abbreviations: AA: anaplastic astrocytoma, ATRX: transcriptional regulator ATP: dependent helicase, BC: burzynski clinic, BRCA2: breast cancer 2 gene, BVZ: bevacizumab, CCND2: cyclin D2 gene, CDK4: cyclin-dependent kinase 4, CDKN2A: cyclin-dependent kinase inhibitor 2A gene, CDKN2B: cyclin-dependent kinase inhibitor 2B gene, c-Kit: type of receptor tyrosine kinase (CD117), CR: complete response, CT: computed tomography, EGFR: epidermal growth factor receptor, FANCG: fanconi anemia, complementation group, FBXW7: F-box/WD repeat-containing protein 7, GATA2: GATA binding protein 2, HSD3B1: hydroxy-delta-5-steroid dehydrogenase, 3 beta-and steroid delta-isomerase 1, iFGFR3: fibroblast growth factor receptor 3, IKBKE: inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon, GBM: glioblastoma multiforme, HF1: factor H, IDH1: isocitrate dehydrogenase 1, KDR: kinase insert domain receptor gene, MDM2: mouse double minute 2 gene, MGMT: O(6)-methylguanine-DNA methyltransferase, MRI: magnetic resonance imaging, mTOR: mammalian target of rapamycin gene, NF1: neurofibromatosis type 1 gene, NF2: neurofibromatosis type 2 gene, NOTCH2: transmembrane protein family type 2, PB: sodium phenylbutyrate, PBT: PB and other drugs, PCV: procarbazine, lomustine (CCNU), and vincristine combination, PD: progressive disease, PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, PIK3R1: phosphoinositide-3-kinase, regulatory subunit 1 (alpha), PR: partial response, PTEN: phosphatase and tensin homolog, RT: radiation therapy, SD: stable disease, TMZ: temozolomide, TP53: tumor protein p53 gene, VEGF: vascular endothelial growth factor, VHL: Von Hippel-Lindau. *Follow-up MRI of the head of October 27, 2014 continues to show resolution of contrast-enhancing tumor. There are two small foci of enhancement without increased cerebral blood volume possibly representing post-treatment effect. PET/CT of November 4, 2014 revealed no metabolic activity in the foci of enhancement. **Follow-up MRI of the head of October 27, 2014 shows no significant change in size of enhancing tumor compared to the study of September 16, 2014. There has been increase in vasogenic edema. The patient was off treatment for 26 days for resection of infected craniotomy site. Increased edema represents reaction to re-initiation of treatment.

3.2. Treatment

The patients received treatment with PB, pazopanib, mTOR inhibitors (everolimus or sirolimus), and additional targeted agents. Patient 4 who previously refused chemotherapy was also given TMZ (details in **Table 2**). The patients were treated with combinations of three to six prescription drugs. Of the mTOR inhibitors, 10 patients were prescribed everolimus and one patient received sirolimus. Patient 9 was initially treated with sorafenib, but was switched to pazopanib after she developed hand and foot syndrome (**Table 3**). **Table 3** describes doses of PB, pazopanib, and other targeted agents, and duration of treatment until the first response for responding patients.

3.3. Response and Survival

A total of 54.5% of patients showed objective responses with 18.2% exhibiting CR and 36.3% PR (**Figures 1-6**). In 27.3% of cases, stabilization of disease was achieved. Response rates are presented in **Table 4**. Survival data were not analyzed because a number of patients remain on treatment. Three patients died while on treatment (Patient 1 from preexisting perianal abscess and septicemia, Patient 5 died from pneumonia and Patient 7 from disease progression). One patient (Patient 2) was lost to follow-up. Patient 4 continues to be in complete response and is off treatment for more than 19 months from treatment start. Patient 8 decided to discontinue treatment. Four patients (6, 9, 10, and 11) are still on treatment.

