



# Comparative proteogenomic characterization of glioblastoma

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**Aim:** Glioblastoma multiforme (GBM) carries a dismal prognosis. Integrated proteogenomic analysis was performed to understand GBM pathophysiology. **Patients & methods:** 17 patient samples were analyzed for driver mutations, oncogenes, major pathway alterations and molecular changes at gene and protein level. Clinical, treatment and survival data were collected. **Results:** Significantly mutated genes included *TP53*, *EGFR*, *PIK3R1*, *PTEN*, *NF1*, *RET* and *STAG2*. EGFR mutations noted included EGFRvIII-expression, EGFR-L816Q missense mutation-exon 21 and EGFR fusion (FGFR3-TACC3). *TP53* mutations were noticed in COSMIC hot-spot driver gene and accompany *IDH1* and *ATRX* mutations suggesting low- to high-grade glioma transformation. Proteomics showed higher (53%) EGFR expression than genomic expression (23%). MGMT methylation was present in two-thirds of cases. **Conclusion:** This study identifies a distinct biological process that may characterize each GBM differently. Proteogenomic data identify potential therapeutic targets of GBM.

First draft submitted: 28 January 2019; Accepted for publication: 21 May 2019; Published online: 10 July 2019

**Keywords:** genomics • glioblastoma • proteomics • survival • temozolomide chemotherapy

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor, with a median survival of 14–15 months [1]. GBM is primarily found in the brain, but it can also be found in other areas including the brain stem, cerebellum and spinal cord. A landmark Phase III study by Stupp *et al.* demonstrated that postsurgical radiotherapy with concomitant temozolomide (TMZ) chemotherapy (also known as the Stupp regimen) increases median survival by 2.5 months and results in a 37% reduction in death compared with radiotherapy treatment alone. This study also showed a direct relationship between methylation of the *MGMT* gene and TMZ response rate [2]. *MGMT* is a gene involved in the repair of highly mutagenic DNA changes. Thus, any damage to it leads to a cascade of uncontrolled mutations, which ultimately allows for subsequent targeting by alkylating agents like TMZ [3,4]. But despite these advancements in treatments and better characterization of the genomic landscape, GBM still carries a highly aggressive and catastrophic prognosis. This is largely due to the poorly understood pathophysiology of GBM, as well as the lack of identifiable biologic targets to guide new therapies. There remains an unmet need to further unravel the pathophysiology of GBM and identify new biologic targets.

Recently, genomic profiling and The Cancer Genome Atlas project sequenced more than 600 genes from approximately 200 human samples [5]. GBM was also systematically studied by the Cancer Genome Atlas Research Network (TCGA) in detail. As a result of these studies, it was found that GBM has a complex genomic/proteomic signaling network, which is key to its rapid growth and differentiation. This network has the ability to adapt in response to certain targeted molecular treatments. Thus, the need for a comprehensive catalog of molecular alterations is of paramount importance. We hope that this understanding will help drive future investigations to further our understanding of GBM, as well as develop new patient-specific therapies [6].

## Methods

### Patients

Samples were obtained from 17 consecutive, newly diagnosed and pathologically confirmed GBMs in patients seen during 2017–2018 at Saint Luke's Hospital on the Plaza in Kansas City, MO, USA. Inclusion criteria were

histopathologic confirmation of GBM, as well as the availability of proteomic and genomic analysis results. Patients without available genomic data were excluded. All patients in the sample had primary grade IV GBMs.

After initial diagnosis, standard treatment was initiated. This included optimal surgical resection and subsequent adjuvant radiotherapy and chemotherapy (with TMZ) at a daily dose of 150–200 mg/m<sup>2</sup> of body surface area for 5 days. This scheme was then repeated on a 28-day cycle [4]. Both clinical and diagnostic/radiological evaluation were subsequently used to determine disease progression as well as incidence of relapse. MRI of the brain before and after treatment was utilized in diagnostic evaluation and assessment of treatment response. The tumor samples utilized for proteogenomic analysis were those obtained at time of initial biopsy and diagnosis; no samples from recurrent tumors were analyzed.

Tumor specimens were collected surgically for all 17 patients with newly diagnosed GBM. For each patient, an initial sample for intraoperative frozen section was obtained. Subsequently, optimal tumor resection was performed. For patients undergoing total or near-total resection, tumor samples in their entirety were sectioned and reviewed for histopathology. For some patients, tissue samples were obtained as separate fragments. If complete or partial resection was not possible, core biopsies were obtained and then analyzed. Details regarding surgical practices utilized and tumor samples obtained have been included as [Supplementary Table 1](#). Sections with histopathological confirmation of GBM were then sent for proteogenomic analysis.

Tumor cells for analysis were obtained through a pathologist-directed laser microdissection system by methods defined previously by Hembrough *et al.* [7]. Areas for microdissection were included in the original individual analysis report as an image. Analysis was performed using molecular fingerprinting by GPS Cancer™, NantWorks (CA, USA). DNA and RNA were extracted from preserved tissues. DNA sequencing libraries were prepared for each tumor sample and a matched-normal sample. Tumor versus matched-normal variant analysis was performed using NantOmics Contraster analysis pipeline to determine somatic and germline single nucleotide variants, insertions, deletions and identify highly amplified regions of the tumor genome. RNA-sequencing libraries were similarly prepared for the tumor sample. Sequencing using the Illumina platform in a NantOmics clinical laboratory improvement amendments and certified authorization profession-certified sequencing laboratory was performed. Quantitative proteomic analysis as well as genomic analysis was reported [8].

MGMT methylation and other biomarkers (chemotherapy response markers, chemotherapy resistance markers, prognostic markers) were scanned from tumor and normal tissues using the laser microdissection system. Genomics were analyzed to look for alterations in known oncogenes, tumor-suppressor genes, potentially treatable genes, tumor mutation burden, variants and disruptive germline alterations (including frame-shifting, insertions, deletions, nonsense and missense). Gene type was obtained by using data from the COSMIC Cancer Gene Census. Clusters of mutations were discovered using OncodriveCLUST on the Five3 variant calls made on more than 5000 TCGA tumor exomes [9,10]. Secondary screening for cancer predisposition was completed according to ACMG's recommendations for incidental findings [11]. Microsatellites consisting of homopolymer repeats were analyzed for a statistically significant increase in the number of length polymorphisms in tumor and normal sample to identify instability. The percentage of unstable loci is calculated for the tumor and matched-normal. The differential is then determined by subtracting the percentage of unstable loci in the normal sample from the percentage of unstable loci calculated in the tumor. A tumor is considered to demonstrate microsatellite instability when the differential exceeds the threshold. The number of length polymorphisms for each microsatellite locus was computed across approximately 5000 blood and solid normal exomes sequenced by TCGA comprising 18 different cancer types [12]. Loci covered by fewer than 30 reads were excluded from the analysis. Potential functional fusions were identified by using transcriptome aligned RNA sequencing data, using clusters of spanning reads between two transcripts where one of the transcripts belongs among the 74 commonly found genes in oncogenic fusions.

