

Review Article

Case comparison and literature review of glioblastoma: A tale of two tumors

Gustavo Mendez¹, Alp Ozpinar¹, Jeffrey Raskin¹, Sakir H. Gultekin², Donald A. Ross^{1,3}¹Departments of Neurological Surgery, and ²Pathology, Oregon Health & Science University, ³Section of Neurological Surgery, Operative Care Division, Portland Veterans Administration Hospital, Portland, OR, USA

E-mail: Gustavo Mendez - mendezg@ohsu.edu; Alp Ozpinar - ozpinar@ohsu.edu; Jeffrey Raskin - raskin@ohsu.edu; Sakir H. Gultekin - gultekin@ohsu.edu;

*Donald A. Ross - rossdo@ohsu.edu

*Corresponding author

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Abstract**Background:** Diagnosis of glioblastoma multiforme (GBM) includes a heterogeneous group of tumors. We describe two cases with histopathologically and molecularly similar tumors, but very different outcomes. We attempt to illustrate the need for improved prognostic markers for GBM.**Case Description:** Two patients with similar molecular profiles were retrospectively identified. The following markers were assessed: O⁶-methylguanine DNA methyltransferase (MGMT) methylation, isocitrate dehydrogenase (IDH) 1 and 2 status, epidermal growth factor receptor (EGFR) amplification, phosphatase and tensin homolog (PTEN) status, Ki-67, p53, and 1p/19q status. Each patient was assigned a Karnofsky performance score at presentation. Case 1 (62-year-old male) was a right temporal lobe glioblastoma with a molecular profile of amplified *EGFR*, normal *PTEN*, no *IDH1/2* mutation, 28.7% MGMT promoter methylation, 5-20% Ki-67, 1p deletion, and 19q intact. The patient underwent resection followed by radiation therapy and 2 years of chemotherapy, and was asymptomatic and tumor free 5 years post diagnosis. Tumor eventually recurred and the patient expired 72 months after initial diagnosis. Case 2 (63-year-old male) was a right frontal white matter mass consistent with glioblastoma with a molecular profile of amplified *EGFR*, absent *PTEN*, no *IDH1/2* mutation, 9.9% MGMT promoter methylation, 5-10% Ki-67, and 1p/19q status inconclusive. A radical subtotal resection was performed; however, 2 weeks later symptoms had returned. Subsequent imaging revealed a tumor larger than at diagnosis. The patient expired 3 months after initial diagnosis.**Conclusion:** The need for formulating more robust means to classify GBM tumor subtypes is paramount. Standard histopathologic and molecular analyses are costly and did not provide either of these patients with a realistic appraisal of their prognosis. Individualized whole genome testing similar to that being reported for medulloblastoma and other tumors may be preferable to the array of tests as currently utilized.**Key Words:** 1p/19q deletion, epidermal growth factor receptor amplification, glioblastoma, isocitrate dehydrogenase1, isocitrate dehydrogenase1 mutation, O⁶-methylguanine DNA methyltransferase methylation and expression, prognosis, phosphatase and tensin homolog deletion**Access this article online****Website:**www.surgicalneurologyint.com**DOI:**

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INTRODUCTION

While astrocytoma grade IV or glioblastoma multiforme (GBM) is a defined histopathologic diagnosis,^[4] molecular oncologic evidence now strongly indicates that a GBM diagnosis includes a heterogeneous group of tumors.^[26] Efforts to identify molecular markers that more clearly define an individual patient's prognosis and treatment susceptibility are ongoing.^[14] Batteries of molecular tests are now available and are reported along with the routine histopathologic analysis of malignant glial tumors. These may include testing for epidermal growth factor receptor (*EGFR*) amplification, p53 mutations, phosphatase and tensin homolog (*PTEN*) deletions, O⁶-methylguanine DNA methyltransferase (*MGMT*) promoter methylation status, 1p/19q deletions, isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) mutations, and Ki-67 labeling index. While results have been helpful in directing treatment and estimating prognosis, they are often not sufficient for informed decisions regarding individual patients. Molecular tests currently provide insight into tumor sensitivity to alkylating chemotherapy and can therefore help drive patient management, but even in cases where ideal molecular profiles predict a robust response to chemotherapy and radiation, accurate prognosis continues to be elusive.

