

Comprehensive Genomic Profiling of Recurrent Classic Glioblastoma in a Patient Surviving Eleven Years Following Antineoplaston Therapy

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Abstract

Most patients with recurrent glioblastoma (RGBM) die within 6 months regardless of treatment. In phase II studies of Antineoplaston A10 and AS2-1 injections (ANP), our investigators have reported objective responses and long-term survival in RGBM. Using a next-generation sequencing (NGS) based assay of 343 cancer-related genes and introns, comprehensive genomic profiling of tumor tissue obtained from a RGBM patient (who remains alive and well) was performed 11 years after diagnosis and permitted assignment of the patient's RGBM to the classical subgroup. The most important genomic alterations included amplification of epidermal growth factor receptor (EGFR), cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A/B), loss of phosphatase and tensin homolog (PTEN) and telomerase reverse transcriptase (TERT) mutation. An analysis of the signaling networks and other targets of ANP therapy was recently performed and presented in this publication. Based on our findings, patients with classical RGBM have a reasonable possibility of responding to ANP therapy and experiencing long-term survival. It is proposed that this subgroup of RGBM patients be enrolled in a genomics-driven clinical trial of ANP therapy.

Keywords: Antineoplastons A10 and AS2-1, genomic profiling, glioblastoma survival, phase II clinical trials, recurrent glioblastoma, targeted therapy

1. Introduction

Among primary malignant brain tumors, glioblastoma (GBM) carries the worst prognosis (Ostrom et al., 2014). Most patients with recurrent GBM (RGBM) following surgery, radiation therapy (RT), and chemotherapy die within 6 months. (Weller et al., 2013). In phase II studies of Antineoplaston A10 and AS2-1 injections (ANP), our investigators have reported objective responses and long-term survival in RGBM (Burzynski, Janicki, & Burzynski, 2014; Burzynski, Burzynski, & Janicki, 2014). ANP are peptides, amino acid derivatives, and carboxylic acids, which were initially isolated from the blood and urine of healthy subjects and reproduced synthetically for the purpose of preclinical and clinical research (S. R. Burzynski, Janicki, & G. S. Burzynski, 2014; S. R. Burzynski, G. S. Burzynski, & Janicki, 2014; Burzynski, 1969; Burzynski, 1986; Burzynski, 2006; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2014, pp 2051-2061; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2014, pp. 565-577; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2014, pp. 977-988; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2015, pp. 334-344; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2015, pp. 28-38; S. R. Burzynski, Janicki, & G. S. Burzynski, 2015; S. R. Burzynski, Janicki, G. S. Burzynski, & Marszalek, 2014, pp e433-e439; S. R. Burzynski, G. S. Burzynski, Janicki, & Marszalek, 2015; S. R. Burzynski, G. S. Burzynski, Marszalek, & Janicki, 2015). Comprehensive genomic profiling was not practiced when the phase II studies of ANP therapy were conducted. However, there have since been exciting advances in molecular research and classification of GBM (Phillips et al., 2006; Verhaak et al., 2010; Van Meir et al., 2010; Sturm, et al., 2012; Bhat et al., 2013; Sturm et al., 2014). Four different molecular subtypes of GBM have been identified: classical, neural, proneural, and mesenchymal (Verhaak et al., 2010; Brennan et al., 2013). The proneural subtype has been further divided into cytosine/phosphate, and guanine (CpG) island methylator phenotype (G-CIMP)-positive and G-CIMP-negative (Noushmehr et al., 2010).

We previously reported on a RGBM patient who has experienced long-term survival following ANP therapy, as a

Special Exception patient, in our Protocol BT-21 (S. R. Burzynski, G. S. Burzynski, & Janicki, 2014). The patient presented in this article was referred to as Patient 2 in that publication. Recently, through the initiative of this patient's local neuro-oncologist, comprehensive genomic profiling of the patient's tumor tissue was conducted. This article analyzes the results and considers the implications of this comprehensive genomic profiling.