'My Cancer Genome' is an online tool that considers disease-relevant human genes, drugs, drug–gene interactions and potential druggability, including 835 drug–gene interactions between 226 drugs and 169 target genes [13,14]. Our proteogenomic analysis utilized this resource and reported potentially activating alterations (missense mutations, in-frame deletions and amplifications) in this panel of 169 'druggable' genes; a druggable or 'treatable' gene is defined as a gene against which at least one of 226 FDA approved or investigational drugs have demonstrated activity. This includes reporting the presence of genomics-based targets indicated on drug labels for 21 FDA-approved drugs [15,16]. Alterations that disrupt a gene were not considered treatable; as the genes must be functional for a drug to have an effect [10]. Variants produced from sequencing data of tumor sample (vs matched normal) were also scanned for evidence of resistance biomarkers for six drugs including cetuximab, dasatinib, imatinib mesylate,

Table 1. Patient characteristics (n = 17).

Age at diagnosis	Years:
– Median	– 56
– Minimum	– 20 <sup>†</sup>
– Maximum	– 72 <sup>†</sup>
Gender	Number of patients
– Male	– 8
– Female	– 9
Overall survival	Months
– Minimum	– 5
– Maximum	– 28 <sup>†</sup>
– Range	– 23
Clinical status	Number of patients
– Living	– 13
– Deceased	– 4
Number of relapses	Number of patients
– Zero	– 12
– One	– 4
– Two	– 1
No. of treatment lines	Number of patients
– One	– 12
– Two	– 4
– Three	– 1
No. receiving:	Number of patients (%)
– Temozolomide	– 17 (100)
– Avastin	– 5 (29)
– Irinotecan	– 4 (23.5)
– Carboplatin	– 1 (0.06)
– Lomustine	– 1 (0.06)
– Radiotherapy	– 17 (100)
– Surgical resection	– 17 (100)
Patients with:	Number of patients:
– High mutation burden	– 1
– <i>TP53</i> mutation	– 5 (29%)
– <i>EGFR</i> mutation	– 7 (41.2%)
– Proteomic MGMT	– 13 (76%)
– Genetic cancer predisposition screen positive	– 1 ( <i>MSH6</i> , frameshift mutation)
<sup>†</sup> Living.	

panitumumab, crizotinib and tamoxifen [16]. Known tumor genes were classified as tumor suppressors or oncogenes using data available from Cosmic Cancer Gene Census [14].

### Data analysis

Retrospective analysis of patients' biomarkers, *MGMT* methylation, mutation burden, microsatellite instability and fusion findings were completed and then correlated to their respective 'overall survival' (OS). OS was defined as duration of survival after the initial diagnosis was made. 'Progression-free survival' (PFS) was defined as the time during and after treatment when the disease did not progress, or when death was caused by any other reason aside from the tumor itself. Alteration-driven treatment after thorough review from different literatures was provided to the appropriate patients and their responses were observed. Baseline demographic data including age at the time of diagnosis as well as gender was retrospectively collected from patients' medical records.

### Results

As summarized in Tables 1 & 2, the dataset contains clinical and proteogenomic data from 17 patients. The same technological and laboratory modalities were utilized for each of these patients.

#### Patient characteristics

17 patients newly diagnosed with GBM were included in the study. Nine (n = 9) patients were females (53%). The median age was 56 (range 20–72 years). Clinical and demographic characteristics of the study population are summarized in Table 1.

All patients underwent surgical resection: 9/17 patients had gross total resection, 4/17 patients had near total or partial resection and the remaining 4/17 had biopsy samples taken either stereotactically or via Burr-hole. Despite

Table 2. Biomarkers and survival status of patients (n = 17).

Patient number	Proteomic MGMT	Proteomic EGFR	G-EGFR	G-p53	OS (months)	Current status
1	Present		Present		7	Alive
2	Present	Present			15	Alive
3	Present			Present	8	Alive
4	Present		Present		10	Alive
5	Present			Present	11	Alive
6			Present		10	Deceased
7	Present		Present		11	Alive
8				Present	28	Alive
9	Present	Present			11	Alive
10	Present	Present			11	Alive
11	Present	Present	Present		12	Alive
12	Present	Present			25	Alive
13		Present		Present	5	Deceased
14	Present	Present	Present		17	Deceased
15	Present	Present		Present	10	Deceased
16			Present		10	Alive
17	Present	Present			8	Alive

Amplification and overexpression of the *EGFR* gene is a distinct feature of GBM, noticed in 40% of these tumors. Table shows discrepancy between frequency of proteomic expression (9/17) and genomic mutation of *EGFR* (7/17), with overlap between the two in only 2/17 cases. No clear relationship has been found between the p53 pathway with treatment and outcome of GBM.

GBM: Glioblastoma multiforme; G-*EGFR*: Presence of *EGFR* mutation on genomic analysis; G-*P53*: Presence of *TP53* mutation on genomic analysis; OS: Overall survival.

standard therapy being given to all patients, 5/17 relapsed. Following relapse, four of these patients were then treated with second-line therapy with Avastin and Irinotecan. One patient was treated with TMZ and Avastin.

13 out of 17 (76%) patients were alive at time of completion of this study. The median OS was found to be 12.29 months. Five months was the lowest survival observed. For this patient, genetic aberrations included: *TP53* missense mutation, *SPEN* nonsense mutation and *H3F3A* missense mutation.

### Genomic alterations

Pathogenic gene mutations detected in study participants included *TP53*, *EGFR*, *NOTCH1*, *RAD21* and *SYNE1* (missense mutations); *SPEN*, *DEPDC5*, *STAG2*, *TPR*, *USP9X*, *MAGED1*, *ARHGAP5*, *CTDNBP1*, *ARID1A* and *BCOR* (nonsense mutations); *PTEN* and *ATRX* (frame-shift mutations); and *PIK3R1*, *CHD8* and *CSMD3* (in-frame deletions).

Likely pathogenic mutations noted were *IDH1*, *RET*, *PPP2R1A*, *PGM5*, *ZNF117*, *ACVR1*, *EPHA6*, *ZNF479*, *ZNF117*, *ZNF181*, *ZFP2*, *MS4A8*, *IL5RA*, *MKRN1*, *CD163*, *ATPB3*, *KLK8*, *COBLL1*, *CHD8*, *GRXC1*, *ABCBI* and *H3F3A* (missense mutations); *KIAA1109*, *NBPF1*, *AMBRA1*, *COL5A2* and *RBM10* (splice site mutation) and *CHD3* (in-frame deletion).