Here we describe two patients with histopathologically and molecularly similar tumors, but very different outcomes. We attempt to illustrate the need for a wider range of GBM molecular markers as is the case for individualized whole genome testing reported for medulloblastoma.^[23]

MATERIALS AND METHODS

Tumor molecular characterization

Histopathologic analysis and immunohistochemical (IHC) staining were performed by the Oregon Health & Science University (OHSU) Pathology Department, Section of Neuropathology. Fluorescence *in situ* hybridization (FISH) assays were performed by the OHSU Knight Diagnostic Laboratories (KDL) Research Cytogenetics Laboratory and mutation analyses and methylation assays were completed by the OHSU KDL.

IDH1, IDH2 status

DNA was extracted and purified from paraffin-embedded tumor tissue. Exon 2 of the *IDH1* gene and exon 4 of the *IDH2* gene were amplified by polymerized chain reaction (PCR) and the product subjected to single-strand sequencing on a pyrosequencer (Biotage, Charlotte, NC, USA). The estimated sensitivity of this method is detection of mutations in *IDH1* even if just 5% of mutant alleles are available in the DNA sample.^[29]

MGMT methylation status

DNA was extracted from paraffin-embedded tumor samples with subsequent pyrosequencer-based analysis of 10 cytosine-phosphate-guanine (CpG) sites. The pyrosequencing method used by the OHSU Pathology Translational Research Laboratory is modified from Dunn.^[8]

Glioma FISH panel

EGFR/CEP7 probe set was used to identify *EGFR* amplification. Fixed, paraffin-embedded tumor tissue was treated according to standard protocols and 100-200 interphase cells were scored. Institutional cutoff for amplification was ≥ 2.2 *EGFR: CEP7*. 1p/19q deletion status was evaluated by using 1p36, 1q25 probes for chromosome 1p, and 19q13, and 19p13 probes were used to test for deletion of chromosome 19q or monosomy of chromosome 19. *PTEN/CEP10* was used to identify chromosome 10q deletion or monosomy 10. Ki-67 and p53 IHC stains were performed as detailed by Pallini *et al.* in 2008.^[24]

Literature review

A United States National Library of Medicine/Medline/PubMed search was conducted to identify studies focusing on association of molecular marker expression and prognosis in GBM. Search terms included: Glioblastoma, grade IV astrocytoma, IDH mutation, *MGMT* expression and/or methylation, *EGFR* amplification, 1p/19q status, and *PTEN* deletion. Articles related to lower-grade brain malignancy, smaller institutional experiences, or studies not found in peer-reviewed journals were excluded. In reviewing pertinent articles, focus was placed on papers providing survival projections based on specific molecular markers, papers defining molecular characteristics of high-grade gliomas, and meta-analyses.

DISCUSSION

Although GBM is currently a histopathologic diagnosis, molecular classification schemes are pioneering a transition toward more accurate subtyping and prognostication. The future of the field has moved ever closer toward defining the oncogenetic signature, correlating genotype with phenotype, and ultimately attempting to tailor therapy to individual tumor marker expression. Verhaak's study on genomic analysis introduced four subsets of GBM: proneural, neural, classical, and mesenchymal.^[35] Each sub-classification of GBM was associated with a particular set of genetic changes that dictated a particular phenotype, with classical phenotype best approximating a primary GBM, that is a GBM occurring *de novo* (high-level *EGFR* amplification and chromosome 10 loss), and proneural representing a phenotype more consistent with a secondary GBM, or a GBM that has developed from a lower-grade glioma [high-level platelet-derived growth

factor receptor α (PDGFRA) amplification and *IDH1* point mutations] enriched with an oligodendrocytic signature. In analyzing the two cases we present, we followed this paradigm and focused on the expression of five molecular markers: *EGFR*, *PTEN*, *IDH1/2*, *MGMT* promoter, and *p53*.