3.4. Safety and Adverse Events

Toxicities related to PB, pazopanib, and other targeted agents are listed in **Table 5**. Grade 3 toxicities included hypertension, mucositis, and cerebral hemorrhage. Among Grade 2 toxicities, the most common were thrombocytopenia and mucositis. These toxicities, suspected to cause such events, were reversible with a reduction of drug dosages. Because of the combination treatment, it can only be speculated as to which drugs were responsible for toxicity.

Table 3. Doses of targeted medications and duration of treatment of responding patients until first response.

Patient	Targeted drugs (daily dose/duration)										
	PB	Pazopanib	Everolimus	Sirolimus	Dasatinib	Vorinostat	Erlotinib	Sorafenib	Lapatinib	Bevacizumab	Response (date)
1	12 g/9 w	200 mg/1 w 400 mg/6 w	10 mg/ 3 w			200 mg/3 w 300 mg/ 8.5 w	150 mg/ 3 w			2.5 mg/kg × 1 5 mg/kg × 4	PR Nov 30, 2010
2	3 g/2 w 6 g/6 w 9 g/6 w 12 g/12 w	200 mg/9 w		1 mg/ 14 w 3 mg/ 2.5 w	50 mg/ 13 w		100 mg/ 12.5 w				SD May 17, 2012
4	12 g/ 7.3 w	200 mg/ 5.5 w	5 mg/ 6 w		50 mg/ 1 w		100 mg/ 5 w			10 mg/kg × 3	CR Apr 8, 2013
5	18 g/ 6 w	200 mg/1 w 400 mg/5 w	10 mg/ 6 w						750 mg/ 5.5 w	5 mg/kg × 1 10 mg/kg × 1	PR Jul 11, 2013
6	12 g/ 16 w	200 mg/ 15 w	10 mg/ 15.5 w				100 mg/6 w 150 mg/9.5 w				SD Feb 19, 2014
8	18 g/ 12 w	200 mg/ 6 w	5 mg/ 3 w		50 mg/ 7 w					10 mg/kg × 4	SD Sep 3, 2014
9	10 g/ 10 w	200 mg/ 3.5w	5 mg/ 9.5 w		50 mg/ 9.5 w			200 mg/ 5.5 w		8 mg/kg × 3	PR Aug 28, 2014
10	12 g/ 5.5 w	200 mg/ 5 w	5 mg/ 5.5 w		50 mg/ 4.5 w					10 mg/kg × 3	CR Sep 4, 2014
11	12 g/ 4 w	200 mg/ 4 w	5 mg/ 4 w		50 mg/ 3.5 w					10 mg/kg × 3	PR Sep 16, 2014

Abbreviations: CR: complete response, PB: sodium phenylbutyrate, PR: partial response, SD: stable disease, w: weeks.

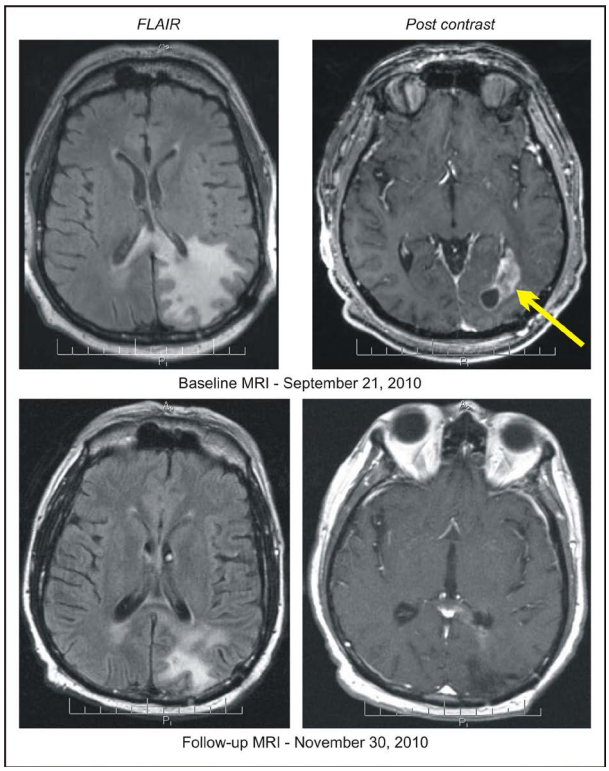

Figure 1. Patient 1, baseline and follow-up MRI of the head indicating partial response.