2. Patient and Methods

Patient 2 was admitted in September 2004 for ANP therapy, at Burzynski Clinic (BC), under Protocol BT-21 as a Special Exemption patient, which was permitted by the Food and Drug Administration (FDA) based on poor performance status. This phase II study was conducted under IND 43,742, sponsored by Burzynski Research Institute, Inc., and supervised by an independent Institutional Review Board (BRI-IRB). The details of the study were recently published (S. R. Burzynski, Janicki, & G. S. Burzynski, 2014; S. R. Burzynski, G. S. Burzynski, & Janicki, 2014). In December 2014, comprehensive genomic profiling of the patient's tumor tissue was performed by Foundation Medicine, Inc. of Cambridge, MA, using a next-generation sequencing (NGS) based assay of 343 cancer-related genes and introns. The list of the genes is located at www.foundationone.com. The assay development, validation and control procedure was described in detail in a prior publication (Frampton et al., 2013). The clinical genomic profiling assay does not report heterozygosity or homozygosity. The assessment of EGFR and other gene amplification via this assay is done by quantitative estimation of the degree of amplification. Fluorescent in situ hybridization (FISH) for gene amplifications relies on a comparison between hybridization to the gene of interest and hybridization to the centromere, ie the HER2/CEP17 ratio is used to gauge HER2 FISH. The amplification of EGFR is quantitatively estimated as 12x in this case. CDKN2A/B is homozygously deleted in this case (100% of the gene is lost). Frampton et al. (2013) contains a detailed description of the methodology used and its validation for clinical use. The methods for data analysis including pathway analysis were described before (Burzynski & Patil, 2014). The tumor tissue analyzed had been preserved in a paraffin block dated May 26, 2004, which correlates with the patient's second tumor resection.

3. Results

3.1 Case Study

In March 2004, Patient 2, a 59-year-old Caucasian male and retired U.S. Marine Corps Lieutenant Colonel, developed severe headaches with diminished coordination and was found by magnetic resonance imaging (MRI) scan to have a contrast-enhancing tumor in the left frontal lobe. In April 2004, the patient was treated with a near-total tumor resection. Histologic examination of the submitted tumor specimen revealed a GBM. One month later, the patient underwent a second tumor resection and placement of Gliadel wafers. Subsequently, the patient received RT to a total dose of 6,000 cGy in 30 fractions and three cycles of temozolomide (TMZ) followed by isotretinoin. The tumor recurred despite this combination therapy. On the MRI scan, there was a doubling in tumor size compared to the baseline evaluation. With documented failure of the patient's initial therapy, Patient 2 was admitted to BC for ANP therapy under Protocol BT-21 as a Special Exemption patient. ANP therapy began in September 2004 and was interrupted after 7.5 months because of an infection of the patient's infusion catheter. At that time, a positron emission tomography (PET) scan showed no evidence of active tumor and ANP therapy was permanently discontinued. MRI performed one year post ANP therapy revealed an approximate 60% decrease in tumor size. MRI scans four years after discontinuation of ANP therapy confirmed resolution of the tumor. Repeated MRIs through February 2014 have shown no tumor recurrence. This case study was recently published (S. R. Burzynski, G. S. Burzynski, & Janicki, 2014).

3.2 Results of Comprehensive Genomic Profiling

In December 2014, comprehensive genomic profiling of the Patient 2's tumor tissue was performed by Foundation Medicine, Inc. of Cambridge, MA, using a NGS based assay of 343 cancer-related genes and introns. The tumor tissue analyzed had been preserved in a paraffin block dated May 26, 2004, which correlates with the patient's second tumor resection. This was a high quality specimen, scored at 78% by computational purity modeling (Figure 1).