IDH1 and *2* are metabolic isozymes, which catalyze the conversion of isocitrate to α -ketoglutarate and produce reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the tricarboxylic acid (TCA) cycle. *IDH1* and *IDH2* are also active outside the TCA cycle and are localized in the cytosol and peroxisome. The roles of these enzymes in gliomagenesis have yet to be elucidated. It has been posited that the high frequency of *IDH* mutation in secondary GBM, low-grade glioma, and oligodendrogliomas implies that *IDH* plays a role in early gliomagenesis. The shared high frequency of *IDH* mutation in lower-grade lesions may also suggest existence of a different stem cell that acts as a precursor to tumors of astrocytic and oligodendrocytic characteristics. A series of biopsies from the same glioma patients found that neither *TP53* mutation nor *1p/19q* co-deletion preceded *IDH* mutation, supporting the notion that *IDH* has an integral role in early events of gliomagenesis.^[37]

A recent meta-analysis^[41] investigated the association between the *IDH* mutations and both progression-free survival (PFS) and overall survival (OS). The study also examined the relationship of *IDH* mutations and expression of other known GBM markers. The meta-analysis included 10 studies totaling 2190 cases and found that *IDH1* or *IDH2* mutations were more frequent in World Health Organization (WHO) grade II and III gliomas (59.5%) and in secondary GBM (63.4%), while the frequency in primary GBM (7.13%) was significantly lower. A strong association was also identified between *IDH* mutation and *MGMT* hypermethylation, *1p/19q* co-deletion, *TP53* gene mutation, and a mutual exclusivity was noted with *EGFR* amplification.^[41]

Favorable glioma survival figures have been a hallmark of patients with *IDH* mutations: improved OS [hazard ratio (HR) = 0.33; 95% confidence interval (CI): 0.25-0.42; $P_{\text{heterogeneity}} = 0.204$] and PFS (HR = 0.38; 95% CI: 0.21-0.68; $P_{\text{heterogeneity}} = 0.000$).^[41] One group looking at secondary GBM found that median survival in patients with an *IDH1* mutation was 31 months, while patients with wild-type (WT) *IDH1* tumors had a median survival of 15 months.^[26] A prospective study of 301 patients with primary GBM identified a sub-population of long-term survivors (defined as survival >36 months). For this group, the three most important prognostic factors for surviving at least 36 months were *MGMT* status, *IDH1* or *IDH2* status, and age. Moreover, the rate of *IDH1* or *IDH2* mutation in this cohort was markedly high (34%) compared to the 4.3% observed in GBM patients who

survived <36 months.^[11] This study also found the same association of *IDH1* mutation to other molecular markers cited in the meta-analysis. However, one novel finding was that in a subset of long-term survivors who lived beyond 36 months, further survival was less predictable based on *IDH* mutation status and more predictable based on *MGMT* promoter methylation, WT *TP53*, and lack of *EGFR* amplification.^[11] In an attempt to elucidate the mechanism of enhanced survival in patients with glioma featuring *IDH1* or *IDH2* mutations, some have proposed that the mutation confers a two-fold advantage. *In vitro* studies have demonstrated decreased proliferation rates and more contact-dependent migration of cells in glioma lines transfected with *IDH1* mutations. Secondly, disruption of normal NADPH replenishment pathways consequent to *IDH* mutations results in higher susceptibility to oxidative stress and, therefore, increased responsiveness to radiation and chemotherapy.^[41] As neither of the patients we present had an *IDH* mutation, this did not aid in distinguishing their disparate prognoses.

