Moving toward molecular classification of diffuse gliomas in adults

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ABSTRACT

Diffuse gliomas are a heterogenous group of neoplasms traditionally classified as grades II to IV based on histologic features, and with prognosis determined mainly by histologic grade and pretreatment clinical factors. Our understanding of the molecular basis of glioma initiation, tumor progression, and treatment failure is rapidly evolving. A molecular profile of diffuse gliomas is emerging. Studies evaluating gene expression and DNA methylation profile have found multiple glioma subtypes and an association between subtype and survival. The recent discovery of isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) mutations in glioma has provided reproducible prognostic biomarkers and novel therapeutic targets. Glioblastomas that exhibit CpG island hypermethylator phenotype, proneural gene expression, or *IDH1* mutation identify a subset of patients with markedly improved prognosis. Accumulated evidence supports the stratification of both low-grade and anaplastic diffuse gliomas into prognostic groups using 1p/19q codeletion and *IDH* mutation status. A classification scheme incorporating clinical, pathologic, and molecular information may facilitate improved prognostication for patients treated in the clinic, the development of more effective clinical trials, and rational testing of targeted therapeutics.
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GLOSSARY

AA = anaplastic astrocytoma; **AG** = anaplastic glioma; **AML** = acute myelogenous leukemia; **AO** = anaplastic oligodendroglioma; **AOA** = anaplastic mixed oligoastrocytoma; **EGFR** = epidermal growth factor receptor; **EORTC** = European Organisation for Research and Treatment of Cancer; **G-CIMP** = CpG island hypermethylator phenotype; **GB** = glioblastoma; **GBO** = glioblastoma with oligodendroglial features; **GSC** = glioma stem cell; **IHC** = immunohistochemical; **LGA** = low-grade astrocytoma; **LGG** = low-grade glioma; **LGO** = low-grade oligodendroglioma; **LGOA** = low-grade mixed oligoastrocytoma; **LOH** = loss of heterozygosity; **PDGFR** = platelet-derived growth factor receptor; **Rb** = retinoblastoma; **RPA** = recursive partitioning analysis; **RTOG** = Radiation Therapy Oncology Group; **TCGA** = the Cancer Genome Atlas.

Diffuse gliomas comprise the second most common primary CNS neoplasms, behind meningiomas, and account for 80% of primary, malignant brain tumors.[1] WHO classification of diffuse gliomas is based on a grading scheme from II to IV based on histomorphology, proliferation, and the presence of microvascular proliferation or necrosis. Diffuse gliomas are traditionally separated by histology into 3 categories: astrocytomas, including glioblastoma (GBs), oligodendrogliomas, and a poorly reproducible group termed mixed oligoastrocytomas.[2]

GBs comprise 53.9% of all gliomas and are the most common primary CNS malignancy in adults.[1] GBs are differentiated histologically from other diffuse astrocytomas by the presence of microvascular proliferation or necrosis. GBs can be partitioned into primary GB, which arise de novo, and secondary GB, which arise by progression from grade II or III astrocytomas. Primary GBs typically occur in patients over 50 years of age and are characterized by overexpression or mutation of EGFR, loss of heterozygosity (LOH) of chromosome 10q, and *PTEN* mutations. Secondary GBs usually occur in younger patients and are characterized by *TP53* and isocitrate dehydrogenase 1 (*IDH1*) mutations, overexpression of platelet-derived growth factor receptor (PDGFR), and abnormalities of the retinoblastoma (Rb) pathway.[3]

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Diffuse WHO grade II (low-grade glioma [LGG]) and WHO grade III (anaplastic glioma [AG]) include low-grade and anaplastic astrocytoma (LGA and AA), low-grade and anaplastic oligodendroglioma (LGO and AO), and low-grade and anaplastic mixed oligoastrocytoma (LGOA and AOA). Diffuse LGGs and AGs in aggregate comprise approximately 15% of gliomas.[1] Accumulated evidence strongly suggests that astrocytomas and oligodendrogliomas are separate clinical and molecular entities with different prognoses and treatment responses.[4–7]

The WHO criteria for classification and grading of diffuse gliomas have limitations. In preceding decades, the principal determinants of prognosis were clinical, pathologic, and pretreatment factors. Using recursive partitioning analysis (RPA), tumor grade combined with age, performance score, and extent of surgical resection assigns diffuse glioma patients into RPA classes associated with survival.[8,9] Although negative correlation exists between increasing WHO grade, RPA class, and survival, significant heterogeneity in clinical behavior among tumors with the same grade and clinical features is observed.