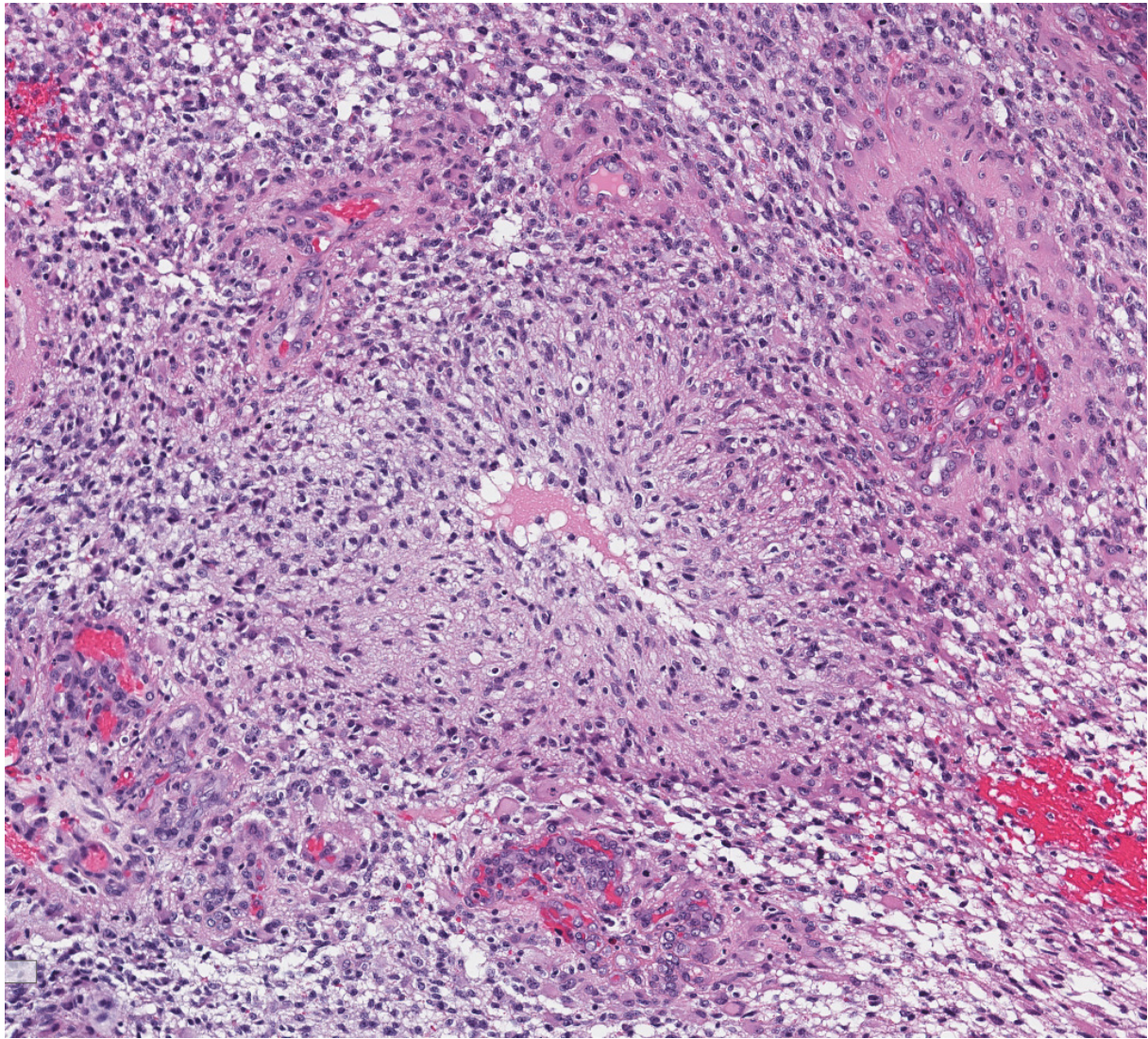


Figure 1. HE staining representing the area used for comprehensive genomic profiling

It demonstrates marked mitotic activity, tumor necrosis, and microvascular proliferation, consistent with a diagnosis of glioblastoma.

The most important genomic alterations included epidermal growth factor receptor (EGFR) amplification, phosphatase and tensin homolog (PTEN) mutation, R159fs21, cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A/B) loss, and mutation of telomerase reverse transcriptase (TERT) promoter-124C>T (Table 1). There were no alterations of platelet derived growth factor receptor, alpha (PDGFRA) and isocitrate dehydrogenase 1 (IDH1). There were nine additional genetic mutations and one gene rearrangement of unknown significance (Table 1). The comprehensive genomic profile permitted classification of Patient 2's RGBM as the classical subtype (Van Meir et al., 2010; Sturm et al., 2012, 2014). Based on the genetic profile, targets for ANP therapy in the patient's RGBM have been proposed (Figures 2 and 3).

