

Updates in prognostic markers for gliomas

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Abstract

Gliomas are the most common primary malignant brain tumor in adults. The traditional classification of gliomas has been based on histologic features and tumor grade. The advent of sophisticated molecular diagnostic techniques has led to a deeper understanding of genomic drivers implicated in gliomagenesis, some of which have important prognostic implications. These advances have led to an extensive revision of the World Health Organization classification of diffuse gliomas to include molecular markers such as isocitrate dehydrogenase mutation, 1p/19q codeletion, and histone mutations as integral components of brain tumor classification. Here, we report a comprehensive analysis of molecular prognostic factors for patients with gliomas, including those mentioned above, but also extending to others such as telomerase reverse transcriptase promoter mutations, O⁶-methylguanine-DNA methyltransferase promoter methylation, glioma cytosine-phosphate-guanine island methylator phenotype DNA methylation, and epidermal growth factor receptor alterations.

Key words

gliomas | molecular markers | prognostic tools

Gliomas have an incidence of approximately 5 cases per 100 000 persons.¹ Historically, gliomas have been classified according to the microscopic, immunohistochemical, and ultrastructural similarity of the tumor cells with the presumed normal cells from which they arose.

Grading has been based on features such as mitoses, necrosis, and microvascular proliferation. The combination of histologic features and tumor grade has provided important prognostic information. In adults, diffuse gliomas have been categorized into the diagnoses of astrocytoma (World Health Organization [WHO] grade II or III), oligodendroglioma (WHO grade II or III), and glioblastoma (WHO grade IV). The overall survival (OS) for grades II, III, and IV astrocytomas is approximately 6–8 years, 2 years,

and 15 months, respectively. In addition to the association with poorer prognosis, higher tumor grade is correlated with more advanced age. Increasingly, genetic biomarkers have become essential components of integrated pathologic diagnoses, and their use has transformed the paradigm of brain tumor classification.

In 2016, the WHO classification schema for adult gliomas was significantly updated to incorporate important new findings on the genomics of diffuse gliomas. In this update, the approach to brain tumor classification was expanded to include both histopathologic and molecular features,² thus integrating phenotypic and genotypic information. Unlike prior editions of the WHO classification, molecular information is now considered integral to the definition of adult

gliomas. In particular, the revised edition now requires integrated pathologic diagnoses that include information about the mutation status of the isocitrate dehydrogenase (*IDH*) genes and about 1p/19q codeletion. Currently, adult gliomas generally fall into 3 major groups: *IDH* mutant with 1p/19q codeletion, *IDH* mutant with 1p/19q intact, and *IDH* wildtype.

In recent years, additional molecular alterations have been discovered that are likely to have important clinical implications for glioma prognostication. To handle the accelerating pace of scientific discovery and the need for clarification and new guidelines for practicing diagnosticians between WHO updates, a consortium has been established, cIMPACT-NOW³ (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy), to facilitate consensus review of new diagnostically relevant information and determining how such information should be used in clinical practice and how it can be incorporated into future updates of the WHO CNS tumor classification.

Molecular Prognostic Markers

IDH Mutation

Recurrent mutations in the metabolic gene *IDH1* were discovered initially in the context of a genomic evaluation of glioblastoma (GBM),⁴ in which a heterozygous point mutation changed arginine to histidine at amino acid 132 in approximately 20% of the tumors analyzed. Further work demonstrated that mutations at R132 can be detected in up to 80–90% of grade II and III gliomas, including both astrocytic and oligodendroglial subtypes, and in a small subset of GBMs. While the vast majority of mutations involve the R132H substitution, noncanonical variants including R132C, R132S, R132G, and R132L also occur.⁵ The mutations tend to be found in younger patients.⁶ Much less commonly, in less than 1% of *IDH* mutant gliomas, a mutation of the related *IDH2* gene has been observed at an analogous arginine, amino acid 172.^{5,6} *IDH2* mutations tend to occur in oligodendrogliomas.

IDH1 and *IDH2* are NADP⁺-dependent metabolic enzymes that are critical for the oxidative carboxylation of isocitrate to alpha-ketoglutarate, with *IDH1* functioning in the cytoplasm and *IDH2* working within the context of the Krebs cycle within the mitochondria. Mutation at R132 (or R172 in *IDH2*) results in the acquisition of a neoenzymatic activity that promotes conversion of alpha-ketoglutarate to R-2-hydroxyglutarate (2-HG).⁷ This change leads to accumulation of exceedingly high levels of the 2-HG metabolite which can promote tumorigenesis. Tumor evolution studies suggest that mutation of *IDH* is an early event in gliomagenesis.⁸