Accumulating evidence suggests that *IDH* mutation status and gene expression profiling provide prognostic information that extends beyond that provided by WHO classification and other prognostic biomarkers such as 1p/19q chromosomal codeletion and methylation of the promoter region of the methylguanine methyltransferase (*MGMT*) gene. It is becoming increasingly evident that diffuse gliomas can be meaningfully separated into prognostic groups based on molecular profiling. In this review, the clinical, diagnostic, and therapeutic implications of an emerging molecular classification of diffuse gliomas are provided.

1P/19Q CODELETION The pathologic criteria for classifying diffuse gliomas as "pure" astrocytoma oligodendroglioma or mixed oligoastrocytoma are not universally agreed upon, and are prone to significant subjectivity and interobserver variability.[10] This can lead to poor reproducibility, diagnostic uncertainty, and perhaps explain the variability of treatment patterns observed in day-to-day clinical practice.[11] Thus, there is an opportunity to integrate molecular classifiers to better delineate tumor subtypes with more uniform outcomes.

Unbalanced translocation of chromosomes 1 and 19 with deletion of 1p and 19q (1p/19q codeletion) is present in 70% or more of oligodendroglial tumors. Using strict histologic criteria for classifying oligodendroglial and astrocytic tumors, the proportion of LGOs with 1p/19q codeletion may be over 90%.[7] Historically, oligoastrocytomas have rates of *TP53* mutation, 1p/19q codeletion, and survival outcomes intermediate between astrocytomas and oligodendrogliomas.[12] However, due to the difficulty in reproducibly diagnosing oligoastrocytoma, 1p/19q codeletion is often considered to be the objective molecular definition of oligodendroglial lineage, with tumors that lack 1p/19q codeletion considered astrocytic. This approach is strengthened by the observation that *TP53* mutation, a marker of astrocytic lineage, and 1p/19q codeletion are mutually exclusive in the vast majority of cases.[13] GB with oligodendroglial features (GBO) is a WHO-recognized GB variant[2]; however, this entity remains controversial, and is poorly reproducible, similar to mixed oligoastrocytomas.[14,15]

1p/19q codeletion is associated with improved prognosis in LGGs and AGs regardless of treatment modality and is a reproducible prognostic biomarker.[6,16–19] In a retrospective study, a trend toward improved survival outcomes in AOs with 1p/19q codeletion treated with PCV (procarbazine, CCNU, vincristine) compared to temozolomide (TMZ, Temodar, Merck & Co., NJ) was reported.[4] Long-term follow-up data from the European Organisation for Research and Treatment of Cancer (EORTC)[5] and Radiation Therapy Oncology Group (RTOG)[20] trials testing radiotherapy vs radiotherapy plus adjuvant or neoadjuvant PCV in AOs were recently presented and the results suggest that 1p/19q codeletion is both prognostic and predictive of improved outcomes with PCV chemotherapy.[21,22] Given the range of survival outcomes and challenge of reproducibly classifying astrocytomas, mixed oligoastrocytomas, and oligodendrogliomas, 1p/19q codeletion has become an important biomarker in the day-to-day management of LGGs and AGs.

MGMT PROMOTER METHYLATION O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that repairs O^6 alkyl guanine adducts. The 5′ promoter region of *MGMT* contains a CpG island, and methylation of CpG islands in the *MGMT* promoter region results in epigenetic silencing of gene transcription. The DNA repair mechanism of MGMT and the cytotoxic effects of TMZ overlap as TMZ alkylates the O^6 position on guanine.[23]

In a retrospective study of the EORTC-NCIC trial of concurrent chemoradiotherapy plus adjuvant TMZ vs radiotherapy, 45% of patients with available tissue had *MGMT* promoter methylation. These patients had significantly prolonged OS compared to patients with unmethylated *MGMT* promoter regions (18.2 vs 12.2 months). *MGMT* promoter methylation was associated with improved survival outcomes regardless of treatment arm. In the TMZ treatment arm, 46% of patients with a methylated *MGMT* promoter survived 2 years, vs 13% of unmethylated patients. In the radiotherapy arm, 22% of patients with a methylated *MGMT* promoter survived 2 years, vs less than 2% of patients with unmethylated tumors.[24] A subsequent retrospective study found 30% of patients with GB with *MGMT* promoter methylation treated with upfront radiotherapy alone survived 2 years, vs only 16% for patients with unmethylated tumors treated similarly.[25] In EORTC 26951 (radiotherapy vs radiotherapy plus procarbazine, CCNU, and vincristine chemotherapy), *MGMT* promoter methylation was associated with improved outcomes in patients with AO treated with radiotherapy alone or radiotherapy plus chemotherapy.[26]