Susceptibility to alkylating chemotherapy is a key feature seen in gliomas with *MGMT* promoter region hypermethylation status.^[39] *MGMT* codes for the sole DNA repair protein that removes *O*⁶-methylguanine from DNA. The *MGMT* protein removes the cytotoxic *O*⁶-alkylguanine adduct, and via this mechanism promotes resistance to anti-glioma alkylators including temozolomide (TMZ) and bis-chloroethylnitrosourea (BCNU).^[32] Wiewrodt *et al.* found a statistically significant difference in PFS and OS for primary and recurrent glioblastoma patients based on the degree of *MGMT* expression.^[39] Tumors exhibiting greater than 30 fmol/mg *MGMT* activity had a decreased PFS and OS, compared with tumors exhibiting *MGMT* activity below this threshold. Adjuvant therapy with radiation following surgery did not result in significant difference in PFS or OS. The subgroup of patients receiving radiation with concomitant alkylating chemotherapy showed a significant difference in PFS but not OS between low and high *MGMT* expression tumors (threshold level 30 fmol/mg protein).^[39] Overall, the data demonstrate that patients expressing less than 30 fmol/mg protein *MGMT* in the primary tumor show a significantly better therapeutic response to combined radiation and chemotherapy than the patients exhibiting *MGMT* expression beyond threshold. The same study also demonstrated that *MGMT* expression increases as the glioma progresses or recurs.^[39]

Another group defined a threshold for comparing survival in newly diagnosed GBM by using the median percentage of cells staining for *MGMT* protein in tumor samples. Looking at a retrospective cohort of 418 GBM subjects, patients with <30% staining had a PFS of 10.9 months and an OS of 20.5 months, compared

with a PFS of 7.8 months ($P < 0.0001$) and an OS of 16.7 months ($P < 0.0001$) among patients with $\geq 30\%$ staining.^[20]

In addition to MGMT protein expression, it is important to consider epigenetic modifications to MGMT, e.g. CpG methylation status. Correlative studies have shown that patients with tumors displaying MGMT promoter hypermethylation or low expression of MGMT protein are more likely to benefit from TMZ treatment, when compared to patients with tumors displaying unmethylated MGMT or high MGMT expression.^[12,19] A British study by Dunn and colleagues tracked survival in glioblastomas treated with TMZ and radiotherapy based solely on the degree of MGMT promoter methylation. They reported that methylation $>35\%$ had median PFS of 19.2 months, OS of 26.2 months, and 2-year survival of 59.7%.^[8] In contrast, unmethylated samples displayed median PFS of 8.3 months, OS of 11.1 months, and 2-year survival of 0%. By their metrics, the patient (Case 1) with 28% methylation would have had a PFS of 11.8 months, OS of 15.5 months, and 2-year survival of 34.2%, whereas the patient (Case 2) with 9.9% methylation would have had a predicted PFS of 7.5 months, OS of 11.3 months, and 2-year survival of 13.3%.^[8]

While the results of Dunn's^[8] methylation analysis are compelling, multiple studies have found that hypermethylation status is a poor prognostic factor since patients with epigenetically silenced MGMT tend to accumulate more deleterious genetic mutations as well (p53 and K-ras).^[3,16,17] In addition to evaluating outcomes based on MGMT protein expression, Lalezari's recent study also probed promoter methylation.^[20] Their results demonstrated that patients with low MGMT immunohistochemical expression (below 30%) and increased methylation had better survival results than patients with no methylation and increased immunohistochemical MGMT expression (above 30%). They further demonstrated that patients with high protein expressions have poor outcomes despite the presence of methylation. Analyzing the mechanism of transcriptional silencing of MGMT, they concluded that on the basis of concurrent analyses of MGMT immunohistochemistry and promoter methylation by methylation specific PCR and bisulfite sequencing (BiSEQ), optimal assessment of MGMT status as a prognostic biomarker for newly diagnosed GBM treated with radiotherapy and TMZ should take into consideration both protein expression and methylation status. Using both evaluations, the most favorable outcome for patients with GBM was observed in the context of simultaneous methylation and low MGMT protein expression. If measuring methylation status by methylation-specific PCR, the best outcome cohort ($<30\%$ MGMT protein expression and methylated promoter) demonstrated a 21-month median survival, while the worst outcome group ($\geq 30\%$ MGMT protein