Table 1. Results of comprehensive genomic profiling of Patient 2

	Gene	Genomic Alteration Detected	Gene Product	Function	Functional Consequence
Variants of known significance	EGFR	Amplification – 12 times	Epidermal growth factor receptor	Receives signal from the environment and passes biochemical messages to the cell to grow and divide	Activation
	PTEN	Mutation R159fs21	Inositol phosphatase	Tumor suppressor negatively regulating PI3K/AKT/mTOR pathway	Activation
	CDKN2A/B	100% Loss	Tumor suppressor proteins p16INK4a, p14ARF, p15INK4b	p15INK4b and p16INK4a bind to and inhibit CDK4 and CDK6. p14ARF stabilizes p53 through MDM2 inhibition.	Activation
	TERT	Mutation of promoter – 124C>T	Catalytic subunit of the telomerase complex	Maintaining appropriate chromosomal lengths	Activation
Variants of unknown significance	ARID1B	Mutation P607L	Component of SWI/SNF chromatin remodeling complex	Regulation of histone acetylation	Activation
	BLM	Mutation R643H	RecQ helicase-like protein	DNA double-strand break repair. Promotion of activity of p21 tumor suppressor	Inactivation
	FAT1	Mutation S3500T	Tumor suppressor	Inhibition of WNT	Inactivation
	GNAS	Mutation Q8P	Adenylate cyclase -stimulating G alpha-protein	Activation of RAS pathway	Activation
	HGF	Mutation N127I	Hepatocyte growth factor	Activation of signaling cascade through c-MET receptor	Activation
	MAG12	Mutation R1370P	Membrane associated guanylate kinase	Upregulation of PTEN	Inactivation
	MLL3	Mutation L4219V	Component of trithorax complex	Regulation of histone methylation	Activation
	NOTCH3	Mutation T1272N	Receptor for Notch signaling pathway	Regulation of proliferation, apoptosis and differentiation progress	Activation
	PIK3R2	Mutation D613N	Phosphatidylinositol 3-kinase	Component of PI3K/AKT/mTOR pathway	Activation
	PTPN11	*Rearrangement	Protein tyrosine phosphatase, type 11	Signaling molecule that regulates growth, differentiation and oncogenic transformation	Activation

**The information on the kind of rearrangement was not provided by Foundation Medicine, Inc. and it's significance is not completely clear.*

Abbreviations: ARID1B=AT-rich interactive domain-containing protein 1B; BLM=Bloom syndrome; CDKN2A/B=cyclin-dependent kinase inhibitor 2A and 2B; FAT1=fat atypical cadherin 1; EGFR=epidermal growth factor receptor; GNAS=guanine nucleotide binding protein, alpha stimulating; HGF=hepatocyte growth factor; MAG12=membrane-associated guanylate kinase 12; MLL3=mixed-lineage leukemia 3; NOTCH3=Notch homolog protein 3; PIK3R2=phosphoinositide-3-kinase, regulatory subunit 2 (beta); PTEN=phosphatase and tensin homolog; PTPN11=tyrosine-protein phosphatase non-receptor type 11; TERT= telomerase reverse transcriptase.