MGMT promoter methylation was a stratification factor in RTOG 0525 and 0825; both trials tested standard upfront chemoradiotherapy plus adjuvant TMZ vs dose-intense TMZ or TMZ plus bevacizumab (Avastin, San Francisco, CA). In RTOG 0525, *MGMT* promoter methylation was prospectively confirmed as prognostic in patients treated with standard or dose-intense TMZ. As TMZ is an MGMT substrate, it was theorized that dose-intense regimens could overwhelm the DNA repair capacity of MGMT. However, *MGMT* promoter methylation was not associated with improved outcomes in the dose-intense TMZ treatment arm in patients with or without *MGMT* promoter methylation.[27]

In a nonrandomized, phase II trial of patients with newly diagnosed GB 70 years and older with Karnofsky performance scores 70 or less treated with standard dosing of TMZ without radiotherapy, *MGMT* promoter methylation was associated with significantly prolonged progression-free and overall survival.[28] A phase III trial, NOA-08, randomized patients with GB over age 65 to either TMZ or radiotherapy alone after surgical resection. *MGMT* promoter methylation was associated with improved survival outcomes in the TMZ-treated patients and patients with unmethylated tumors had improved outcomes when treated with radiotherapy.[29] These results suggest *MGMT* promoter methylation may be a predictive biomarker in certain patient subsets.

The role of *MGMT* promoter methylation as a predictive biomarker of temozolomide sensitivity is controversial. The accumulated evidence clearly supports *MGMT* promoter methylation as a prognostic marker of improved survival outcomes. *MGMT* promoter methylation is frequently associated with other prognostic biomarkers: 1p19q codeletion, *IDH* mutations, gene expression, and DNA methylation signatures. Promoter methylation may be an epiphenomenon related to these or other factors ultimately mediating improved survival outcomes.

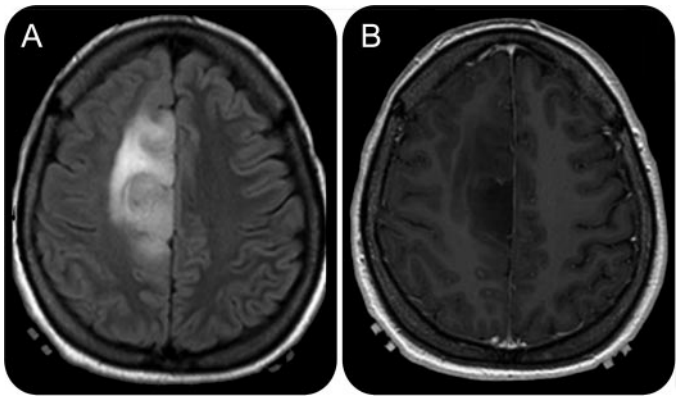
ISOCITRATE DEHYDROGENASE MUTATIONS

Using genome-wide sequencing, *IDH1* mutations were found in 18/149 (12%) of GB samples. These patients were noted to have prolonged overall survival compared to patients with wild-type *IDH1*.[30] A subsequent analysis of 445 glioma tissue samples (grades I to IV), medulloblastomas, and 494 non-CNS tumor samples found *IDH1* or *IDH2* mutations in over 80% of grade II and III oligodendrogliomas and astrocytomas, *IDH1* mutations in 80% of secondary GBs, and no *IDH1* or *IDH2* mutations in non-CNS solid tumors.[31] *IDH* mutations, especially $IDH1^{R132H}$, appear to be unique to gliomas. *IDH1* or *IDH2* mutations have also been discovered in a subset of cytogenetically normal acute myelogenous leukemia (AML), intrahepatic cholangiocarcinoma, and central cartilaginous neoplasms.[32–34] The discovery of *IDH* mutations is novel as this metabolic pathway was not previously implicated in oncogenesis.[35] IDH catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, generating NADPH. Glial cells have high baseline levels of α-ketoglutarate due to uptake of glutamate and glutamine, which are ultimately metabolized to α-ketoglutarate.[35,36]

Whether mutant IDH results in a loss of tumor suppressor function or acts as an oncogene is a source of intense research. The exact mechanisms associated with tumorigenesis and improved prognosis are yet to be defined. Mutant IDH enzyme produces decreased cytoplasmic levels of α-ketoglutarate and NADPH. Resulting decreased cytosolic α-ketoglutarate may stabilize hypoxia inducible factor-1α (HIF-1α) facilitating cellular proliferation.[35,36]

Alternatively, mutant IDH1 may be oncogenic by a gain of neomorphic enzymatic activity and the ability to convert α-ketoglutarate to D-2-Hydroxyglutarate (2HG).[35] 2HG may act as an oncometabolite by antagonizing α-ketoglutarate-dependent enzymes such as ten-eleven-translocation 2 (TET2), which is responsible for histone and DNA methylation.[37] *IDH1* mutation appears to be a necessary molecular event to establish the glioma hypermethylation phenotype, discussed subsequently.[38] 2HG is found in micromolar cytoplasmic concentrations of mutated cells and provides