Key tumor-suppressor genes detected included *TP53*, *SPEN*, *FBXO11*, *ARID1A*, *PIK3R1*, *BCOR*, *NOTCH1*, *ATRX*, *PTEN*, *CDKN2A*, *NF1*, *STAG2*, *RBI* and *RAD21*. Known oncogenes identified included *EGFR*, *RET*, *IDH1*, *SOX2*, *GNAS*, *H3F3A*, *ACVR1*, *PPP2R1A*, *RPL5* and *ATP2B3*. These findings are summarized in Table 3, which depicts major types of mutations for each of the 17 patients included in the study and whether these were pathogenic, likely pathogenic or of unknown clinical significance. It also identifies potential 'treatable' genes. Table 4 summarizes the frequency of major mutations seen and highlights the major pathways these genes are involved in. Such pathways may be altered as a result of these mutations.

The TP53 pathway was dysregulated in 5/17 patients (29%). One patient had *p53* mutation in PI3K pathway. At the RNA level, *p53* mutations were noticed in COSMIC hotspot driver gene and accompany *IDH1* and *ATRX* mutations in two patients. This suggests the transformation from low- to high-grade glioma. Three patients had coexisting *RET*, *SPEN* and *CDK2NA* mutations, respectively, with a *p53* mutation. In contrast to proteomic expression, we noticed significant heterogeneity of *EGFR* expression on genomic platform. *EGFR* alterations were noticed in 7/17 patients (41.2%). Two of them showed *EGFR* variant III fusion. These mutations were accompanied by DNA amplification with multiple mutation allele frequencies. One patient had *L816Q EGFR*

Table 3. Alterations in top-ranked known oncogenes, tumor suppressor genes and/or treatable genes detected for each of 17 samples.

Genetic mutation	Patient																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Nonsense	ARHGAP5 <sup>‡</sup>	SPEN <sup>‡</sup>	ARID1A <sup>‡</sup>	DEPDC5 <sup>‡</sup>	GRHR	CTDNEP1 <sup>†</sup>	USP9X <sup>‡</sup>	COL3A1	PDZD2	RPS16	TPR <sup>‡</sup>	STAG2 <sup>‡</sup>	KDMAE	RAVER2	ORS2N1	PCDH1X	MAGED1 <sup>‡</sup>
	MEGF8		BCOR <sup>‡</sup>	ABCB1 <sup>†</sup>		KRT38B	CDKN2A <sup>‡</sup>			KEL	DNAH6	PIEZO2	RPL5	SAGE1	CHUK		RAB5A
			DPT	HYDIN		VATIL	NFI <sup>‡</sup>					NBPFI5		COX6B2	HMCN1		ZSWIM1
			ARGFX	MGME1		HAPLN1	THSD7B					TTTC3		USP17L11			ELMOD3
			PDEADIP	HECW2		PHFZ1A	SCAF11										CFTR
			RTKN	SRGAP1		MUC19	RARGEF										COL7A1
						JMJD4											COL9A1
						CEPB3											
						NTSDC1											
						PHLDA2											
Missense	GNAS <sup>†</sup>	TP53 <sup>‡</sup>	ACVR1 <sup>†</sup>	WDR90	NOTCH1 <sup>‡</sup>	TP53 <sup>†</sup>	M54A8 <sup>†</sup>	KLK8 <sup>†</sup>	1DH1 <sup>†</sup>	TP53 <sup>†</sup>	PIK3R1 <sup>‡</sup>	EGFR <sup>‡</sup>	PPR2R1A <sup>†</sup>	RAD21 <sup>‡</sup>	IDH1 <sup>†</sup>	SYNE1 <sup>‡</sup>	EGFRp.R324L <sup>‡</sup>
	ZNF479 <sup>†</sup>	H3F3A <sup>†</sup>	FAM47A	ABCA7	EGFR <sup>‡</sup>	EPHA6 <sup>†</sup>	IL5RA <sup>†</sup>	ZNF181 <sup>†</sup>	TP53 <sup>†</sup>	PHLDA1	NLGN1	CHD8 <sup>†</sup>	EGFR <sup>‡</sup>	ATP2B3 <sup>†</sup>	TP53 <sup>†</sup>	PGC5 <sup>†</sup>	EGFRp.G598V <sup>‡</sup>
	POTEF	PTPRK	MAP3K4	CD42BPB	FBX011	KIAA1109 <sup>†</sup>	MKRN1 <sup>†</sup>	DCAF12L2	PRKRIR	TBX3	FAM83A	GRXCR1 <sup>†</sup>	EVPL	RBBP6	CD163 <sup>†</sup>	ZNF117 <sup>†</sup>	EGFRp.R108K <sup>†</sup>
	TNFRSF10C	FBX011	BIRC6	TNRC18	ZNF335	RET <sup>‡</sup>	OR2T33	MYLK4	DFNA5	KDM5C	DHCR24	KIAA0907	NOTCH2	WRN	SCML4	KMT2C	EGFRp.T363A <sup>†</sup>
	DOCK2	DLG2	NR2E1	RADIL	SPATA2L	NBPF1p.L943V <sup>†</sup>	TMEM132D	NAV1	AQP5	BCOR	COBL1	CD5L	FAM47B	MME	CTDNBP1	SRSF1	ZNF117
	ADAMTS10	CASR	CSNK1A1	LPAR1	XKR7	NBPF1p.N114S <sup>†</sup>	RYR2	MEN1	ZNF469	OR2A25	FAM72D	PPM1K	BLM	ZNF91	SP7	DOPEY1	ZFP2
	NUMA1	ANP32E	BTD	JAG2	VWF	AMBRA1 <sup>†</sup>	NETO1	MUC7	RRN3	GPC3	SIGLEC9	TTTC37	KRT72	CENPB	CFH	HNF4G	OR2M7
	OGT	UIMC1	TPTE	SLC39A12	CD19p.R514C	NBPF1p.S340F <sup>†</sup>	ZFH33	ATRX	ATAD3B	COG1	TSNAN8	AQP10	ARAP3	DCAF12L2	TLR4	HCN1	SYNE1
	DACH1	TMCC1	DNAH5	SIGLEC7	PRR23A	ZNF91	CPBB2	GCMT1	PCNT	FLNA	UGT2A2	RB1	ZNF469	NR4A3	ERCC6	CDC27	KCNK9
	CPME7	NOV	BCAR3	SPATA13	SLC30A4	LEPR-pf898L	COG3	COG3	NCF1	TRIM3	AMPH	RIMS2	TBXAS1	OPLAH	GTF2B	ZNF705B	HNF1A
	ZNF91	SH2D4B	ASCL1	DOK5	LOXL4	LEPR-pH924P	CACNA1C	GPR12	TRIM49B	VCX3A	F5	LRWD1	TARS	GEM	CNTNAP3	CDH1	
		MYO18B	SALL3	PAD16	MCCp.G20S	NPH52	SVLp.P809A	SPOPL	DNAH14	MAP1B	MAP1B	NIPBL	CENPB	RRN3	PLEC	ZNF107	
	TDRD6	FGF2	ZNF92	LHX5	GOLGA6B	DIDO1	SVLp.L948R	DNAAF1	PCDH87	KRT7	PCDH87	KRT7	FBXL18	TUBA3D	METTL4	DIP2C	
	CEP290	INO80D	KCNH2	SLC37A4	SIC37A4	DIDO1	TMPPRS13	SVOP	LRP2	TYWIB	ZNF831	CROCC	ZNF257	FLG	TBX22	DNM2	
	CYP7B1	TRIM22	SULT1A4	MCCp.G23S	MCCp.G23S	DIDO1	TMPPRS13	SVOP	PAXIP1	MUC12	LIN37	FLG	TBX22	HERC2	AOAH		
	TTN	W5C2D	TERF1	NHSL1	GCNT2	RGPI	GCNT2	RGPI	EEF2	OR6B3	CRNN	ZNF708	DDY28	LAMA3	PCDHA4		
	CACNB4	HOXA3	GANC	PER1	CSMD1	PHEZ0											