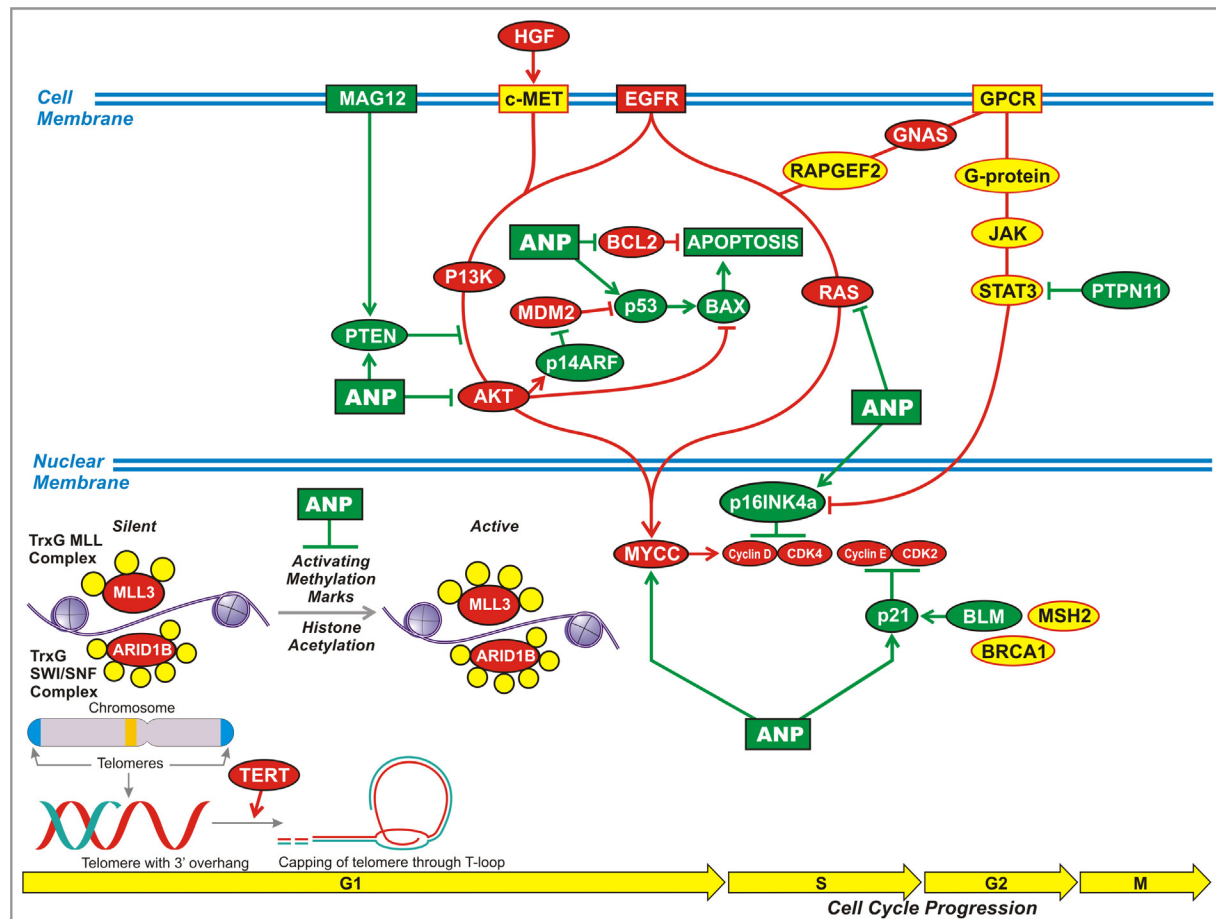


Figure 2. Proposed molecular mechanisms involved in GBM of Patient 2 and targets for ANP

The mechanisms are limited to genes analyzed by Foundation Medicine, Inc. The main inhibitory affects of ANP are directed against the PI3K/AKT/PTEN and RAS pathways, prevention of apoptosis and restoration of the G1-S checkpoint.

Abbreviations: AKT=protein kinase B; ANP=antineoplastons A10 and AS2-1; ARID1B=AT-rich DNA interacting domain-containing protein 1B; BAX=BCL-2 associated X protein; BCL2=B cell lymphoma 2; BLM= Bloom syndrome; BRCA1=breast cancer type 1 susceptibility gene; CDK2=cyclin-dependent kinase 2; CDK4=cyclin-dependent kinase 4; c-MET=mesenchymal epithelial transition factor; Cyclin D=protein in the cyclin family; Cyclin E=protein in the cyclin family; EGFR=epidermal growth factor receptor; GNAS=guanine nucleotide binding protein, alpha stimulating; GPCR=G-protein-coupled receptors; G-protein=guanine nucleotide-binding proteins; HGF=Hepatocyte growth factor; JAK=Janus kinase; MAG12=membrane associated guanylate kinase 12; MLL=mixed-lineage leukemia; MLL3=mixed-lineage leukemia 3; MSH2=DNA mismatch repair protein homolog 2; MYC=MYC oncogene protein; p14ARF=alternate reading frame protein product of the CDKN2A locus; p16INK4a=Inhibitor of CDK6; p21=p21 tumor suppressor protein; p53=p53 tumor suppressor protein; PI3K=phosphoinositide 3 kinase; PTEN=phosphatase and tensin homolog; PTPN11=tyrosine-protein phosphatase non-receptor type 11; RAPGEF2=Rap guanine nucleotide exchange factor 2; RAS=RAS oncogene protein; SNF=sucrose non-fermenting component of chromatin remodeling complex; STAT3=signal transducer and activator of transcription 3; SWI=faulty watering-type switch component of chromatin remodeling complex; TERT= telomerase reverse transcriptase; TrxG=trithorax group protein.