Figure 1 Representative MRI of an *IDH1* mutant glioblastoma



Fluid-attenuated inversion recovery sequence (A) of an *IDH1* (R132H) mutant glioblastoma (mutation positive on immunohistochemistry and sequencing of *IDH1* gene) which is located in the medial frontal lobe and does not enhance with gadolinium (B).

a tumor-specific biomarker. Noninvasive means of diagnosis and monitoring treatment response may be possible as 2HG is detectable on magnetic resonance spectroscopy.[39,40]

Primary GBs make up the majority of grade IV tumors while secondary GBs account for 10% or less.[3] *IDH1* mutations are found in 73%–88% of secondary GBs, but only 3%–7% of primary GBs.[3,41] GBs with *IDH1* mutations are phenotypically and genotypically distinct.[42] Over 60% of *IDH1* mutant GBs are localized in the frontal lobe and the peak incidence occurs in the third decade of life. *IDH1* mutant GBs share radiographic features with grade II and III gliomas. *IDH1* mutant GBs are often nonenhancing, associated with a lesser extent of edema, and often have cystic or diffuse components more often than *IDH1* wild-type GBs (figure 1). *IDH1* mutant GBs also have a higher frequency of *MGMT* promoter methylation and p53 mutation. Overall survival ranges from 24 to 36 months in *IDH1* mutant vs 9 to 15 months in *IDH1* wild-type GBs.[42–45] *MGMT* promoter methylation is 3 to 4 times more common than *IDH1* mutation in GBs, and the relative contribution of each to prognosis has not been compared prospectively.

IDH1 or *IDH2* mutations occur in 70% or more of LGGs and AGs.[31] The majority of LGGs with *TP53* mutations or 1p/19q codeletion have *IDH* mutations.[46] *IDH2* mutations are rare and occur mainly in oligodendrogliomas.[31] A total of 90% to 100% of 1p/19q codeleted gliomas also harbor *IDH1* or *2* mutations.[46,47] *IDH* mutations are strongly associated with 1p19q codeletion and *MGMT* promoter methylation in AOs.[19] *IDH* mutations have a robust statistical effect on survival outcome in LGGs and AGs.[18,45,48] When controlling for factors such as age, extent of resection, *MGMT* promoter methylation, and 1p/19q codeletion, only *IDH1* mutation was significantly associated with OS in a retrospective study of LGGs from the German Glioma Network.[18]

IDH wild-type LGGs and AGs have inferior survival outcomes compared to *IDH*-mutated tumors. Although comprising only a minority of LGGs, *IDH* wild-type tumors have a significantly worse prognosis compared to *IDH*-mutated LGGs, with a median PFS of only 1.4 years (vs 4.7 years), and with only 14% of patients surviving 5 years (vs 42%) in 1 study; a second study showed an OS of 150 vs 60 months for *IDH1*-mutated vs wild-type tumors.[18,49] *IDH* wild-type LGGs are often "triple-negative," lacking 1p/19q codeletion, *TP53* mutation, and *IDH* mutation.[49] In 1 study of 382 patients with AA or GB, *IDH1* mutation was the most prominent prognostic factor, followed by age, histology (AA or GB), and *MGMT* promoter methylation status, suggesting that *IDH1* mutation may be a more significant prognostic factor than histologic classification as either AA or GB.[44]

MULTIGENE SETS The explosion of genomic datasets made available by high-throughput microarray technologies have revolutionized understanding of different types of cancer. Global genomic profiling is a promising approach for developing molecular subtype classifications, multigene clinical predictors, new target identification, and predictive markers for targeted therapeutics. The most well-known project, the Cancer Genome Atlas (TCGA), encompasses DNA, mRNA, microRNA, and epigenetic profiling (DNA methylation).[50] Recent gene expression profiling has revealed multiple glioma subtypes with different clinical outcomes.