While a complete understanding of the mechanism of tumor promotion is still under study, the supra-physiologic levels of 2-HG detected in *IDH* mutant glioma are implicated in the inhibition of a number of 2-oxoglutarate (2-OG)-dependent enzymes. These proteins include hydroxylases, histone demethylases, and DNA repair enzymes which have pleiotropic effects, including effects on cellular epigenetic state.⁹ Consistent with these effects, *IDH* mutant gliomas exhibit a typical pattern of hypermethylation of cytosine-phosphate-guanine (CpG) islands, commonly referred to

as the CpG island methylator phenotype (CIMP).^{10,11} The epigenetic patterns promoted by the presence of the *IDH* mutation is proposed to lock glioma cells in a less differentiated or stem cell–like state, thereby rendering them prone to acquisition of additional genomic alterations that are known to promote tumorigenesis, including *TP53* mutation and loss of chromosome arms 1p and 19q.^{9,12,13}

Of all of the known glioma-associated molecular alterations discovered to date, the presence or absence of an *IDH* mutation has the largest prognostic significance. *IDH* mutations are noted in the vast majority of grade II and grade III gliomas,⁶ which are associated with improved survival compared with GBM. Further, there is evidence from retrospective analyses that *IDH* mutation status is as strong a predictor of survival as histologic grading that differentiates high-grade astrocytomas. In a retrospective analysis of nearly 400 anaplastic astrocytomas (AAs) and GBMs, the presence of an *IDH* mutation more strongly predicted OS than did histologic grade. The authors demonstrate in a multivariate model incorporating age, *IDH* mutation status, extent of surgical resection, histologic diagnosis, and *MGMT* status that the presence of *IDH* mutation was the dominant prognostic factor (relative risk, 2.7; 95% CI: 1.6–4.5). Simultaneous evaluation of histology and *IDH* mutation by Kaplan–Meier analysis revealed improved progression-free survival (PFS) and OS for both *IDH* mutant AA and GBM compared with *IDH* wildtype AA and GBM.¹⁴

There is a relationship between the presence of an *IDH* mutation and improved prognosis in grade II and grade III diffuse glioma as well. When 271 grade II diffuse gliomas were evaluated based on a number of clinical and molecular parameters, mutation of *IDH* was significantly associated with increased OS on univariate analysis ($P = 0.002$), as well as in a multivariate model incorporating known clinical factors such as age, performance status and extent of resection, and chromosome 1p/19q status ($P = 0.003$).¹⁵ Likewise, in an analysis of 552 tumors that consisted of both grade II and grade III gliomas, *IDH* mutant gliomas exhibited prolonged OS compared with *IDH* wildtype gliomas, regardless of grade. Indeed, only a modest effect of grade on OS was observed in the *IDH* mutant tumors analyzed.¹⁶

The favorable prognostic profile of *IDH* mutations was also demonstrated in a comprehensive genomic analysis of 293 grade II and grade III gliomas performed by The Cancer Genome Atlas (TCGA) Research Network. Patients without an *IDH* mutation exhibited a significantly shorter OS compared with those with an *IDH* mutation, with a median of 1.7 years for *IDH* wildtype glioma patients. This is in contrast to an OS of 6.3 years for patients with *IDH* mutation and no 1p/19q codeletion (astrocytic gliomas) or 8 years for patients with *IDH* mutation and 1p/19q codeletion (oligodendroglial gliomas).¹⁷ Additionally, in a recent study of grade III glioma patients treated with radiotherapy and either temozolomide (TMZ) or nitrosourea, *IDH* mutation status was found to be a significant prognostic factor for both OS (hazard ratio [HR] 0.42) and PFS (HR 0.59).¹⁸

Low-grade gliomas that are wildtype for *IDH* mutation were found to be genomically heterogeneous and to have independent prognostic factors.¹⁹ Out of 718 grade II and III gliomas that were genotyped for *IDH* mutations, 166 wildtype cases were found. These tumors were genotyped for epidermal growth factor receptor (*EGFR*) and