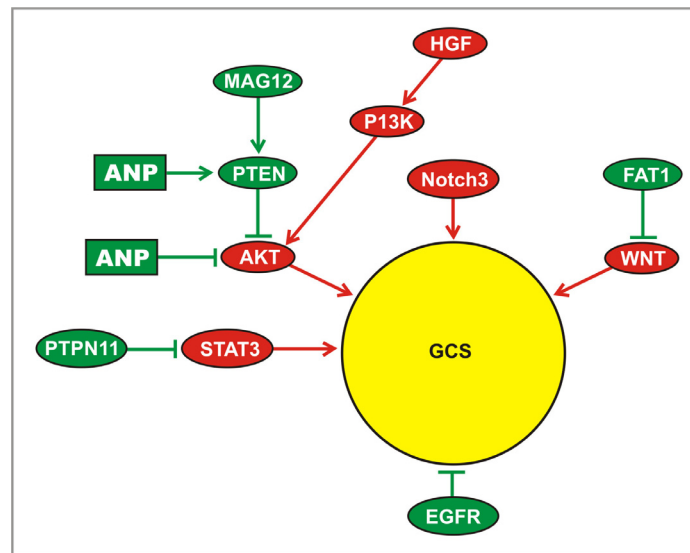


Figure 3. Proposed molecular mechanisms involved in the maintenance of GCS in GBM in Patient 2 and targets for ANP

ANP interferes with signal transmission through the PI3K/AKT/PTEN pathway.

Abbreviations: AKT=protein kinase B; ANP=antineoplastons A10 and AS2-1; EGFR=epidermal growth factor receptor; FAT1=fat atypical cadherin 1; GCS=glioma stem cells; HGF=hepatocyte growth factor; MAG12=membrane-associated guanylate kinase 12; NOTCH3=Notch homolog protein 3; PI3K=phosphoinositide 3 kinase; PTEN=phosphatase and tensin homolog; PTPN11=tyrosine-protein phosphatase non-receptor type 11; STAT3=signal transducer and activator of transcription 3; WNT=wingless integration protein.

4. Discussion

The most important molecular features of classical GBM are EGFR mutation, amplification or overexpression, PTEN loss or mutation, CDKN2A loss, and Notch homolog protein (Notch) or Hedgehog signaling (Shh) pathway activation (Van Meir et al., 2010; Sturm et al., 2012; Sturm et al., 2014). All of these alterations were seen in Patient 2's RGBM. The significance of these alterations is discussed below, as is the significance of other alterations found in the patient's tumor.

The EGFR gene encodes the epidermal growth factor receptor, a cell surface receptor. Ligands bind to EGFR, producing a chemical message which exerts control on the growth rate of cells. EGFR amplification is identified in 44% to 66% of cases of GBM (Huang et al., 2007; Brennan et al., 2013; Furnari et al., 2015). In GBM, EGFR variant III (EGFRvIII) occurs in 40% of cases. This mutation affects the extracellular domain of the protein and produces a constitutively active receptor (Huang et al., 2007). In a clinical trial with erlotinib, which is an EGFR kinase inhibitor and has sensitivity to EGFRvIII, there was no evidence of efficacy in GBM (Mellinghoff et al., 2005; Bastien et al., 2015).

PTEN tumor suppressor gene negatively regulates the phosphoinositide 3 kinase (PI3K)/ protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway. Loss of function of PTEN causes inhibition of apoptosis and promotes neoplastic growth (Simpson & Parsons, 2001). Loss of PTEN is very common in GBM with homozygous loss occurring in 70% of cases and loss at the other allele ("second hit") in 40% of cases (Furnari et al., 2007). Loss of function due to truncation mutations, as seen in Patient 2's GBM, have been reported in 22% of GBMs (Brennan et al., 2013; Das et al., 2003; Wang et al., 2008; Kim et al., 2011). Loss of PTEN activates the PI3K/AKT/mTOR pathway. EGFR (erlotinib) and mTOR (everolimus) inhibitors have been used in the treatment of these patients, but thus far with no success (Doherty et al., 2006; Wang et al., 2006; Courtney et al., 2010; Kesavabhotla et al., 2012). Based on preclinical data, poly ADP ribose polymerase (PARP) inhibitors such as olaparib are candidates for clinical trials (Juric et al., 2014; Fritsch et al., 2014).