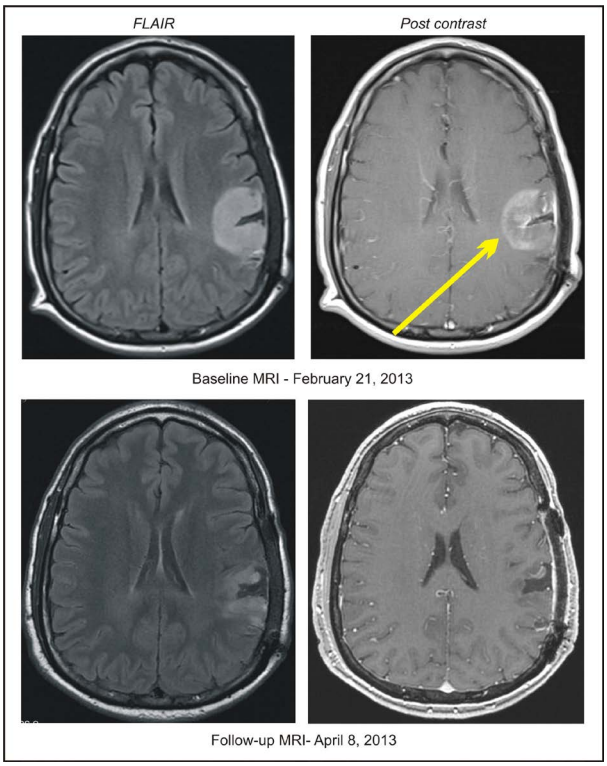

Figure 2. Patient 4, baseline and follow-up MRI of the head indicating complete response.

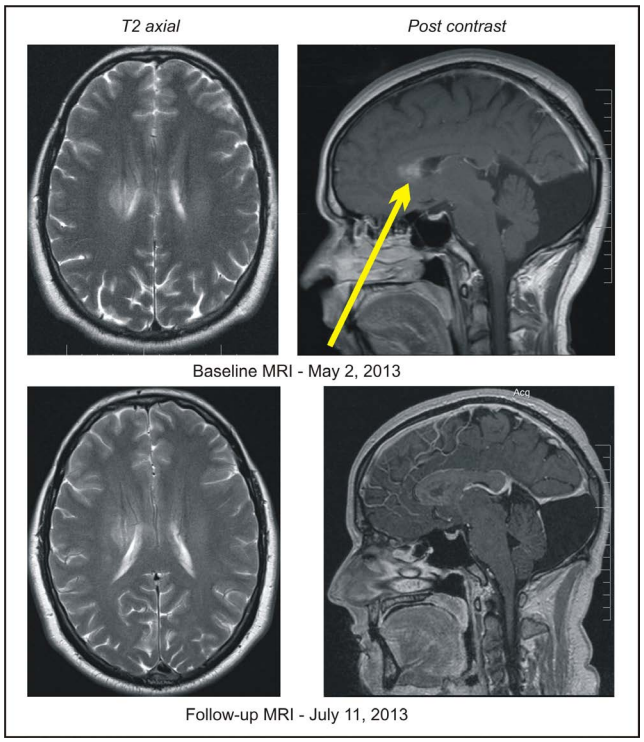

Figure 3. Patient 5, baseline and follow-up MRI of the head indicating partial response.