Table 1 Summary of prognostic molecular markers in glioma

Molecular Marker	Functional Significance	Frequency	Prognostic Value	Diagnostic Evaluation
<i>IDH</i> mutations	Lead to accumulation of oncometabolite 2-HG ⁷ , which inhibits 2-OG dependent enzymes and alters epigenetic state	80–90% grade II and III gliomas, 12% of GBMs	Favorable prognosis <i>Grades II–III</i> : HR 7.5 for <i>IDH</i> mutant vs <i>IDH</i> wildtype tumors <i>Grades III–IV</i> : RR 2.7 ¹⁴	Routinely performed IHC, sequencing for IHC-negative samples, imaging
<i>1p/19q</i> codeletion	Deletion of tumor suppressor genes, candidates include <i>CIC</i> ³³	Defines tumors of oligodendroglial lineage	Favorable prognosis and predictive of response to chemotherapy <i>Grade II</i> : PFS 62 vs 48 vs 20 months for <i>IDH</i> mutant and <i>1p/19q</i> codeleted, <i>IDH</i> mutant alone and <i>IDH</i> wildtype respectively ³⁸ <i>Grade III</i> : Median OS 14.7 vs 2.6 years for codeleted and non-codeleted tumors treated with RT + chemotherapy ³⁹	Routinely performed FISH, aCGH, SNP arrays
<i>MGMT</i> promoter methylation	Silencing of <i>MGMT</i> expression, which mediates resistance to alkylating agents	40% of gliomas, more common in lower grade <i>IDH</i> mutant	Favorable prognosis and predictive of response to temozolomide <i>Grade IV</i> : Median OS 18.2 vs 12.2 months for methylated vs unmethylated tumors irrespective of treatment ⁴⁶ Median OS 21.7 vs 12.7 months for methylated vs unmethylated tumors among patients who received treatment with TMZ ⁴⁶	Routinely performed qMSP, IHC
G-CIMP methylation	Silencing of tumor suppressor genes and mismatch repair proteins	8.8% of GBMs 55% of <i>IDH</i> mutant gliomas	Favorable prognosis <i>IDH</i> mutant tumors: median OS 7.2 vs 2.7 years for G-CIMP high vs low respectively ⁶²	Not routinely performed
<i>TERT</i> promoter mutations	Reactivation of telomerase and telomere maintenance	80% of <i>IDH</i> wild type GBMs	Poor prognosis <i>Grades II–III</i> : HR 11.74 for <i>TERT</i> mutant only tumors vs tumors with <i>TERT</i> , <i>IDH</i> mutation and <i>1p/19q</i> codeletion ³⁴	Not routinely performed but detected in sequencing panels
<i>EGFR</i> alterations	Constitutive activation of <i>EGFR</i> pathway, involved in cell proliferation, apoptosis control, cell invasion	<i>EGFR</i> amplification: 40–50% of <i>IDH</i> wildtype GBMs <i>EGFR</i> vIII: 50% of <i>EGFR</i> -amplified tumors	High <i>EGFR</i> expression confers a poor prognosis <i>Grade IV</i> : HR 1.57 for high expressing tumors vs low expressing ones ⁸²	Not routinely performed but detected in sequencing panels
<i>BRAF</i> V600E mutations	Constitutive activation of MAPK pathway, which controls cell proliferation, differentiation, apoptosis and migration	10–15% of pilocytic astrocytomas, 5–10% pediatric gliomas, 34% of glioneuronal tumors, <2% adult gliomas	Favorable prognosis in young patients <i>All gliomas</i> : HR 0.51 for pediatric patients and 0.43 for younger adults (age <35) ⁹¹	Not routinely performed but detected in sequencing panels
Histone mutations, H3K27 mutation can occur in histone H3.1 or H3.3	Regulation of transcription mediated by reduction of H3K27 methylation	Defines diffuse midline gliomas, H3K27 mutant, predominantly pediatric	Poor prognosis <i>Pediatric gliomas</i> : median OS 1.04 vs 6.1 years for H3K27 mutant tumors vs wildtype ones ⁹⁴ <i>Adult gliomas</i> : median OS 19.6 months, comparable to <i>IDH</i> wildtype tumors ⁹⁶	Not routinely performed but detected in sequencing panels

Abbreviations: CIC, protein capicua homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B; MAPK, mitogen-activated protein kinase.

myeloblastosis (*MYB*) amplifications as well as telomerase reverse transcriptase (*TERT*) promoter, H3 histone family 3A (*H3F3A*), and proto-oncogene B-Raf (*BRAF*) mutations. The most favorable prognostic factor was found to be *MYB* amplification, whereas *EGFR* amplification and *H3F3A* mutation conferred an unfavorable prognosis.