Each column depicts genetic mutation profile for individual patients with glioblastoma multiforme, classified by the type of mutation. Mutations are further classified as pathogenic or likely pathogenic, suggesting these mutations likely have a role in tumorigenesis. Availability of an FDA-approved or investigational drug targeting a potentially 'treatable' gene is also depicted.

- <sup>†</sup>Pathogenic.
- <sup>‡</sup>Likely pathogenic.
- <sup>§</sup>Treatable genes detected in this cohort.

**Table 3. Alterations in top-ranked known oncogenes, tumor suppressor genes and/or treatable genes detected for each of 17 samples (cont.).**

Genetic mutation	Patient																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Frame shift	NPAS2	TRIM37	DDX10	FOXD1	FOXD1	C5MB3 <sup>‡</sup>	USP3	ADAMT57	ATRX <sup>‡</sup>	PTEN <sup>†</sup>	PAPSS2	PTEN <sup>†</sup>	HMCN2	ATRX <sup>‡</sup>	LRRK1	ANAPC4	
	RAD51AP2	SPATA31D1	ANKLE1	SLC16A9	CCDC121	CCDC121	C3		NKX2-2	CDKN2A <sup>‡</sup>	ZZEF1	WDHD1					
			NLRP5		DYNC2H1	ADAMT57	TP11		TRIM64B	VGLL3		KHSRP				CCDC65	
									HGC6.3p. Q151Tfs <sup>†7</sup>	GOLGA4							
									HGC6.3p. Q151Hfs <sup>†7</sup>	PIGP							
									GABRG2	FOXD1							
									SAA1	MSI2							
									SAA2-SAA4	CRCP							
Splice site								CHD9		LYPD4						HELO	STAG2 <sup>‡</sup>
								WDR45B									
								LRRC37B									
Amplification																	
Fusion																	
Truncation																	
Inframe deletion																	

Each column depicts genetic mutation profile for individual patients with glioblastoma multiforme, classified by the type of mutation. Mutations are further classified as pathogenic or likely pathogenic, suggesting these mutations likely have a role in tumorigenesis. Availability of an FDA-approved or investigational drug targeting a potentially 'treatable' gene is also depicted.

<sup>†</sup> Pathogenic.

<sup>‡</sup> Likely pathogenic.

<sup>§</sup> Treatable genes detected in this cohort.

Table 4. Frequency of detected mutations in known tumor-suppressor genes and known oncogenes and RNA fusions along with potentially altered pathways.

Mutations	Frequency	Pathway
Known tumor-suppressor genes:		
– <i>TP53</i>	5/17	p53 pathway
– <i>PIK3R1</i>	3/17	PI3K signaling
– <i>FBXO11</i>	3/17	Ubiquitination
– <i>ATRX</i>	3/17	Genome integrity
– <i>CKDN2A</i>	2/17	Rb pathway
– <i>PTEN</i>	2/17	PI3K signaling
– <i>TBX3</i>	2/17	Transcription repression
– <i>BCOR</i>	2/17	Transcription corepression, BCL6 pathway
– <i>STAG2</i>	2/17	Cell division, chromatids separation
– <i>SPEN</i>	1/17	DNA repair and mitosis
– <i>ARID1A</i>	1/17	Chromatin remodeling
– <i>RB1</i>	1/17	RB pathway
– <i>NOTCH1</i>	1/17	Ligand-activated transcription
– <i>NOTCH2</i>	1/17	Transcriptional coactivation
– <i>NF1</i>	1/17	MAPK signaling
– <i>MEN1</i>	1/17	Transcription regulation
– <i>GPC3</i>	1/17	Wnt pathway
– <i>KDM5C</i>	1/17	Transcription regulation/chromatin remodeling
– <i>BLM1</i>	1/17	DNA repair
– <i>RAD21</i>	1/17	RB1 pathway
– <i>KMT2C</i>	1/17	Transcriptional coactivation
Known oncogenes:		
– <i>EGFR</i>	7/17	
– <i>EGFR</i> variant III (RNA fusion)	2/17	RTK signaling
– <i>EGFR</i> Amplification	3/17	RTK signaling
– <i>EGFR</i> p.L62R	1/17	RTK signaling
– <i>EGFR</i> (p.L861Q)	1/17	RTK signaling
– <i>EGFR</i> (p.N842K)	1/17	RTK signaling
– <i>IDH1</i>	2/17	Metabolism
– <i>RET</i>	1/17	MAPK and PI3K signaling
– <i>GNAS</i>	1/17	GPCR pathways
– <i>PPP2R1A</i>	1/17	GSK3β, Akt and mTOR signaling
– <i>SOX2</i>	1/17	Transcription regulation
– <i>ACVR1</i>	1/17	BMP/TGF-β signaling pathway
– <i>H3F3A</i>	1/17	Post-translational modification
– <i>RPL5</i>	1/17	Ribosomal formation
– <i>ATP2B3</i>	1/17	Calcium transport
RNA fusions:		
– <i>EGFR</i> variant III	2/17	RTK signaling
– <i>FGFR3-TACC3</i>	2/17	RTK signaling
– <i>NOTCH1-AGPAT2</i>	1/17	Notch signaling
– <i>EGFR-SEPT14</i>	1/17	RTK signaling
Others:		
– High-tumor exonic mutational burden	1/17	Mismatch repair
– <i>MSH6</i> frameshift detected	1/17	DNA repair
– <i>MGMT</i> methylation	13/17	DNA repair
– Proteomic <i>EGFR</i>	9/17	Proteomic expression higher than genomic expression

missense mutation on exon 21 which is commonly seen in lung cancer patients, who respond specifically to EGFR inhibitors.