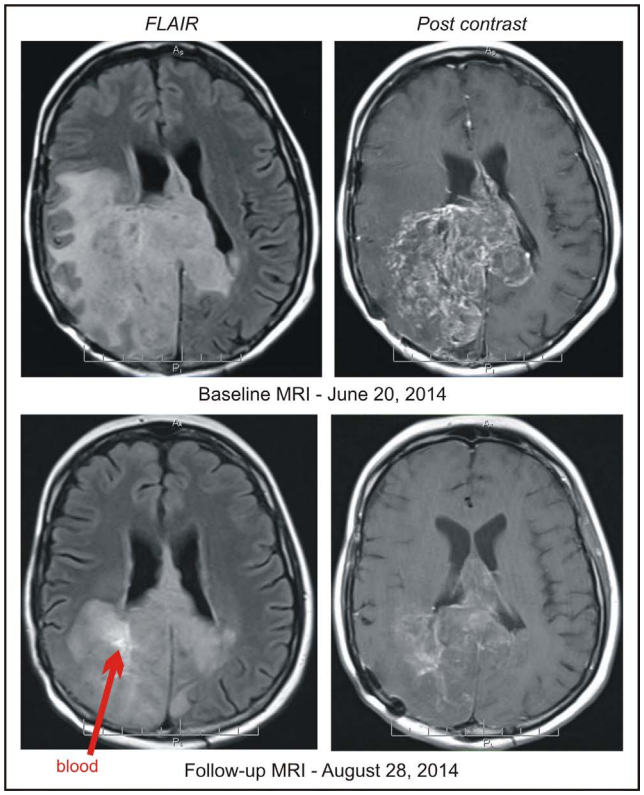

Figure 4. Patient 9, baseline and follow-up MRI of the head indicating partial response.

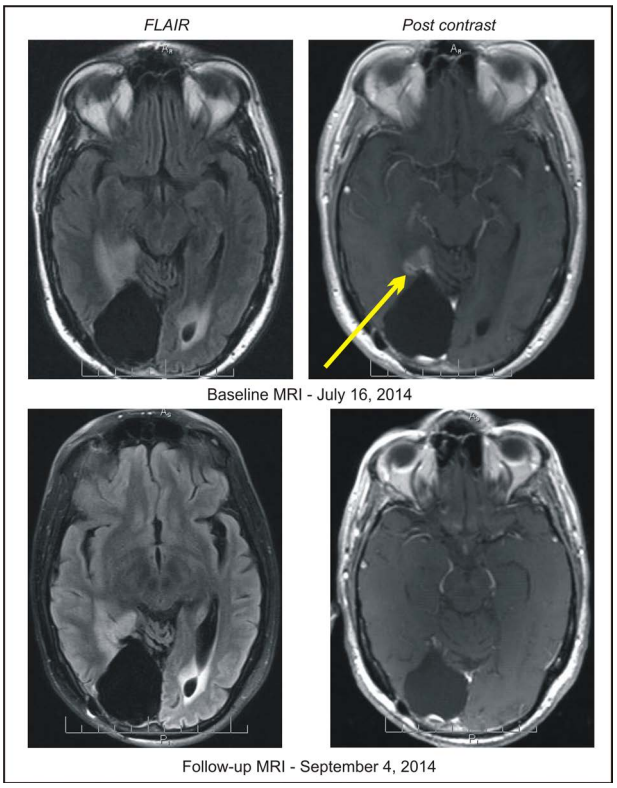

Figure 5. Patient 10, baseline and follow-up MRI of the head indicating complete response.