expression and unmethylated promoter) showed median survival of 14.5 months. Methylation determined by BiSEQ yields a best outcome group ($<30\%$ MGMT expression and ≥ 3 sites methylated) median survival of 20.5 months and low outcome group ($\geq 30\%$ MGMT expression and <3 sites methylated) median survival of 11.9 months.^[20] The study also found that methylation was correlated with reduced protein expression, although low expression occurred frequently in the absence of methylation.^[20]

Along with MGMT expression and gene methylation, one other area of study has focused on the interaction between MGMT and other biomarkers. German trials have probed the prognostic versus predictive role of MGMT as a function of either IDH or 1p/19q status. A study by Wick *et al.* found that in WHO grade III/IV gliomas, MGMT promoter methylation is prognostic for patients with *IDH1* mutations, conferring longer PFS independent of treatment modality. In the cohort with WT *IDH1*, MGMT methylation proved to be a predictive marker of response to alkylating chemotherapy, but not prognostic of survival. In contrast, this type of relationship was absent when analyzing 1p/19q co-deletions and MGMT status.^[38]

Prognosis based on *EGFR* gene amplification and/or *EGFR* overexpression continues to be debated, as some studies find direct association with poor prognosis^[1,7,9,30,40] while others find no significant effect on survival.^[6,25,36] A more nuanced approach to analyzing the relationship of *EGFR* amplification and survival was employed by Hobbs *et al.* who determined that GBM behavior and sensitivity to treatment was a function of the degree of *EGFR* amplification, not just presence of amplification.^[13] Using FISH to analyze biopsy specimens from 532 newly diagnosed patients, the group was able to classify patients into three categories based on the ratio of *EGFR* copy number to chromosome 7 ploidy (expressed as the ratio of *EGFR* to centromeric enumeration probe for chromosome 7): no amplification (*EGFR*: CEP7 <2), low-to-moderate amplification (*EGFR*: CEP7 = 2-20), and high-level amplification (*EGFR*: CEP7 >20). Surprisingly, the group with the longest survival was the high-level amplification cohort, registering a median survival of 11 months, while the no-amplification group had a median survival of 7.9 months and the low-to-moderate amplification sub-category had a median survival of 7.7 months.^[13] This non-linear association of degree of amplification to median survival may be a consequence of genome fragility with high amplification that may counteract the increased infiltrative and angiogenic capacity observed in low-to-moderate levels of *EGFR* amplification, but the relationship has yet to be fully elucidated.

While p53 is a well-documented tumor suppressor involved in various tumorigenesis processes, its role as

a prognostic marker in GBM remains controversial. The role of p53 as a tumor suppressor is intimately linked to its involvement in apoptosis, cell cycle modulation, and metabolism. Just based on p53's known spectrum of activity, it would be reasonable for mutations of p53 in tumor cells to confer resistance to apoptosis. Conversely, it is conceivable that overexpression of WT p53 would enhance radiosensitivity of glioma cells. That said, multiple studies have demonstrated the difficulty of making such an assertion. In fact, the effect of p53 mutations on glioma sensitivity to radiation and chemotherapy remains inconclusive.^[2,10,22] Some have reported that p53 mutation status has no bearing on survival or sensitivity to radiotherapy,^[21,34] while others have shown an association between p53 gene mutations in GBM and improved survival and radiation response.^[28,31] In the German prospective study of *IDH1* or *IDH2* modification in GBM,^[11] p53 modification showed little utility as a prognostic marker until patients had reached the 36-month threshold that defined long-term survival in the study. At that point, the group identified WT TP53 as an important marker of increased survival, second only to MGMT.^[11] In terms of the frequency of mutation, Verhaak's genomic analysis^[35] found a lack of TP53 mutation in classical phenotype GBM (the GBM subtype most similar to primary GBM), with the highest frequency of TP53 mutation and loss of heterozygosity occurring in the proneural subtype.^[35] When comparing two long-term survival groups, Hartmann and colleagues found a higher frequency of TP53 mutations in LTS36 than in the LTS60 (patients surviving 60 months or more).^[11] In isolation, none of the markers are truly predictive of outcome and derive their greatest utility when coupled with other markers, yielding a more comprehensive profile.