The critical role of *IDH* mutation in prognostication has led to routine evaluation of this biomarker in all glioma samples, whenever possible. *IDH* mutations are clinically detected using immunohistochemistry (IHC) with a mutation-specific antibody.^{20,21} In a number of studies comparing IHC with sequencing, the concordance rate between these methods was found to be between 88% and 99%. The main reason for this discrepancy is that IHC fails to detect less frequent *IDH* mutations such as R132C, R132L, R132S, and R132G²² and those in *IDH2*. This prompts the need to evaluate IHC-negative samples using sequencing methods. The *IDH* status of a tumor can also be assessed using imaging techniques such as magnetic resonance spectroscopy,^{23–25} tissue-based analysis such as Raman spectroscopy²⁶ and Fourier-transform infrared spectroscopy,^{26,27} as well as intraoperative testing using mass spectrometry imaging^{26,28} or rapid genotyping assays.^{26,29}

1p/19q Codeletion

The association between heterozygous loss of the short arm of chromosome 1 and the long arm of chromosome 19 (1p/19q codeletion) and improved prognosis of glioma has been appreciated since the 1990s.^{30–32} The 1p/19q codeletion is found in tumors of the oligodendroglial lineage. Several candidate genes that may be lost with this alteration have emerged, including the homolog of *Drosophila capicua* (*CIC*), found to be mutated in up to 50% of oligodendroglial tumors.³³ *CIC* is a high mobility group box for transcriptional repression and promoter targeting. Loss of this tumor suppressor is thought in part to promote transcriptional programs that favor tumor growth.

Loss of 1p/19q co-occurs with mutation of *IDH*.³⁴ Patients with oligodendroglial tumors with this combination of molecular alterations tend to survive the longest of all patients of the glioma subtypes. One of the first studies to demonstrate the importance of 1p/19q codeletion on prognosis was a retrospective analysis of 125 tumors analyzed for the presence of 1p/19q codeletion, in which the median OS of codeleted tumors was 11.9 years (95% CI: 10.4 to not reached), significantly longer than the median survival of 8.1 years for patients with tumors that were 1p/19q intact (95% CI: 4.1–11.2).³⁵

This association of 1p/19q codeletion and prolonged OS has been observed in many other studies since that time, including in large genomic analyses of gliomas^{34,36} and prospective clinical trials. The Radiation Therapy Oncology Group (RTOG) trial 9802 was a large study undertaken to compare the efficacy of radiation treatment combined with a 3-drug chemotherapy regimen (procarbazine, lomustine, and vincristine [PCV]) to radiation treatment alone in patients with grade II gliomas who were deemed to be “high risk” and require adjuvant treatment. Notably, patients with the histologic subtype of oligodendroglioma experienced the longest PFS and OS on subgroup

analysis.³⁷ Although 1p/19q status was available for only a small number of tumors in the trial population, it is known from other studies that the majority of tumors with oligodendroglial histology have 1p/19q codeletion. In a large, phase III randomized study comparing TMZ with radiation therapy for treatment of patients with grade II glioma, the authors undertook an exploratory analysis based on the molecular features of *IDH* mutation and 1p/19q status. Patients with combined 1p/19q codeletion and *IDH* mutation enjoyed the longest PFS regardless of treatment, at 62 months (95% CI: 41–not reached) compared with 48 months (95% CI: 41–55) for *IDH* mutant alone and 20 months (95% CI: 21–26) for *IDH* wildtype.³⁸

The improved prognosis associated with 1p/19q codeletion has further been appreciated in grade III tumors as well. RTOG 9402, comparing PCV plus radiation treatment to radiation treatment alone in patients with grade III oligodendrogliomas and oligoastrocytomas, showed that patients with codeleted tumors lived significantly longer than others regardless of treatment group. In this trial, the median OS for patients with 1p/19q codeleted tumors was 14.7 years in the PCV plus radiation group and 7.3 years for radiation treatment alone, compared with 2.6 and 2.7 years for patients with non-codeleted tumors.³⁹ Altogether, these data support the concept that codeletion of 1p/19q is a prognostically favorable molecular marker associated with longer survival times.

In addition to being a favorable prognostic marker, 1p/19q deletion was found to be predictive of response to chemotherapy in oligodendroglial tumors. In the European Organisation for Research and Treatment of Cancer (EORTC) 26951 study, patients with anaplastic oligodendroglial tumors were assigned to receive radiation alone or in combination with 6 cycles of adjuvant PCV.⁴⁰ There was an overall benefit for combination therapy, which was higher for patients with 1p/19q codeleted tumors. Patients with codeleted tumors did not reach OS in the combination therapy arm, while OS was 112 months in the radiation only arm. For the non-codeleted cohort, OS was 25 months for combination therapy and 21 months for radiation alone. Similar results were seen in the RTOG 9402 trial, where patients with codeleted tumors derived a significantly larger benefit from chemotherapy compared with patients with wildtype tumors.³⁹