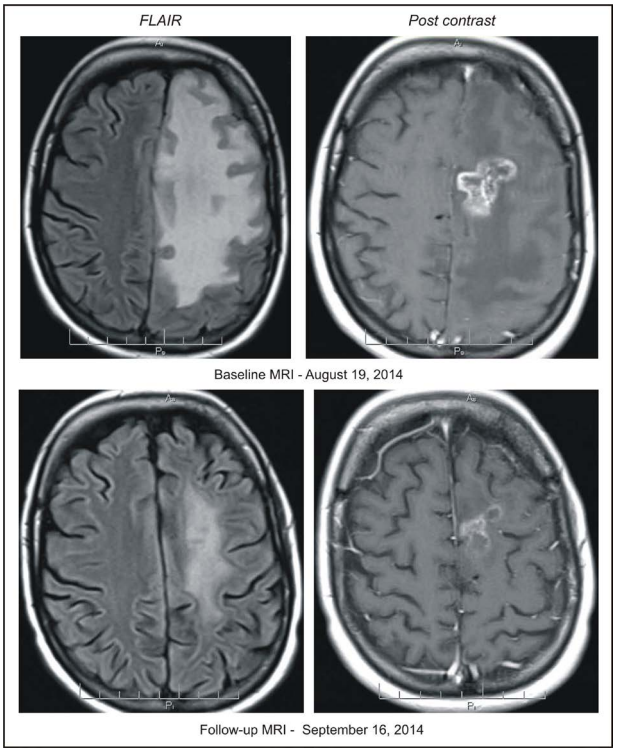

Figure 6. Patient 11, baseline and follow-up MRI of the head indicating partial response.

3.5. Compliance

The preferable treatment regimen consisted of PB, pazopanib, dasatinib, everolimus, and BVZ. This treatment plan was formulated over time and in the cases of patients 1, 4, and 5, dasatinib had not yet been included in the regimen prior to the response to treatment. Patient 1 obtained PR, but opted to discontinue due to the recurrence of a chronic infection. There were a variety of reasons why 6 additional patients did not comply with the regimen, which is listed in **Table 6**. For Patient 2, 3, and 7, economic factors were cited as the reason for treatment discontinuation. In Patient 5, who achieved PR, lapatinib was substituted for dasatinib. Patient 6 did not receive BVZ as his residual tumor did not show hypermetabolic activity on the PET scan. Despite these treatment

Table 4. Response rates.

Diagnosis	N	CR	PR	SD	PD
Glioblastoma multiforme	11	2 (18.2%)	4 (36.3%)	3 (27.3%)	2 (18.2%)

Table 5. Incidence of adverse drug events. N = 11.

Adverse Event	G1	G2	G3	Total
Anemia		1		1
Leukopenia	1			1
Thrombocytopenia		3		3
Hypertension			1	1
Fatigue	2	1		3
Sweating (diaphoresis)	1			1
Rash	2	1		3
Diarrhea	1	1		2
Dysphagia		1		1
Mucositis/stomatitis (clinical exam)	1	3	1	5
Hemorrhage, CNS			1	1
Alkaline phosphatase, SGPT, SGOT	2	1		3
Hyponatremia	1			1
Proteinuria		1		1
Neuropathy: sensory (paresthesia)	1			1
Pain: neck		1		1

Adverse drug events for current patients as of October 4, 2014.

Table 6. Patient compliance with recommended regimen.

	Patient	Non-compliance	Reason for non-compliance	Response
Compliant patients	4	Compliant		CR
	9	Compliant		PR
	10	Compliant		CR
	11	Compliant		PR
Non-compliant patients	1	No dasatinib	Infection	PR
	2	Sirolimus instead of everolimus	Cost	SD
	3	Discontinued everolimus due to lack of insurance coverage	Cost	PD
	5	No dasatinib	Used lapatinib instead	PR
	6	No BVZ	No hypermetabolic lesion by PET scan	SD
	7	Interruption of BVZ	Cost	PD
	8	Interruption of all medications	Pancreatitis, pneumonia	SD

Abbreviations: BVZ: bevacizumab, CR: complete response, PD: progressive disease, PET: positron emission tomography, PR: partial response, SD: stable disease.

deviations, two of the patients obtained PR and two additional patients achieved SD. Two other patients developed PD. Of note is that all four patients who were compliant with the regimen obtained OR. For this reason, when future clinical trials are conducted, improved response rates may be impacted due to better treatment compliance.

4. Discussion

Numerous chemotherapy and targeted therapy regimens have been evaluated for the treatment of patients with recurrent GBM [3] [21]. Some studies demonstrated minor improvement in PFS, but no significant increase in survival [21] [22]. A successful therapy for recurrent GBM is thus, still desperately required [3] [21] [22].

The authors of this article suggest that treatment consisting of a combination of available and approved drugs, including PB, pazopanib, dasatinib, everolimus, and BVZ is currently an option.