The first study using multigene sets to define molecular subtypes of high grade gliomas discovered 3 subtypes: proneural, mesenchymal, and proliferative.[51] A similar analysis of the TCGA dataset confirmed the proneural and mesenchymal subtypes, and also identified neural and classic subtypes.[52] The proneural subtype is associated with PDGFR amplification, *IDH1* mutations, and overexpression of genes related to neural and glial development. The mesenchymal subtype is associated with increased expression of angiogenic peptides, neurofibromatosis 1 gene (*NF1*) loss or mutation, and overexpression of genes related to motility, the extracellular matrix, and cell adhesion. The TCGA neural and classic subtypes are associated with epidermal growth factor receptor (EGFR) mutation or amplification, and the classic subtype is associated with PTEN loss. Tumor grade is associated with the proneural and mesen-

Table 1 Gene expression profile, characteristic mutations, and survival of glioblastoma subtypes[a]

	Genetic/epigenetic profile		Characteristic gene mutations	Percent of all GB (approximate)	Median OS, mo
	DNA meth	Gene exp			
Glioblastoma (group 1)	G-CIMP (+)	Proneural[b]	*IDH1 mut*[b]	10	36-48[c]
Glioblastoma (group 2)	G-CIMP (−)	Mesenchymal	*NF-1 mut*	30	12[c,d]
Glioblastoma (group 3)	G-CIMP (−)	Neural, classical, proneural[e]	*EGFR mut*	60	15[c,d]

Abbreviations: DNA meth = DNA methylation profile; *EGFR mut* = epidermal growth factor receptor gene mutation; G-CIMP = CpG hypermethylator phenotype; GB = glioblastoma; gene exp = genetic expression profile; *IDH1 mut* = isocitrate dehydrogenase 1 gene mutation; *NF-1 mut* = neurofibromatosis type 1 gene mutation; OS = overall survival.
[a] Glioblastoma subtypes separated into 3 groups based on DNA methylation and gene expression profile. Characteristic gene mutations enriched within each group are annotated. Group 1 has markedly improved survival outcomes compared to groups 2 and 3.
[b] A small percentage of G-CIMP (+) GBs have non-proneural genetic signatures or are *IDH1* wild-type.
[c] References 42 and 57.
[d] References 51 and 52.
[e] Non-mesenchymal, G-CIMP (−) GBs including proneural, neural, and classical genetic subtypes have similar outcomes to mesenchymal GBs.

chymal subtypes. Grade II and III diffuse gliomas are predominantly proneural while GBs represent a mix of all subtypes. At recurrence, some proneural tumors undergo a proneural to mesenchymal gene expression transformation.[51]

The proneural and mesenchymal subtypes are the best characterized across studies and are differentiated by clinical outcomes. Proneural GBs have improved survival outcomes compared to GBs with mesenchymal signatures. The other subtypes (e.g., neural, classic) are not clearly associated with different survival outcomes. However, the importance of differentiating among these subtypes may lie in the enrichment of characteristic gene mutations or amplified signaling pathways. For example, the enrichment of *NF1* mutations in mesenchymal GBs and EGFR mutations in classic GBs may be important for designing personalized clinical trials in molecularly selected patient populations.[51,52]