Treatable genes were identified. Three out of 17 patients showed *PIK3R1*, 7/17 showed *EGFR* and 1/17 showed *RET* as treatable genes. These are summarized along with potential targeted therapeutic agents in Table 5.

### Proteomic expression

Biomarkers analyzed in this study included hENT1, ERCC1, TUBB3, MGMT, PDL1, EGFR, FGFR1234, HER3, AXL, IDO1 and RRM1 proteins. KRAS, p16 and tissue Ki-67 were also analyzed. Only a single patient demonstrated high mutation burden, and this patient is still currently alive with an overall survival of 8 months. 9 out of 17 patients had proteomic expression of EGFR out of which only two showed its coinciding genomic expression. Mutation burden was found to be low in all patients except for one. 13 out of 17 (76%) biopsies showed MGMT methylation. Table 6 provides a detailed proteomic profile that was available for 13/17 patients. This includes presence or absence of different biomarkers on analysis including chemotherapy response markers,

**Table 5. Treatable genes detected and potential targeted therapy options.**

Treatable gene	Targeted treatment
<b>PIK3R1:</b>	
– p.T576del	BKM120 (investigational drug)
– p.G376R	
– p.R465del	
<b>EGFR amplification</b>	Vandetanib, gefitinib, erlotinib, cetuximab, panitumumab, afatinib (FDA approved for other indications) TXL647, MM151, SYM004, MEHD7945A, CO-1686, AZD8931, necitumumab, nimotuzumab, icotinib, dacomitinib (investigational)
<b>EGFR variant III (RNA fusion)</b>	Dacomitinib, rindopepimut, aNK-EGFR
<b>EGFR p.L62R</b>	Vandetanib, gefitinib, erlotinib, cetuximab, panitumumab, afatinib (FDA approved for other indications) Nimotuzumab, icotinib, dacomitinib, AZD8931 (investigational)
<b>EGFR (p.L861Q)</b>	Vandetanib, gefitinib, erlotinib, cetuximab, panitumumab, afatinib (FDA approved for other indications) TXL647, MM151, SYM004, MEHD7945A, CO-1686, AZD8931, Necitumumab, Nimotuzumab, Icotinib, Dacomitinib (investigational)
<b>EGFR (p.N842K)</b>	Vandetanib, gefitinib, erlotinib, cetuximab, panitumumab, afatinib (FDA approved for other indications) Nimotuzumab, icotinib, dacomitinib (investigational)
<b>RET (p.H840–841delinsQT)</b>	Vandetanib, sunitinib, regorafenib, sorafenib, cabozantinib

FDA: Food and Drug Administration.

**Table 6. Proteomic landscape of patients with glioblastoma multiforme (n = 13/17).**

	Proteins	Patient number (n = 13)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Targeted therapy response markers	EGFR	D <sup>†</sup>	D	D <sup>†</sup>	D	D	D <sup>†</sup>	D <sup>†</sup>	D	D <sup>†</sup>	D <sup>†</sup>	D	D <sup>†</sup>	D <sup>†</sup>
	ALK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	AR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	HER2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDL1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D <sup>†</sup>	ND	ND
	ROS1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	RET	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chemotherapy response markers	hENT1	D <sup>†</sup>	ND	D <sup>†</sup>	ND	D <sup>†</sup>	ND	ND	ND	ND	D <sup>†</sup>	ND	D	D <sup>†</sup>
	Fr-alpha	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	TOPO1	D	D	D	D	D	D	D	D	ND	ND	D	ND	D
	TOPO2A	ND	D	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND
	TYMP	ND	ND	ND	ND	ND	ND	D	ND	ND	D	D	D	ND
Chemotherapy-resistance markers	MGMT	D <sup>‡</sup>	D <sup>‡</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>
	ERCC1	ND <sup>†</sup>	D <sup>‡</sup>	D <sup>‡</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	ND <sup>†</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	ND <sup>†</sup>	ND <sup>†</sup>
	TUBB3	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>	D <sup>‡</sup>
	RRM1	D	D	D	ND	D	ND	ND	ND <sup>†</sup>	D	ND <sup>†</sup>	D	D	D <sup>‡</sup>
Clinical trial response markers	FGFR-1234	ND	D <sup>†</sup>	ND	ND	ND	ND	ND	ND	ND	D <sup>†</sup>	ND	ND	ND
	Her3	ND	D <sup>†</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	AXL	ND	ND	ND	ND	ND	ND	D <sup>†</sup>	ND	ND	D <sup>†</sup>	ND	ND	D <sup>†</sup>
	IDO1	ND	ND	ND	ND	ND	ND	ND	D <sup>†</sup>	ND	ND	ND	ND	ND
	IGF1R	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MET	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MSLN	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Prognostic markers	KRAS	D	D	ND	D	D	ND	ND	ND	ND	D	D	ND	ND
Other	P16	ND	ND	D	ND	ND	ND	D	ND	ND	ND	D	ND	ND

Patients (n = 13/17). Table shows presence or absence of biomarkers on proteomic analysis that may indicate chemotherapy response or resistance, availability of targeted therapy or clinical trials. Biomarkers are further classified on the basis of whether their absence or presence is of clinical advantage.

<sup>†</sup>Beneficial.

<sup>‡</sup>Unlikely beneficial, otherwise uncertain clinical significance.

D: Detected; ND: Not detected.



chemotherapy resistance markers, prognostic markers and biomarkers against which FDA-approved drugs may be available (for GBM or non-GBM indications) or may currently be in clinical trial phase.

## Discussion

Understanding the pathogenesis of GBM and its genomic landscape is paramount. This is especially true considering it is the most prevalent primary brain tumor in adults and carries a particularly malignant and aggressive course in the majority of cases. The current standard of care includes maximal surgical resection, radiation therapy and subsequent chemotherapy with an alkylating agent such as temozolomide. Despite this multidisciplinary treatment approach, median survival is still quite short at 14.6 months. This is a 3–4 months increase in median survival without any type of treatment [17,18].