In addition to 1p/19q codeletion, other chromosomal copy number changes were found to correlate with prognosis in low-grade gliomas. For example, in a study of 231 low-grade gliomas, 25% of tumors were found to have loss of heterozygosity (LOH) on chromosome 9p and 14% had LOH on 10q.⁴¹ These alterations did not associate with each other. LOH at both of these sites correlated with a poor prognosis (HRs for PFS were 1.46 for 9p LOH and 1.49 for 10q LOH, while HRs for OS were 0.98 for 9p LOH and 2.53 for 10q LOH). Expanding further on the relationship between copy number variation (CNV) and prognosis, a recent retrospective study examining >300 astrocytic *IDH* mutant tumors (grades II–IV) noted a strong, significant association between high CNV load and prognosis. Notably, when the specific regions exhibiting CNV were analyzed, the strongest association with OS was observed for homozygous deletion of cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A/B*), found on chromosome

9p21, with IDH mutant patients with *CDKN2A/B* deletion living for much shorter times following diagnosis compared with those without the deletion.⁴²

As mentioned above, the 2016 WHO classification for nervous system tumors established molecular diagnostic requirements for some intracranial malignancies. For example, the presence of both an *IDH* mutation and the 1p/19q codeletion is necessary for a diagnosis of oligodendroglioma. Therefore, analysis of 1p and 19q is routinely done as part of clinical practice using fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH) microarrays, single nucleotide polymorphism (SNP) microarrays, or next-generation sequencing techniques.⁴³

MGMT Promoter Methylation

O⁶-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that removes alkyl groups from the O⁶ position of guanine, which is the critical site modified by alkylating chemotherapeutics.^{44,45} This activity allows MGMT to effectively reverse the damage induced by TMZ, the chemotherapy used as standard of care for GBM and frequently used in treatment of grade III and grade II gliomas. Therefore, high levels of MGMT activity can render tumors resistant to alkylating agents. Interestingly, approximately 40% of gliomas exhibit epigenetic modification of the *MGMT* gene promoter in the form of methylation, which leads to decreased MGMT expression and enhanced sensitivity to TMZ and other alkylating agents.⁴⁴

MGMT promoter methylation serves as both a predictive and prognostic molecular marker in glioblastoma. From the landmark clinical trial that established temozolomide and radiation as standard of care for GBM, methylation of the *MGMT* promoter was found to be a clear predictive biomarker for tumors that were most sensitive to treatment with TMZ.^{45,46} Interestingly, the investigators also noted that there was a significant difference in OS for patients with tumors exhibiting *MGMT* methylation compared with those without, regardless of treatment received. The median OS for patients with *MGMT* methylation was 18.2 months (95% CI: 15.5–22), compared with 12.2 months (95% CI: 11.4–13.5) in patients without methylated *MGMT*.⁴⁶ Analysis of the *MGMT* promoter methylation status of patients enrolled in RTOG 0525, which compared dose-dense TMZ administration to standard, monthly temozolomide, corroborated the association with methylation status and prognosis. Patients whose GBMs did not have *MGMT* methylation exhibited more rapid disease progression following diagnosis and a higher risk of death, particularly within the first 2 years following diagnosis (HR 1.87; 95% CI: 1.46–2.17).⁴⁷

Despite the clear correlation between TMZ treatment and improved survival in patients with methylated *MGMT*, it is worth noting that patients with glioblastoma with unmethylated *MGMT* promoters appear to derive some benefit from TMZ. There was a 31% risk reduction for death in patients with unmethylated *MGMT* promoters who received both radiation and TMZ, compared with unmethylated patients who received radiation alone, though this difference was not statistically significant.⁴⁶ A similar trend of extended OS in patients with unmethylated *MGMT* promoters treated with both radiation and TMZ compared with

radiation alone was observed in a randomized trial of elderly patients with GBM.⁴⁸ Together, these data suggest the presence of a marginal benefit from TMZ in patients with unmethylated GBM.

However, the prognostic value of *MGMT* promoter methylation is so strong that trials are currently under way to determine whether alternative therapies could be beneficial for patients with *MGMT* unmethylated GBM. For example, the EORTC 26082 study compared the standard of radiation plus TMZ with radiation plus temsirolimus, an inhibitor of mammalian target of rapamycin (mTOR), in *MGMT* unmethylated patients. In this study, however, patients receiving temsirolimus did not have a superior 1-year survival compared with patients receiving TMZ.⁴⁹ Additionally, the phase III study CheckMate 489 is aimed at investigating the efficacy of nivolumab and radiation compared with TMZ and radiation in newly diagnosed, *MGMT* unmethylated GBMs. Additionally, the Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGHT) is a biomarker-based study looking at the role of alternative adjuvant therapies to TMZ in these patients. The 3 experimental arms include adjuvant neratinib, abemaciclib, or CC-115 (dual inhibitor of mTOR kinase and DNA-dependent protein kinase).⁵⁰