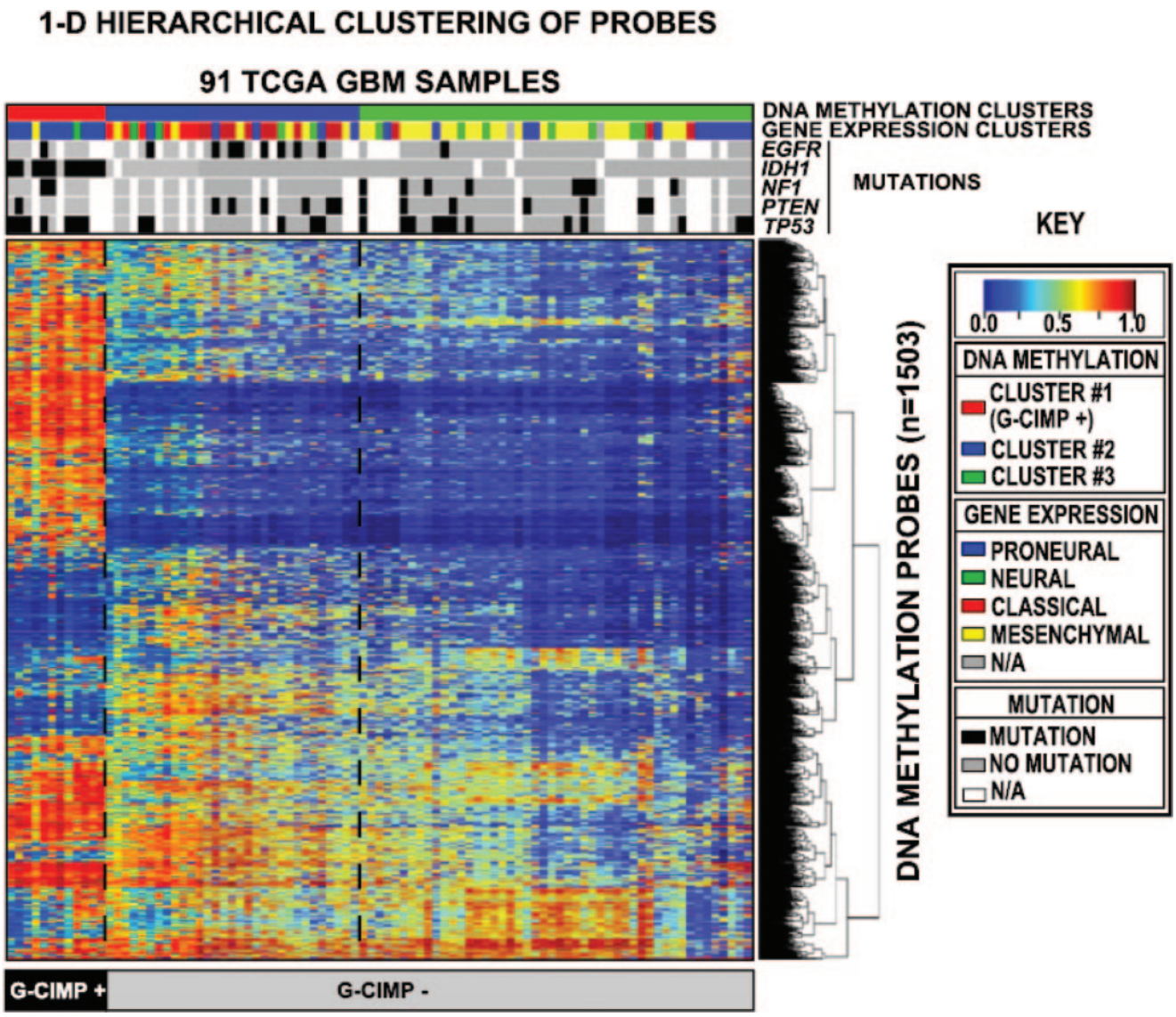
GBs with a mesenchymal genetic signature overexpress genes associated with a glioma stem cell (GSC) phenotype.[53] GSCs are intrinsically resistant to radio- and cytotoxic chemotherapy; a stem-cell like phenotype may account for a proportion of the patients with poor response to the current standard of care in newly diagnosed GB.[54] Targeting pathways that regulate mesenchymal and GSC phenotypes, such as STAT3, TGF-β, and CEBP-β/δ,[55,56] may be more effective strategies in mesenchymal GBs.

Changes in DNA methylation are a hallmark of some cancers with global hypomethylation alternating with hypermethylation of CpG islands located in gene promoter regions. A study from the TCGA evaluated CpG island methylation patterns in gliomas. A distinct CpG island hypermethylator phenotype (G-CIMP) was found (figure 1). G-CIMP tumors are seen in younger patients and make up the majority of WHO grade II and III gliomas. These patients experience significantly improved outcomes compared to non-G-CIMP tumors.[57]

G-CIMP GBs have improved survival outcomes.[42,57] *IDH1* mutant and proneural GBs appear to segregate almost exclusively into the G-CIMP phenotype (table 1, figure 2). G-CIMP status further separates clinical outcomes in proneural GBs; G-CIMP, proneural GBs have improved survival outcomes compared to non-G-CIMP, proneural GBs, which have similar survival outcomes to other GB subtypes.[57] G-CIMP, proneural GBs with *IDH1* mutations appear to maintain their G-CIMP status at recurrence and do not undergo proneural to mesenchymal transformation.[42,57] A few G-CIMP GBs have nonproneural genetic signatures or are *IDH1* wild-type; these rare exceptions are challenging with regard to both classification and prognostication. Given the clustering of positive prognostic biomarkers in G-CIMP GBs (table 1, figure 2), G-CIMP status may be the most robust prognostic biomarker in GB; this assertion requires prospective evaluation.

Similar to GBs, DNA methylation and genetic signature can identify LGGs and AGs with improved prognoses. In 2 retrospective studies, proneural AOs and LGOs had improved outcomes compared to oligodendrogliomas with nonproneural gene expression.[58,59] The accumulated evidence suggests that oligodendrogliomas with 1p/19q codeletion and *IDH* mutations typically also have *MGMT* promoter methylation, a proneural genetic signature, and a CpG island hypermethylator phenotype.[47,60] A retrospective analysis of EORTC 26951 (radiotherapy plus or minus adjuvant chemotherapy in AOs) found G-CIMP status to be the most robust prognostic factor when considering clinical features, 1p/19q codeletion, *IDH* mu-