Data obtained from proteomic and genomic analysis in this study, among others, have concluded that each tumor has its own individual pathophysiologic profile. The future in treatment methodology will include identification of aberrations at a cellular level, which can allow for targeted therapies based on each tumor's particular proteogenomic identity. This has been accomplished to some degree in solid and hematologic malignancies, with directed treatment strategies against EGFR in non-small-cell lung cancer (NSCLC) and ERBB2 in breast cancer. However, this approach has not been successful in the treatment of GBM thus far.

In one study by Blumenthal *et al.*, 13 patients with GBM were treated based on results of genome sequencing. This included the utilization of EGFR tyrosine kinase inhibitors (TKI) afatinib and erlotinib. The study also treated one patient who had CDK4/6 amplification with Palbociclib. However, despite genomic sequencing and the identification of targetable genes, no significant response to tailored therapy was noticed [19]. This study showed that our understanding and knowledge of the molecular pathogenesis of GBM remains very limited, highlighting a need to continue our research in this domain.

Our study focused on identifying tumor heterogeneity at the cellular and molecular level for each of the 17 patients. Thus, by correlating their proteogenomic alterations with clinical course and radiographic disease advancement, we can understand how a particular tumor profile affects both prognosis and survival. Ultimately, the objective of research in the field of GBM aims to define the molecular basis of glioblastoma evolution, so that specific therapies may be developed and tailored to an individual's treatment. Our study aimed to delineate the essential biomarkers that can affect clinical outcome. Of note, this study did not aim to correlate GBM phenotype and pathologic structure with its mutation profile.

While tumor heterogeneity evidently exists between different individual patients, it is important to note that it also occurs within different areas of a single patient's tumor. In a prior study comparing primary and recurrent samples of low-grade gliomas, it was seen that in 43% cases, half the mutations seen in the primary tumor were undetectable in recurrent samples [20]. In our study, proteogenomic analysis was obtained to identify biomarkers that are of prognostic and predictive significance. Prognostic biomarkers aid in providing a more complete clinical picture regarding the overall survival with and without standard treatment following initial diagnosis. Predictive biomarkers help understand the potential benefits of a specific therapeutic intervention. Clinical data evaluated in the study included survival status, overall survival, relapse-free survival, treatment provided, number of relapses and treatment offered following a relapse. This information is summarized in Table 2. Corresponding genetic biomarkers were reviewed to assess for both prognostic and predictive significance.

The first biomarker of interest is MGMT, a DNA repair protein coded for by the *MGMT* gene. Alkylating agents, such as TMZ, result in the alkylation of this gene at O<sup>6</sup> position of guanine. Such alkylation results in double-stranded DNA breaks and ultimately ends with apoptosis of the tumor cell. This action is counteracted by the DNA repair effect of the MGMT protein, leading to a less effective response to treatment [21]. MGMT protein was detected in samples from 13 of the 17 patients (76%) included in the study.

Amplification and overexpression of the *EGFR* gene is a distinct feature of GBM and is noticed in 40% of these tumors. On the other hand, it is rare in low-grade gliomas. There are two types of *EGFR* mutations: wild-type and EGFR variant III with the latter being the most common. EGFRvIII occurs due to deletion of exons 2 through 7 of the *EGFR* gene, resulting in an in-frame deletion in its extracellular domain [22]. Studies have shown EGFRvIII to be more tumorigenic than the wild-type form [23]. The EGFR regulates cell proliferation via signal transmission by binding EGF and TNF- $\alpha$ . EGFRvIII on stimulation results in the activation of intracellular pathways such as PTEN resulting in increased tumor proliferation and survival [24]. EGFRvIII variant is a potential target for chimeric antigen receptor T-cell (CAR-T) therapy [25]. CAR-T is a form of immunotherapy, which uses T lymphocytes that have been genetically altered, and allows for high binding affinity and specificity to tumor

antigens. It is currently FDA approved for the treatment of acute childhood lymphoblastic lymphoma and B-cell non-Hodgkin's lymphoma [26]. Of particular interest were mutations in the *EGFR* gene that have previously been reported in patients with lung adenocarcinoma and have had a promising response to anti-EGFR antibodies in those patients. Response to EGFRvIII inhibitors has been very low in GBM. This suggests that complex molecular pathways need to be targeted within the EGFR system. There are also additional challenges pertaining to pharmacokinetics as any drug-targeting GBM needs to be able to cross the blood–brain barrier and then, should be able to stay in the brain parenchyma long enough to fulfill its action [27]. There has been conflicting data regarding the response to Erlotinib (TKI) in patients with coexpression of EGFRvIII and PTEN [28,29]. The discordance observed in EGFR expression on genomic and proteomic analysis shows the complex relationship between signaling pathways and molecular alterations.

*TP53* is a tumor-suppressor gene that codes for a protein involved in the regulation of cell cycle, differentiation and death. Such mutations are more common in secondary GBM in comparison to primary GBM (65 vs 28%) [30]. Loss-of-function of normal p53 function from TP53/MDM2/MDM4/p14ARF alteration leads to clonal expansion of glioma cells [31]. Due to the incomplete understanding of its complexity, no clear relationship has yet been found between the p53 pathway and targetable treatments as well as outcomes of GBM [32].

*IDH* gene codes for isocitrate dehydrogenase, an intramitochondrial enzyme with three intracellular forms: of these, the IDH1 enzyme is involved in the production of NADPH from oxidative decarboxylation of isocitrate. NADPH has a role in protecting cells from oxidative stressors and damage. Mutations in the *IDH1* gene lead to increased tumor proliferation by activation of VEGF-mediated angiogenesis. In patients with GBM, the presence of these mutations has been noted to improve the intracellular response to TMZ when compared with individuals with the wild-type *IDH1* gene [33]. In a prior genomic analysis, *IDH1* gene mutations were detected in 12% of GBM tumors. It was also identified in more than 70% of patients with grade II and III astrocytomas and oligodendrogliomas, as well as in GBMs that develop from lower-grade lesions [34]. The presence of *IDH1* mutation has been associated with improved OS when compared with *IDH* wild-type [34]. In addition, in comparison to *IDH1* wild-type tumor, presence of *IDH1* mutation is associated with longer time between diagnosis of low grade or anaplastic glioma and eventual progression to GBM (66 vs 16 months). With respect to *IDH1* mutation's role in recurrent GBM, however, prior studies have shown no significant difference in PFS and OS between *IDH1* mutation and *IDH1* wild-type tumors [35]. In our study, two patients were found to have an *IDH1* mutation (variant p. R132H). These two patients currently have a PFS with TMZ at 11 and 28 months. Also, in each case, the tumor pathology revealed the presence of both astrocytoma and GBM.