In phase II studies of GBM with ANP, we identified a small group of patients with unusually long overall survival. Eligible patients in RGBM study exhibited an OR of 16.6% and increased OS compared to other studies. Unfortunately, only a small percentage of the patients obtained long-term OS. Understanding the spectrum of the effects of constituents of ANP on the targets in the GBM genome, we understood that control of GBM may require more than two agents [17]. PB is a drug with a similar spectrum of activity as ANP, but does not have as prominent an effect, since it is orally administered. Nonetheless, we chose to use PB together with selected drugs for treatment of patients who, except for one case, had recurrent GBM.

A key study conducted by the European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada Clinical Trials Group in patients with newly-diagnosed GBM provided the rationale for the current standard-of-care [4] [5]. Since 2005, this standard-of-care consists of tumor resection followed by standard RT with concomitant TMZ (75 mg/m^2/d) for seven weeks and adjuvant TMZ (150 - 200 mg/m^2/d) on 5 day therapy every 28 days. Based on these recommendations, a patient may expect a median OS of 14.9 months or a median PFS of 6.7 months. However, the recurrence rate is still over 90%, median OS is less than 18 months, and survival at 5 years is less than 2% of patients [3] [5]. Clinical studies on RGBM with chemotherapy and targeted therapy were recently compiled and published in excellent reviews [3] [21]. However, there has been only very modest progress in treatment of GBM and specifically there are no current standard recommendations for RGBM [3] [14] [21] [22].

Based on research with PB from private practice patients and our experience in phase II studies with ANP, we propose a treatment plan for the upcoming phase I/II clinical studies of PB in combination with targeted agents, as well as with ANP [14] [17]. This treatment approach appears to address basic biological mechanisms in GBM, such as the control of growth, survival, invasion and migration of neoplastic cells, vascular effects, metabolism, maintenance and function of neoplastic stem cells, main signaling pathways, cell cycle mechanisms, apoptosis, autophagy, and drug resistance (**Figure 7**). The medications proposed regulate vascular endothelial growth factor (VEGF) signaling (BVZ, pazopanib), Src kinases (dasatinib), mTOR pathway (everolimus) and affect multiple targets of ANP and PB. These mechanisms have recently been discussed in detail elsewhere [14] [17].

The treatment with PB with the combination of the four additional targeted medications resulted in a 54.5% rate of CRs and PRs and a 27.3% rate of SD. As described in **Table 6**, four patients fully complied with the treatment regimen and all achieved ORs. One, whose response was categorized as CR, is now leading a normal life for over one year and seven months since the treatment onset and three years and two months since tumor diagnosis. The remaining three patients are continuing treatment and appear to be improving on an ongoing basis. Molecular profiling did not provide extensive data to classify these patients in biological subgroups. Based on the available information, however, we can assign two patients to the isocitrate dehydrogenases 1 (IDH1) proneural subgroup (CR and PR), two additional patients to the ribosome biogenesis and trna synthetase-associated kinase (RTK1) proneural subgroup (2 PR), two to the classical subgroup (1 SD, 1 PD), one to the mixed tumor protein p53 (TP53) subgroup (PD) and one PR patient to an unclassified subgroup. These data suggest that IDH1 and RTK1 proneural subgroups may be associated with better treatment response. Recently, published data on long-term survival with GBM did not provide the information that would favor a longer survival in these subgroups [23]. None of the genomic characteristics associated with long-term survivals were present among our patients and the IDH mutation does not appear to impart a better survival benefit. Further studies are necessary in this area to afford a better understanding and relationship between genomic signature and treatment responses.