CDKN2A/B encodes three tumor suppressors: inhibitor of cyclin-dependent kinase (CDK)6 (p16INK4a), alternate reading frame protein product of the CDKN2A locus (p14ARF), and cyclin-dependent kinase 4 inhibitor B (p15INK4b) and also supports the cyclin D/retinoblastoma tumor suppressor protein

(CDK4/6/cyclin/Rb) and tumor suppressor protein p53/mouse double minute 2 homolog (p53/MDM2) pathways (Quelle et al., 1995; Sharpless et al., 2005). p15INK4b and p16INK4a support the activity of the Rb tumor suppressor through inhibition of CDK4 and CDK6 (Roussel, 1999). p14ARF inhibits MDM2 which leads to stabilization of p53 (Ozenne et al., 2010).

According to The Cancer Genome Atlas (TCGA) dataset, homozygous deletion of CDKN2A/B occurs in over 50% of GBMs and is associated with a poor outcome (Brennan et al., 2013; Feng et al., 2012). The Cdk4/6 inhibitor palbociclib shows preclinical activity in GBM cell culture (Cen et al., 2012). Theoretically, MDM2 inhibitors would be candidates for clinical trials.

TERT represents a catalytic subunit of the telomerase complex which is necessary for chromosome elongation (Shay & Wright, 2011). TERT promoter mutations, as seen in Patient 2's GBM, are found in over 80% of GBMs (Liu et al., 2013; Nonoguchi et al., 2013). EGFR amplification is frequently associated with TERT promoter mutation and is a poor prognostic factor in GBM (Nonoguchi et al., 2013). Validated therapeutic options for targeting mutated TERT in GBM are not currently available.

The additional genomic alterations seen in GBM affect PI3K/AKT/mTOR and signal transducer and activator of transcription 3 (STAT3) pathways, G1/S phase checkpoint, and chromatin remodeling. The PI3K/AKT pathway is affected through hepatocyte growth factor (HGF) (N127I) and phosphoinositide-3-kinase, regulatory subunit 2 (beta) (PIK3R2) (D613N) mutations (De Bacco et al., 2012; Gheradri et al., 2012; Thorpe et al., 2015). In addition, mutated membrane-associated guanylate kinase 12 (MAG12) (R1370P) affects PTEN (Wu et al., 2000). Reports on activation of the RAS oncogene protein (RAS) pathway by mutated guanine nucleotide binding protein, alpha stimulating (GNAS) in cholangiocarcinoma and in adenocarcinoma of the stomach and duodenum (Hsu et al., 2013; Matsubara et al., 2013), suggest the possibility of a similar effect in GBM. Variants of HGF (mutation N127I), PIK3R2 (mutation D613N), MAG12 (mutation R1370P), and GNAS (mutation Q8P) were seen in Patient 2's GBM (Table 1).

The STAT3 pathway is among the most promising targets for cancer therapy (Hua et al., 2014). GBM with increased signaling along the STAT3 pathway has a more aggressive behavior (Sturm et al., 2014). Inhibition of this pathway resulted in tumor progression in preclinical studies (Carro et al., 2010). STAT3 induces methylation of the CDKN2A promoter which silences this important tumor suppressor (Lee et al., 2012). Activating tyrosine-protein phosphatase non-receptor type 11 (PTPN11) mutants inhibit STAT3 (Zhang et al., 2009). Rearrangement of PTPN11 was seen in Patient 2's GBM (Table 1).

Patients with the mutation of the Bloom (BLM) gene suffer from Bloom syndrome, which predisposes them to many different types of cancer (Rezazadeh, 2013). The gene encodes a RecQ-like helicase, which plays an important part in DNA double-strand break repair and telomerase independent telomere elongation. It also promotes the activity of p21 tumor suppressor. A variant of BLM (mutation R643H) was seen in Patient 2's GBM (Table 1).

Genomic alterations in GBM affect epigenomic changes (Sturm et al., 2012). The trithorax group (TrxG) and polycomb group (PcG) affects modifications of histone tails including methylation and acetylation (Mills, 2010). The loss of TrxG chromatin remodeling proteins predispose to cancer (Mills, 2010). TrxG proteins activate the tumor suppressor activity of p16INK4A and p15INK4B (Mills, 2010). Mutated genes AT-rich DNA interacting domain-containing protein 1B (ARID 1B) (P607L) and mixed-lineage leukemia 3 (MLL3) (L4219V) play an important part in TrxG. Faulty watering-type switch-sucrose non-fermenting component of chromatin remodeling complex (SWI-SNF) (ARID 1B) and TrxG MLL complexes and may compromise the activity of p16INK4A and p15INK4B (Mills, 2010; Wang et al., 2004; Khurshed et al., 2013; Sausen et al., 2013). Variants of ARID 1B (mutation P607L) and MLL3 (mutation L4219V) were seen in Patient 2's GBM (Table 1).