MGMT promoter methylation is more common in lower-grade, *IDH* mutant gliomas.³⁸ This is consistent with data showing a correlation between the presence of an *IDH* mutation and a hypermethylator phenotype (discussed in more detail below). When *MGMT* status was retrospectively investigated in tumors of patients enrolled in the EORTC 22033–26033 trial comparing radiotherapy with dose-dense TMZ, it was noted that 100% of tested *IDH* mutant, 1p/19q codeleted tumors had a hypermethylated *MGMT* promoter compared with 86% of *IDH* mutant, non-codeleted tumors.³⁸ In the *IDH* mutant tumors in this cohort, the presence of *MGMT* promoter methylation was correlated with longer PFS only in the TMZ treatment arm,⁵¹ suggesting that *MGMT* promoter methylation status can be a useful aid for predicting which patients may respond to TMZ. A retrospective review of *MGMT* promoter status in low-grade glioma samples from patients treated with radiation therapy plus TMZ in the context of the single-arm phase II NRG/RTOG 0424 trial also noted a higher frequency of *MGMT* promoter methylation in *IDH* mutant tumors compared with *IDH* wildtype tumors. Though the predictive nature of *MGMT* methylation could not be addressed in this single-arm study, a multivariate analysis highlighted the prognostic importance of *MGMT* methylation in low-grade gliomas, demonstrating prolonged OS and PFS of patients with methylated tumors, independent of *IDH* mutation status.⁵²

MGMT promoter methylation status is currently clinically determined using quantitative methylation-specific PCR (qMSP) and pyrosequencing techniques⁵³ and in some cases using IHC.²⁸

TMZ resistance can develop following therapy, leading to a “hypermutator” phenotype. Resistance results from mutational inactivation of mismatch repair proteins such as mutS homolog (MSH) 2, MSH6, mutL homolog (MLH) 1, PMS2 (postmeiotic segregation increased 2), POLE (polymerase epsilon), and POLD1 (polymerase delta 1), leading to an accumulation of G/T mismatches in the presence of an alkylating agent and increased mutational

burden at a rate of 31.0–90.9 mutations per megabase, most of which are G:C→A:T transitions.⁵⁴ The hypermutator phenotype has been implicated in the progression from low-grade to high-grade gliomas.⁵⁵ In a sequencing study of 23 low-grade gliomas at diagnosis and recurrence, the hypermutator phenotype was found in 6 out of 10 tumors treated with TMZ, all of which had progressed to GBM and had acquired genetic changes in signaling pathways characteristic of this tumor.⁵⁵ The true risk of TMZ-induced hypermutation in glioma progression and its prognostic significance in high-grade gliomas has yet to be determined in larger-scale studies.⁵⁶ The hypermutator phenotype may also have important implications as a biomarker and predictor of response to therapy, as there have been several case reports of hypermutant tumors exhibiting durable responses to checkpoint blockade agents.^{57,58}

G-CIMP DNA Methylation

Global changes in DNA methylation frequently occur in cancer as ways of regulating transcription of oncogenes and tumor suppressor genes. CpG islands are regions of the genome that are high in guanine-cytosine content and commonly occur in promoters. These regions can be transcriptionally silenced by methylation, which either blocks access to transcription factors or recruits methyl-binding proteins that initiate structural chromatin changes.⁵⁹ The CpG island methylator phenotype (CIMP) was first described in 1999 in colonic tumors as a state of global hypermethylation.⁶⁰ This was differentiated from age-related methylation and was thought to lead to transcriptional repression of tumor suppressors such as p16 and mismatch repair deficiency through inactivation of MLH proteins. A similar phenotype was described in a subset of glioblastomas.⁶¹ This phenotype is strongly associated with *IDH* mutations and is frequently found in recurrent tumors. In fact, when introduced into primary astrocytes, the *IDH* mutation was found to be sufficient to cause hypermethylation and the glioma (G)-CIMP phenotype.¹¹

Tumors harboring the G-CIMP phenotype are known to have a favorable prognosis. It is unclear whether this is due to silencing of specific genes induced by methylation or is related to the presence of the *IDH* mutation. Despite the strong correlation between *IDH* mutation and G-CIMP, a subgroup of *IDH* mutant gliomas with a G-CIMP low phenotype was recently discovered.⁶² In this study, *IDH* mutant gliomas were divided into G-CIMP high, G-CIMP low, and 1p/19q codeleted tumors. Among these subtypes, G-CIMP high tumors had the best prognosis, comparable to the codeleted groups (median OS 7.2 years and 7.9 years, respectively), while G-CIMP low tumors had a significantly worse outcome, with a median OS of 2.7 years.⁶² G-CIMP low gliomas may arise from G-CIMP high ones. Intratumoral heterogeneity was found to be a poor prognostic factor in G-CIMP high gliomas. Nine patients with initially G-CIMP high tumors at diagnosis exhibited G-CIMP low recurrences that were all grade IV and portrayed epigenetic changes that resembled *IDH*-wildtype primary GBMs.⁶³ While testing for the G-CIMP phenotype is not routinely performed in the clinical setting,

assessing genome-wide methylation of tumor is becoming increasingly common and should become routine in clinical practice.⁶⁴