Genomic analysis also reveals potential targetable genes, as summarized in Table 5. EGFR amplification and EGFR variant III were detected, which may be sensitive to medications such as an NK-EGFR, dacomitinib and rindopepimut [36]. In fact, dacomitinib is a kinase inhibitor recently approved by the FDA for first-line treatment of metastatic NSCLC in patients with either an *EGFR* exon 19 deletion or an exon 21 L858R mutation. This supports the possibility that these same drugs could be utilized to target the *EGFR* mutations in GBM in future.

Proteomic analysis in one patient in our study group revealed the presence of TOPO1 and hENT1. TOPO1 is a potential targetable protein for treatment with irinotecan and topotecan. hENT1 protein is a possible target for treatment with gemcitabine [37]. After this patient relapsed within 13 months of standard therapy with TMZ, she was treated with irinotecan and demonstrated good response with PFS at 12 months after its initiation.

At this point, the eventual clinical significance of the results of our proteomic and genomic analysis is unclear. However, presently, since molecular therapies are being developed, any information regarding molecular profiling contributes to current efforts to develop therapy tailored against pathogenic molecular processes, even though it is too preliminary to assess definite impact.

The observations made in this study highlight the importance of tumor tissue analysis and increased research in the realm of molecular studies in GBM. Understanding these complex genetic pathways and how they interact will lead to the development of effective treatment strategies that can be tailored to specific patients based on the pathologic makeup of their cancer. It also reminds us of the limitations of the current TCGA data and that there is still much to understand about the pathogenesis of GBM. Hence, this study validates the need for further research to assist in mapping specific gene and protein changes, in order to organize a detailed landscape of this deadly tumor.

## Conclusion

Proteogenomic analysis suggests that the presence of certain biological processes in each GBM will aid to classify them into groups with different biological thumbprints. Meticulous defining of aberrations at the cellular level can potentially enable for the development of targeted therapies based on an individual tumors' 'identity.' Thus, proteogenomic data are paramount to identifying potential future therapeutic targets for GBM.

## Future perspective

There have been many successes in defining molecular profiles of tumors in patients with NSCLC. This has subsequently led to identification of therapeutic targets, either at protein or gene level. These advances with NSCLC fuel hope for similar triumph to be achieved in the future for patients with GBM. As described by Lin *et al.*, retrospective analysis showed that treatment of patients with *EGFR*-mutant metastatic lung adenocarcinoma with EGFR-TKI improved 5-year survival rates from less than 5% to as high as 14.6% [38]. This study also highlighted that certain proteogenomic attributes, such as the presence of an exon 19 deletion, were associated with improved outcomes in NSCLC patients. With continued investigations to extract clinically significant proteomic and genomic data, new diagnostic, prognostic and predictive biomarkers may be identified for GBM in the near future in addition to already known molecular markers such as EGFR, PIK3R1 and PTEN that will serve as targets for a novel generation of therapies against GBM. While limited therapeutic success has been achieved in trials that have used therapies against known oncogenic pathways thus far, as greater information is accumulated regarding GBM pathogenesis and classification of aberrant genes, we anticipate that development of therapies, such as targeted immunotherapies, will be developed over the next few years. Current genomic sequencing techniques allow for accumulation of large pools of data. If applied clinically, this information provides positive prospects to achieve better outcomes in patients with GBM. Given heterogeneity of GBM, it remains essential that all information gathered by a GBM analysis in individual studies is incorporated into our current available knowledge. This can then serve as a resource on which future studies can be built upon.

### Summary points

- Glioblastoma multiforme (GBM) is the most frequent primary brain tumor, which has an aggressive clinical course and extremely poor prognosis. Given limited success of the current standard of care that combines surgery, chemotherapy and radiation, the search for more effective therapies is critical. Thus, understanding the molecular pathogenesis of GBM, and classifying these data into a pool of organized information can allow for the development of novel treatment agents.
- Proteogenomic analysis in our study revealed *TP53*, *EGFR*, *PIK3R1*, *PTEN*, *NF1*, *RET* and *STAG2* as significantly mutated genes in these aggressive tumors.
- *EGFR* alterations seen included EGFRvIII expression, L816Q *EGFR* missense mutation on exon 21 and EGFR fusion (FGFR3-TACC3); all three are potential therapeutic targets of novel agents including EGFRvIII-specific dendritic cell vaccine, EGFR inhibitors and FGFR inhibitors.
- *TP53* mutation was found in 30% of patients, including in COSMIC hotspot driver gene as well as accompanying *IDH1* and *ATRX* mutations suggesting transformation from low- to high-grade glioma.
- One patient had a high mutation burden, currently living after 12 months of diagnosis.
- Proteomics showed significantly higher (n = 9, 53%) EGFR expression than genomic expression (53 vs 23%), suggesting tumor heterogeneity. 75% were methyl guanine methyl transferase-methylated, a predictive marker of response to temozolomide chemotherapy.
- At this point, while the eventual clinical significance of the results of our proteomic and genomic analysis is unclear, any information regarding molecular profiling contributes to current efforts to develop therapy tailored against pathogenic molecular processes.
- Further research is required to completely comprehend the specific genetic and protein changes underlying the development of GBM.

### Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: [www.futuremedicine.com/doi/suppl/10.2217/cns-2019-0003](http://www.futuremedicine.com/doi/suppl/10.2217/cns-2019-0003)

### Acknowledgments

The authors acknowledge NantOmics for providing proteogenomic laboratory analysis.

### Financial & competing interests disclosure

The author S Raza is a member of advisory board and speaker bureau for Amgen, Celgene, Takeda, Novartis and Janssen. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

### Ethical conduct of research

The study was approved by Institutional Review Board at Saint Luke's Health System (MO, USA) and was conducted according to ethical principles for research involving human subjects as stated in the Declaration of Helsinki.