CASE REPORTS

Case 1

A 62-year-old previously healthy male (Karnofsky = 100) presented with an account of riding his bicycle home from work and becoming lost and unable to recall approximately 1 hour of time. Subsequent evaluation for a likely partial complex seizure led to the discovery of a right temporal lobe mass. Stereotactic biopsy was consistent with a WHO Grade IV astrocytoma (glioblastoma). He underwent gross total resection confirmed by magnetic resonance imaging (MRI) followed by adjuvant radiation therapy and 2 years of TMZ chemotherapy, per the Stupp protocol.^[33] Karnofsky score remained 100. Prophylactic levetiracetam was administered post-surgery and continued indefinitely after a discussion of benefits and risks. The patient remained seizure free. He was asymptomatic and tumor free on follow-up (MRI 5 years post-diagnosis). His tumor then recurred, and he underwent a second resection followed by additional

TMZ. The tumor spread subependymally and he expired 72 months after initial diagnosis.

Case 2

A 63-year-old previously healthy male developed headaches, personality changes, and disorientation (Karnofsky = 70). A 17.5 cm³, irregular, peripherally enhancing mass deep in the right frontal white matter with some extension into the corpus callosum was visible on brain MRI. A radical but subtotal resection was performed without complication, removing 77% of the mass and leaving a 4 cm³ mass in the lateral corpus callosum, and with marked improvement in symptoms. Karnofsky improved to 80 postoperatively, with some mild confusion. Pathology was consistent with WHO Grade IV astrocytoma (glioblastoma). Two weeks later, prior to a radiation oncology visit, his symptoms returned. Subsequent MRI revealed a tumor larger than that prior to surgery. Radiation and TMZ were started immediately, per the Stupp protocol.^[33] The patient's clinical status declined rapidly, rendering him unable to continue treatment after 2 weeks of radiation. He was placed on hospice care and expired soon thereafter, only 7 weeks from initial diagnosis.

Both cases shared amplification of *EGFR* and an absence of *IDH* mutations. *PTEN* was normal in Case 1 and absent in Case 2. MGMT methylation was 28.7% in Case 1 and 9.9% in Case 2. Ki-67 proliferation marker was 5-20% in Case 1 and 5-10% in Case 2. Case 1 had 5-10% p53 and Case 2 had <5% p53. Case 1 showed a 1p deletion with 19q intact, while Case 2 had inconclusive status [Table 1].

In reviewing the two cases presented, the molecular profiles were quite similar, with differences noted in *PTEN* status (Case 2 had complete absence of chromosome 10) and degree of MGMT promoter methylation (Case 1 had greater methylation). While presence of *EGFR* amplification and *PTEN* loss in Case 2 make it a candidate for classification as a classical GBM subtype, neither marker has been shown to definitively dictate outcome and, therefore, the rapid decline of Case 2 cannot be attributed solely to these molecular characteristics. In fact, a study of *PTEN* loss versus *PTEN*

Table 1: Tumor molecular characteristics

Molecular marker	Case 1	Case 2
EGFR (amplification)	Amplified	Amplified
PTEN (loss)	Normal	Absent chr 10
<i>IDH1</i> , <i>IDH2</i>	No mutations	No mutations
MGMT promoter (% methylated)	28.7	9.9
Ki-67 (%)	5-20	5-10
P53 (%)	5-10	<5
1p/19q	1p deletion, 19q intact	