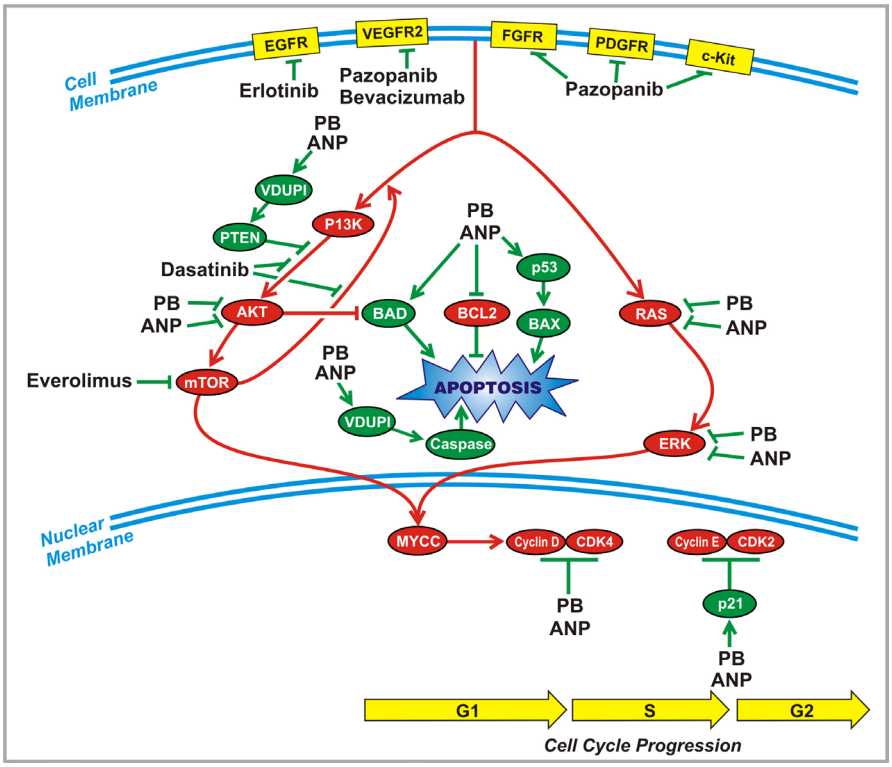

Figure 7. Proposed mechanism of action of PB, pazopanib, and additional targeted agents. The metabolites of PB and ANP affect signal transmission through AKT and RAS pathways, promote apoptosis and interrupt cell cycle progression at G1/S and S/G2 checkpoints.

The authors thus propose new phase I/II clinical trials to be conducted with PB in combination with targeted agents BVZ, pazopanib, dasatinib and everolimus in patients with RGBM after failure of standard surgery, RT and TMZ, evaluating the survival, response to treatment and toxicity. Furthermore, molecular profiling on tumor tissue should be undertaken to identify "genomic signatures" of responders and non-responders. Based on the experience from private practice of the authors, some patients did not tolerate the proposed combination. Such patients may nonetheless still be helped by the substitution of pazopanib with sorafenib, a drug with a similar spectrum of action. As well, erlotinib or lapatinib can be used in cases with specific genomic targets for these drugs.

5. Conclusion

There is no established standard of care for RGBM. Numerous clinical studies with single chemotherapy and targeted agents or their combinations have shown some promising results, but progress has been modest at best. The treatment with PB in combination with targeted agents carries promise for a rapid and durable response in RGBM. The results reported in this paper provide evidence that it may be possible to accomplish a high response rate in RGBM with PB in combination with four targeted agents. The authors propose phase I/II clinical trials with PB in combination with targeted agents: BVZ, pazopanib, dasatinib and everolimus for patients with RGBM after failure of standard surgery, RT and TMZ. Caution should be exercised when combining these agents, since no clinical trials have yet been conducted with such combinations. With proper dose reduction, such treatment appears to be reasonably well-tolerated. Molecular profiling will ameliorate the selection of subgroups of RGBM having favorable genomic signature for future studies.

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Consent

Written informed consent was obtained from patients for publication of this article and accompanying images.

Competing Interests

All authors are employed by Burzynski Clinic. Dr. Stanislaw R. Burzynski and Dr. Gregory S. Burzynski are shareholders and directors, and Dr. Tomasz J. Janicki is the Vice-President of Burzynski Research Institute, Inc. Dr. Stanislaw R. Burzynski is President of Burzynski Research Institute, Inc., Dr. Gregory S. Burzynski is Vice-President of Burzynski Clinic and Dr. Sheldon Brookman is Director of Pharmaceutical Development of Burzynski Clinic.

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