TERT Promoter Mutations

Telomerase reverse transcriptase is the catalytic component of telomerase, which allows for the elongation and maintenance of telomeres at chromosome ends. While telomeres normally shorten with every cell division and allow for a defined lifespan length of any particular cell, cancer cells exhibit aberrant activation of telomerase, which allows for unlimited proliferative capacity. The most frequent mechanism of telomerase activation is through mutations within the promoter of the *TERT* gene, which were found to be the third most common genetic alteration in cancer after mutations in *KRAS* and *TP53*.⁶⁵ These mutations are thought to lead to telomerase reactivation by creating a novel binding site for a transcription factor of the *ETS* family; however the full transcriptional regulation at the mutant locus has yet to be elucidated.⁶⁶ These mutations were first discovered in melanomas⁶⁵ but were later found in a large number of other tumors, including non-small cell lung cancer,⁶⁷ bladder cancer,⁶⁸ hepatocellular carcinomas,⁶⁹ and glioblastomas.⁷⁰

TERT promoter mutations are found in approximately 80% of *IDH* wildtype GBM,^{71,72} as well as in the majority of *IDH* mutant, 1p/19q codeleted oligodendrogliomas.^{34,71} Recent phylogenetic analysis of pre- and posttreatment GBMs suggests that *TERT* promoter mutations are an early event in gliomagenesis.⁷³ In GBM, *TERT* promoter mutations have been associated with worse prognosis compared with that of patients with *IDH* wildtype GBM in a number of studies.^{34,70,74,75} A recent study of 1087 glioma samples subdivided tumors into molecular groups based on 3 genetic alterations: *TERT* promoter mutations, *IDH* mutations, and 1p/19q codeletion.³⁴ Grade II and III gliomas with *TERT* promoter mutations alone harbored the worse prognosis (HR 11.74, 95% CI 6.15–22.41, compared to tumors with all 3 alterations). A similar effect was also seen in GBMs compared to *IDH* mutant tumors but was not significant on multivariate analysis.³⁴ The impact of the mutation on prognosis may be influenced by a common polymorphism rs2853669, age at diagnosis, and extent of resection.^{74,75} There is currently no standard role for detection of *TERT* promoter mutations in the diagnosis of GBM, but this alteration is frequently included on many tumor sequencing panels. Additionally, a rapid genotyping assay was recently developed to genotype tumors for *IDH* and *TERT* promoter mutations intraoperatively.⁷⁶

In gliomas, *TERT* promoter mutations are mutually exclusive with mutations in the alpha thalassemia/mental retardation syndrome X-linked (*ATR*X) gene. This gene codes for a telomere binding protein and confers an alternative lengthening of telomeres phenotype,⁷⁷ characterized by long telomeres which are maintained in a telomerase-independent manner. This suggests that telomere regulation is an important process in the development of gliomas.

*ATR*X mutations were first identified in 31% of pediatric glioma patients and found to co-occur with histone H3

mutations.⁷⁸ They were then identified in grade II and III gliomas and found to co-occur with p53 mutations in this population.⁷⁹ A recent study subdivided 671 grade II and III gliomas into 3 genomically distinct types: type I tumors were characterized by the presence of *IDH* mutations and 1p/19q codeletion, type II tumors had *ATRX* and *TP53* mutations, and type III were the remaining samples. In this study, type II tumors had an intermediate prognosis with HR of 2.06 compared with type I tumors (HR was 3.40 for type III tumors).⁷⁹ While *ATRX* mutation status appeared to influence survival in univariate analysis, this effect was not seen in multivariate analysis in this study. Therefore, the full prognostic effect of *ATRX* mutations in gliomas has yet to be determined.