EGFR: Epidermal growth factor receptor, PTEN: Phosphatase and tensin homolog, IDH: Isocitrate dehydrogenase

retained revealed no significant difference in outcome as the *PTEN* retained group had a median survival of 20.0 months and *PTEN* loss had a median survival of 18.2 months.^[5] Carico and collaborators believed the outcomes to be similar because of an increased sensitivity to TMZ in *PTEN* loss that may balance out any enhanced tumorigenicity.^[5] On the other hand, increased OS in Case 1 cannot be completely due to more pronounced MGMT methylation, given that the literature suggests methylation status is an indicator of sensitivity to alkylating chemotherapy, not an independent marker of prognosis. That said, reports have linked improved outcomes to increased MGMT methylation and a predisposition for pseudoprogression.^[15]

We recognize one of the shortcomings of the study comparison is sample size, with molecular profiles that were not exactly the same. However, the cases shared similarities in critical markers and, as illustrated in Table 2, projecting survival based on current molecular markers is highly variable and is dependent on which individual marker is being used. Extent of resection also differed between the two cases, as one patient had a gross total tumor resection and the other patient received subtotal resection. Degree of resection has been a heavily debated topic in the literature, but a recent study found that while aggressive extent of resection is associated with improved OS, subtotal resections with as little as 78% resection correspond to significant survival benefit.^[27]

CONCLUSION

As evidenced by case comparison, the need for formulating more robust means to classify GBM tumor subtypes

is paramount. Standard histopathologic analysis and molecular testing available for the two cases we present did not allow either patient a realistic appraisal of their prognosis, as is true for many patients. The problem of predicting life expectancy for patients with neurological malignancies is not unique to gliomas, as recent data suggest prognosis for patients with brain metastases is also quite uncertain.^[18] This raises the question of cost versus utility of more extensive molecular routine testing on all patients. Currently, little data have been published regarding the cost of GBM molecular analysis. At our institution, the cost of testing (IDH + EGFR + MGMT + PTEN + p53) is approximately \$3900-\$3950. An estimate from University College London, Department of Neuropathology, in 2013, for a similar panel of markers (1p19q + IDH + BRAF*2 + EGFRviii + MGMT) is approximately \$700-\$800.^[11] In addition to variability in marker selection and cost of testing as noted, it even remains uncertain whether the data acquired from marker expression are worth the incurred expense. Individualized whole genome testing similar to that being reported for medulloblastoma^[23] and other tumors may be preferable to the array of tests as currently utilized.

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Table 2: Molecular markers and projected survival

Reference	Molecular survival prognosticator	Median overall survival (months)
Robertson et al., 2011*	<i>IDH1</i> WT	15
	<i>IDH1</i> mutation	31
Lalezari et al., 2013	<30% MGMT protein expression and methylated promoter (by MSP)	21
	≥30% MGMT protein expression and unmethylated promoter (by MSP)	14.5
	<30% MGMT expression and ≥3 sites methylated (by BiSEQ)	20.5
	≥30% MGMT expression and <3 sites methylated (by BiSEQ)	11.9
Hobbs et al., 2012	<i>EGFR</i> no amplification	7.9
	<i>EGFR</i> low-to-moderate amplification	7.7
	<i>EGFR</i> high amplification	11
Carico et al., 2012	<i>PTEN</i> loss	18.2
	<i>PTEN</i> retained	20

*Robertson's data were derived from cases of secondary GBM. IDH: Isocitrate dehydrogenase, MGMT: O6-methylguanine-DNA-methyltransferase, MSP: methylation-specific polymerase chain reaction, EGFR: Epidermal growth factor receptor, PTEN: Phosphatase and tensin homolog

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