Figure 2 Identification of the CpG island methylator phenotype in glioblastoma



DNA methylation from 91 TCGA glioblastomas was profiled and subjected to unsupervised clustering (statistical methodology, reference 57). Three distinct methylation clusters were identified, with cluster 1 (red top bar) designated as G-CIMP+ due to a high frequency of DNA methylation. The gene expression profile and mutation status of selected genes (*EGFR*, *IDH1*, *NF1*, *PTEN*, and p53) are shown in the bars below each methylation cluster. *IDH1* mutations were found exclusively in G-CIMP+ tumors, and nearly all G-CIMP+ tumors had a proneural gene expression profile. Adapted from Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17: 510–522; with permission from Elsevier. EGFR = epidermal growth factor receptor; G-CIMP = CpG hypermethylator phenotype; GBM = glioblastoma multiforme; *IDH1* = isocitrate dehydrogenase 1 gene; *NF1* = neurofibromatosis 1 gene; *PTEN* = phosphatase and tensin homologue gene; TCGA = The Cancer Genome Atlas; *TP53* = p53 gene.

tation status, and *MGMT* promoter methylation status.[60]

Important practical considerations limiting widespread application of gene expression profiling include feasibility outside of a research setting and cost effectiveness. A 38-gene profile consistently associated with patient outcome was further refined to a 9-gene set prognostic of outcome in GB after controlling for clinical factors and *MGMT* promoter methylation.[53] The 9-gene profile can be performed on formalin-fixed paraffin-embedded tissue and conceptually represents a multigene tool with broad applicability. An important question to answer prospectively is the relative contributions of G-CIMP status, genetic subtype, *IDH1* mutational status, and *MGMT* promoter methylation to prognosis.

MOLECULAR CLASSIFICATION OF DIFFUSE GLIOMAS The accumulated data support molecular stratification of LGGs and AGs using first 1p19q codeletion and then *IDH* mutation status in 1p/19q intact patients. This separates both LGGs and AGs

Table 2 Molecular classification and survival of low-grade and anaplastic gliomas[a]

	WHO grade	Prognostic biomarkers: 1p/19q codel	Prognostic biomarkers: *IDH1* or *2 mut*	Median OS, y
Low-grade glioma (group 1)	2	(−)	(−)	≤5[b]
Low-grade glioma (group 2)	2	(−)	(+)	>7[c]
Low-grade glioma (group 3)	2	(+)	(+)	>12[c]
Anaplastic glioma (group 1)	3	(−)	(−)	≤2[d]
Anaplastic glioma (group 2)	3	(−)	(+)	>5[d]
Anaplastic glioma (group 3)	3	(+)	(+)	>10[e]

Abbreviations: Codel = chromosomal codeletion; *IDH1* or *2 mut* = isocitrate dehydrogenase 1 or 2 gene mutation; OS = overall survival.
[a] Classification of diffuse gliomas based on WHO grade and prognostic biomarkers. Low grade and anaplastic gliomas are divided into groups 1, 2, 3 based on biomarker characteristics. Median OS was estimated based on the current literature. Due to prolonged survival times, median OS has not been reached in many studies of 1p/19q codel or *IDH1* or *2 mut* grade II and III diffuse gliomas. 1p/19q codel, low grade, and anaplastic gliomas have the best outcomes, and are nearly always mutated on *IDH1* or *2*. Low grade and anaplastic gliomas intact on 1p/19q and wild type on *IDH1* and *2* have markedly inferior survival rates.
[b] References 45–48.
[c] References 18, 47, and 48.
[d] References 44, 45, and 47.
[e] References 5, 21, and 47.

into 3 groups with different prognoses (table 2). Molecular stratification in prospective trials using 1p/19q codeletion status, *IDH* mutation status, and possibly other molecular biomarkers (G-CIMP status, genetic subtype) would be the ideal means of evaluating a classification scheme including these biomarkers.

Molecular classification of LGGs and AGs has become standard at our institution; diffuse glioma cases are reported by our neuropathologists using this general format:

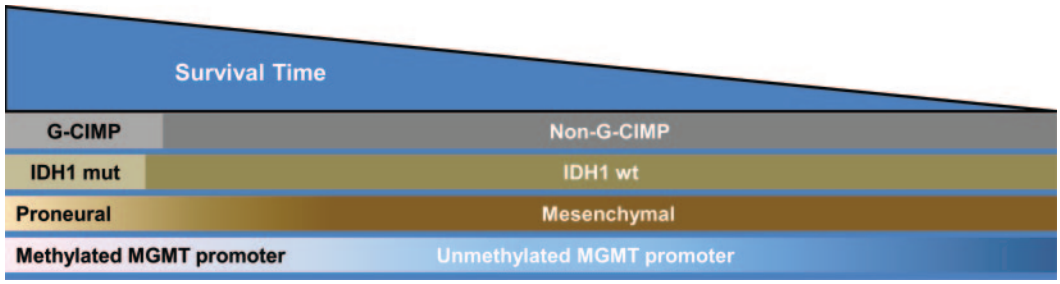
DIFFUSE GLIOMA
WHO GRADE II
IDH: IDH1 R132H MUTANT PROTEIN-POSITIVE
1p/19q status: CO-DELETED

Other pathologic features, including morphologic characteristics such as oligodendroglial or astrocytic phenotype, the results of other relevant immunohistochemical (IHC) stains or diagnostic molecular tests (e.g., p53, EGFR amplification, proliferation indices), and the method of molecular feature determination (e.g., FISH, IHC, CGH) are included in a Comments section. Receiving this data routinely from our neuropathologists informs both clinical decision-making and patient counseling. This format also allows for continued evolution of molecular classification as new robust markers are verified.