Research data coming from many centers indicate that GBM tumors consist of a spectrum of cell populations with different genomic and epigenomic compositions (Furnari et al., 2015; Fine, 2015; Venere et al., 2011). The disease cannot be eradicated without the elimination of rapidly multiplying cells with stem cell-like properties that possess the ability to reconstruct the entire GBM. These cells, also called glioma stem cells (GSC), are refractory to RT and chemotherapy, but may respond to targeted therapy (Bao et al., 2006). In Patient 2's GBM, four signaling pathways support maintenance of GSC: EGFR/HGF/PI3K/AKT, STAT3, Notch3, and wntless integration protein (WNT) (Figure 3) (Sturm et al., 2014; Brennan et al., 2013; Furnari et al., 2015; Courtney et al., 2010; De Bacco et al., 2012; Fine, 2015; Ables et al., 2011; Anastas & Moon, 2013). Possible defense mechanism includes MAG12/PTEN against AKT, PTPN11 against STAT3, and fat atypical cadherin 1 (FAT1) against WNT (Furnari et al., 2015; Simpson & Parsons, 2001; Wu et al., 2000; Hua et al., 2014; Zhang et al., 2009; Anastas & Moon, 2013; Chosdol et al., 2009; Morris et al., 2013).

The comprehensive genomic profile of Patient 2's GBM suggests a central role of signaling pathways, PI3K/AKT and RAS, originating from an amplified EGFR and enforced by HGF/mesenchymal epithelial transition factor (c-MET) and G-protein-coupled receptors (GPCR)/GNAS. The tumor suppressor system may be compromised by the mutation of PTEN and MAG12. The STAT3 pathway contribution to disease progression may be due to lack of down-regulation by rearranged PTPN11. Another important pathway that appears compromised is CDKN4/6/cyclin/Rb, through the loss of CDKN2A/B and possibly through mutations of BLM, ARID1B and MLL3.

Based on analyses of the effect of ANP therapy on the GBM genome, we propose that the main inhibitory effects of ANP therapy are directed against the PI3K/AKT and RAS pathways through the inhibition of AKT and RAS, the up-regulation of PTEN, and restoration of the G1-S checkpoint (Figure 2) (Burzynski SR, Burzynski GS, Janicki TJ, 2014; Burzynski & Patil, 2014). Another important mechanism is promotion of apoptosis mediated through p53. Inhibition of GCS can be explained by the effect of ANP therapy on AKT and PTEN.

While comprehensive genomic profiling constitutes an exciting development in the diagnosis and treatment of GBM, incomplete data on the extremely complex GBM genome are obtained. This article has discussed approximately 2% of the genetic variants in the GBM genome. Additional genetic variants and important pathways in GBM that are affected by ANP therapy have been previously described, including promotion of neoplastic growth and invasion, cell cycle control, cellular metabolism, autophagy, GCS and improved tumor penetration. (Burzynski SR, Burzynski GS, Janicki TJ, 2014; Burzynski & Patil, 2014). It is important to notice that there are many variants of unknown significance listed in Table 1 and the explanation of their effects remains hypothetical. The alterations have been previously observed in peer-reviewed literature or the database Catalogue of Somatic Mutations in Cancer (COSMIC).

5. Conclusion

Large scale genomic and epigenomic analyses have permitted classification of GBM into four major subgroups, each with a different genomic profile. Each of these subgroups may require a different therapeutic approach. Under IND 43,742, Phase II studies of ANP therapy for GBM started in 1995 when comprehensive genomic profiling was not practiced. Among the GBM patients treated are long-term survivors (up to 20 years survival).

Through the initiative of Patient 2's local neuro-oncologist, comprehensive genomic analysis was performed to determine the genetic profile that had responded favorably to ANP therapy. From that analysis, we propose that patients with classical RGBM have a reasonable possibility of responding to ANP therapy and experiencing long-term survival. It is proposed that this subgroup of RGBM be enrolled in a genomics-driven clinical trial of ANP therapy.

Acknowledgments

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Disclosure Statement

The authors have no conflicts of interest to declare.

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