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### References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

- Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pac. J. Cancer Prev.* 18(1), 3 (2017).
- Stupp R, Mason WP, van den Bent MJ *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* 352(10), 987–996 (2005).
- Mjelle R, Hegre SA, Aas PA *et al.* Cell cycle regulation of human DNA repair and chromatin remodeling genes. *DNA Repair (Amst.)* 30, 53–67 (2015).
- Esteller M, Garcia-Foncillas J, Andion E *et al.* Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N. Engl. J. Med.* 343(19), 1350–1354 (2000).
- Parsons DW, Jones S, Zhang X *et al.* An integrated genomic analysis of human glioblastoma multiforme. *Science.* 321(5897), 1807–1812 (2008).
- Brennan CW, Verhaak RGW, McKenna A *et al.* The somatic genomic landscape of glioblastoma. *Cell.* 155(2), 462–477 (2013).
- Hembrough T, Thyparambil S, Liao WL *et al.* Selected reaction monitoring (SRM) analysis of epidermal growth factor receptor (EGFR) in formalin fixed tumor tissue. *Clin. Proteomics* 9(1), 5 (2012).
- **Describes how tissue is prepared for proteogenomic analysis, a topic beyond scope of this article but relevant to methods used in obtaining proteomic and genomic data.**
- Rabizadeh S, Garner C, Sanborn JZ, Benz SC, Reddy S, Soon-Shiong P. Comprehensive genomic transcriptomic tumor-normal gene panel analysis for enhanced precision in patients with lung cancer. *Oncotarget* 9(27), 19223–19232 (2018).
- Forbes SA, Beare D, Gunasekaran P *et al.* COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res.* 45(D1), D777–D783 (2015).
- Tamborero D, Gonzalez-Perez A, Lopez-Bigas N. OncodriveCLUST: exploiting the positional clustering of somatic mutations to identify cancer genes. *Bioinformatics.* 29(18), 2238–2244 (2013).
- Kalia SS, Adelman K, Bale SJ *et al.* Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet. Med.* 19(2), 249 (2017).
- Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin. Chem.* 60(9), 1192–1199 (2014).
- Griffith M, Griffith OL, Coffman AC *et al.* DGIdb: mining the druggable genome. *Nature Methods* 10(12), 1209 (2013).
- Forbes SA, Beare D, Gunasekaran P *et al.* COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res.* 43(D1), D805–D811 (2014).
- NantHealth. NantHealth Precision Insights. (2019) <https://nanthealth.com/precision-insights/>
- US FDA. US FDA Homepage. (2019) [www.fda.gov/](http://www.fda.gov/)
- Stupp R, Hegi ME, Mason WP *et al.* Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised Phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 10(5), 459–466 (2009).

- **Defined current standard of therapy for glioblastoma multiforme (GBM) and associated survival data, which emphasize how essential it is to develop new treatment modalities in order to improve overall survival in patients with GBM.**
18. Moton S, Elbanan M, Zinn PO, Colen RR. Imaging genomics of glioblastoma: biology, biomarkers, and breakthroughs. *Top. Magn. Reson. Imaging*. 24(3), 155–163 (2015).
  19. Blumenthal DT, Dvir A, Lossos A *et al.* Clinical utility and treatment outcome of comprehensive genomic profiling in high grade glioma patients. *J. Neurooncol.* 130(1), 211–219 (2016).
- **This is a prior study in which targeted therapy for GBM patients was clinically applied.**
20. Johnson BE, Mazor T, Hong C *et al.* Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science*. 343(6167), 189–193 (2014).
- **Describes the heterogeneity of GBM and helps highlight the complexity of defining proteogenomic landscape of GBM.**
21. Pegg AE. Repair of O6-alkylguanine by alkyltransferases. *Mutat. Res. Rev. Mutat. Res.* 462(2–3), 83–100 (2000).
- **Describes role of MGMT as a predictive marker.**
22. Hatanpaa KJ, Burma S, Zhao D, Habib AA. Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia*. 12(9), 675–684 (2015).
  23. Batra SK, Castelino-Prabhu S, Wikstrand CJ *et al.* Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant *EGFRvIII* gene. *Cell Growth Differ.* 6(10), 1251–1260 (1995).
  24. Zadeh G, Bhat KL, Aldape K. *EGFR* and *EGFRvIII* in glioblastoma: partners in crime. *Cancer Cell*. 24(4), 403–404 (2013).
  25. O'Rourke DM, Nasrallah MP, Desai A *et al.* A single dose of peripherally infused *EGFRvIII*-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci. Transl. Med.* 9(399), eaaa0984 (2017).
  26. Forsberg MH, Das A, Saha K, Capitini CM. The potential of CAR T therapy for relapsed or refractory pediatric and young adult B-cell ALL. *Ther. Clin. Risk Manag.* 14, 1573 (2018).
  27. Karpel-Massler G, Schmidt U, Unterberg A, Halatsch ME. Therapeutic inhibition of the epidermal growth factor receptor in high-grade gliomas: where do we stand? *Mol. Cancer Res.* 7(7), 1000–1012 (2009).
- **Describes potential for targeted therapy against EGFR mutation, an essential component of our discussion.**
28. Mellinshoff K II, Wang MYM, Vivanco I *et al.* Molecular determinants of the response of glioblastomas to *EGFR* kinase inhibitors. *N. Engl. J. Med.* 353(19), 2012–2024 (2005).
- **Also describes potential for targeted therapy against EGFR mutation, an essential component of our discussion.**
29. Schiff D, Yong WH, Giannini C *et al.* Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J. Clin. Oncol.* 26(34), 5603–5609 (2008).
  30. Ohgaki H, Kleihues P. Genetic alterations and signaling pathways in the evolution of gliomas. *Cancer Sci.* 100(12), 2235–2241 (2009).
  31. Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J. Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? *Nat. Rev. Clin. Oncol.* 10(1), 14 (2013).
  32. Masui K, Cloughesy TF, Mischel PS. Molecular pathology in adult high-grade gliomas: from molecular diagnostics to target therapies. *Neuropathol. Appl. Neurobiol.* 38(3), 271–291 (2012).
  33. Songtao Q, Lei Y, Si G *et al.* *IDH* mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci.* 103(2), 269–273 (2012).
  34. Yan H, Parsons DW, Jin G *et al.* *IDH1* and *IDH2* mutations in gliomas. *N. Engl. J. Med.* 360(8), 765–773 (2009).
  35. Mandel JJ, Cachia D, Liu D *et al.* Impact of *IDH1* mutation status on outcome in clinical trials for recurrent glioblastoma. *J. Neurooncol.* 129(1), 147–154 (2016).
  36. Zahonero C, Aguilera P, Ramirez-Castillejo C *et al.* Preclinical test of dacomitinib, an irreversible *EGFR* inhibitor, confirms its effectiveness for glioblastoma. *Mol. Cancer Ther.* 14(7), 1548–1558 (2015).
  37. Gilbert DC, Chalmers AJ, El-Khamisy SF. Topoisomerase i inhibition in colorectal cancer: biomarkers and therapeutic targets. *Br. J. Cancer.* 106(1), 18 (2012).
  38. Lin JJ, Cardarella S, Lydon CA *et al.* Five-year survival in *EGFR*-mutant metastatic lung adenocarcinoma treated with *EGFR*-TKIs. *J. Thorac. Oncol.* 11(4), 556–565 (2016).
- **Delineates how targeted therapy can markedly change clinical outcomes, which is what we are trying to achieve.**