Results from ongoing phase II and III clinical trials in 1p/19q codeleted and 1p/19q intact LGGs and AGs are anxiously awaited to determine the best therapeutic approach for astrocytic vs oligodendroglial tumors with or without 1p/19q codeletion. Despite inferior outcomes in 1p/19q intact, *IDH* wild-type tumors (table 2), appropriate treatment has not been established. Combined treatment regimens, including radiotherapy, concurrent chemoradiotherapy, or adjuvant chemotherapy, have not been proven to improve survival or have acceptable rates of toxicity.

Figure 3 (see also table 1) demonstrates the relative frequency, overlap, and association with survival

Figure 3 Frequency, overlap, and relative survival glioblastomas (GBs) (includes GB and all GB variants including gliosarcoma) based on molecular profile



Relative frequency of G-CIMP status, gene expression profiles, *IDH1* mutation, and *MGMT* promoter methylation in GBs. *IDH1* mutation and G-CIMP are depicted as discrete categories while gene expression and *MGMT* methylation status are depicted as a continuum. GBs with PN gene expression profiles and *IDH1* mutations cluster almost exclusively within G-CIMP GBs and have improved clinical outcomes (left side of figure, see also table 1). Mesenchymal GBs are exclusively non-G-CIMP and *IDH1* wild-type and have inferior clinical outcomes (see also table 1). G-CIMP = CpG hypermethylator phenotype; *IDH1 mut* = isocitrate dehydrogenase 1 gene mutation; *IDH1 wt* = isocitrate dehydrogenase 1 wild-type gene.

outcomes between gene expression profile, DNA methylation signature, *IDH1* mutation, and *MGMT* promoter methylation in GB. Incorporating these biomarkers into clinical trials may prevent over- or underestimating benefit of a particular agent by balancing GBs with different molecular profiles in different treatment arms. This paradigm may also allow determination of benefit in specific subgroups of patients with a particular molecular profile. Extrapolation from the genes and pathways differentially activated will facilitate rational clinical trial design of targeted therapeutics in molecularly defined patient subgroups. In an application of this molecular-based paradigm, the 9-gene profile[53] was integrated into the stratification of the ongoing phase III clinical trial RTOG 0825 (TMZ vs TMZ plus bevacizumab) in newly diagnosed GB.

A minority, approximately 10%, of GBs have a favorable biomarker profile, including proneural gene expression, G-CIMP phenotype, *IDH1* mutation, and *MGMT* promoter methylation (table 1). The majority of GBs, however, are non-G-CIMP, nonproneural, *IDH1* wild-type, and are not separated by clinical outcomes (table 1). There is no alternative treatment for patients with a favorable or unfavorable molecular profile. The current standard of care should be routinely administered to all patients with GB unless within the confines of a clinical trial. At present, the technology to profile gene expression and DNA methylation is expensive and not widely available. Further development and prospective validation of molecular profiles capable of classifying GB with relevance to both prognosis and treatment is necessary before widespread application is justified.

DISCUSSION Molecular profiling, including gene expression, DNA methylation, key mutations, and cytogenetic events can separate diffuse gliomas into prognostic groups. Incorporation of this information when classifying diffuse gliomas will better account for the clinical, pathologic, and molecular heterogeneity observed among all tumor grades. Targeting patients with poor prognosis or specific molecular profiles will become increasingly feasible if such a classification can be standardized. Significant challenges remain. Further stratification by molecular means (e.g., separating LGGs into 3 groups as in table 2) will potentially shrink the number of patients available for clinical trials. Prospective studies with large groups of patients requiring multi-institution collaboration will be necessary to effectively validate prognostic biomarkers before more widespread inclusion into a classification scheme is warranted. Collaboration will be even more important to design the next generation of clinical trials in select groups of patients with similar molecular profiles.

AUTHOR CONTRIBUTIONS

Brett J. Theeler wrote and revised the manuscript. Gregory N. Fuller revised the manuscript. W.K. Alfred Yung revised the manuscript. John F. De Groot wrote and revised the manuscript.

DISCLOSURE

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