EGFR Alterations

Amplification of EGFR is considered a hallmark alteration of GBM and is observed in ~40–50% of primary (*IDH* wildtype) GBM.⁸⁰ About one half of EGFR-amplified GBM express a constitutively activated variant of EGFR known as EGFRvIII, which further dysregulates the EGFR pathway. This variant contains a deletion in exons 2–7 of the *EGFR* gene, which leads to expression of a constitutively active protein.⁸¹ A recent meta-analysis of 10 articles involving 1074 patients demonstrated that high EGFR expression is associated with poor prognosis in GBM patients, with the pooled HR for OS of 1.57 (95% CI: 1.15–2.14, $P = 0.004$).⁸² The presence of the EGFRvIII variant was not found to alter prognosis in patients with *EGFR*-amplified tumors.⁸¹

Despite the high frequency of EGFR alterations in gliomas, these tumors show little clinical response to tyrosine kinase inhibitors that have proven effective in other cancers.⁸³ This is likely due to the fact that *EGFR* alterations display a significant amount of intratumoral heterogeneity in GBM.⁷³ Single cell sequencing technology allowed us to understand that different *EGFR* alterations, such as the vIII variant and carboxy-terminal deletions, were found in different subclonal populations within the same tumor.⁸⁴ Of note, while EGFR amplification status of the tumor tends to remain stable over time, expression of EGFRvIII can change. In 15 primary and recurrent tumor pairs that exhibited EGFRvIII expression within the primary specimen, only 8 (53%) of paired tumors continued to maintain EGFRvIII expression at recurrence.⁸⁵

Recently, novel EGFR-targeted therapeutic agents have started to show promise. The monoclonal antibody–drug conjugate depatuzumab mafodotin (depatux-m, ABT-414) completed phase I studies in patients with recurrent, EGFR-amplified GBM and in newly diagnosed GBM given with concurrent TMZ with encouraging results.^{86–88} A phase III study (RTOG 3508) looking at the efficacy of adding depatuzumab to both radiation and TMZ and Adjuvant TMZ in newly diagnosed GBMs has completed accrual. Additionally, a phase I study of 10 patients treated with chimeric antigen receptor T cells directed against the EGFRvIII protein shows that this treatment is well tolerated, and 1 patient had stable disease at 18 months post-infusion.⁸⁹ However, rindopepimut, a vaccine targeting the EGFRvIII mutation in patients with EGFRvIII-expressing, newly diagnosed GBM, failed to improve survival when added to standard therapy of radiation and TMZ in a large phase III trial.⁹⁰

BRAFV600E Mutations

BRAF encodes for the B-Raf protein kinase, which is involved in growth-promoting pathways. Mutation of the *BRAF* gene at the V600E hotspot results in constitutive activation of B-Raf and has been detected in a number of cancer types, including in glial tumors, such as pilocytic astrocytoma and glioneuronal tumors in the pediatric population as well as in diffuse gliomas, glioneuronal tumors,²⁷ and GBM in adults. Although the influence of the BRAF V600E mutation on prognosis in gliomas is not entirely clear, there is a suggestion from the literature that the significance of the alteration is dependent on the age of the patient. In a recent meta-analysis reviewing 11 articles describing ~1300 patients with gliomas, the authors found no prognostic relationship of BRAF V600E in patients over 35 years of age. In pediatric patients and younger adults (<35 y), the presence of the mutation is associated with improved survival, with pooled HR of 0.51 (95% CI: 0.34–0.79) for pediatric cases and 0.43 (95% CI: 0.20–0.93)⁹¹ for younger adults. In GBM, the presence of the BRAF V600E mutation has been associated with epithelioid GBM on histopathologic analysis,⁹² which has been reported to have more aggressive behavior and poorer prognosis.⁹³ Further investigation is required to better understand how BRAF V600E may influence outcome. Detection of the alteration has a therapeutic role, as many cancer types with BRAF V600E have been shown to respond to BRAF inhibitors.

Histone Mutations

Mutation of the histone H3 proteins is found in a subset of high-grade gliomas known as diffuse midline glioma, H3 K27M-mutant, as described in the update to the WHO classification of CNS tumors.² These tumors are generally found in the pons, thalamus, and spinal cord, in both adults and children, where they are generally associated with a poor prognosis.⁹⁴ The presence of the histone mutation is mutually exclusive with *IDH* mutations but can co-occur with mutations in receptor tyrosine kinase/Ras/phosphatidylinositol-3 kinase pathways.⁹⁵ The median age at diagnosis of histone-mutated tumors in adults is the early 30s,⁹⁶ compared with a median age of 64 for GBM. In pediatric patients, tumors with H3K27 mutations were found to have an overall worse prognosis independent of anatomical location. The median OS was 1.04 years for mutant tumors versus 6.1 years for wildtype ones.⁹⁴ Characterization of a small series of adult patients with histone-mutated gliomas suggests that the H3 K27M-mutation is also associated with poor prognosis in older age groups, with median OS of 19.6 months, similar to an OS of 17 months that was observed in *IDH* wildtype gliomas in this cohort.⁹⁶

Conclusions

The genomic analysis of adult gliomas has led to insight into the underlying pathways that lead to tumor formation. In addition, some of the molecular alterations discovered have clinical and prognostic relevance. We have discussed the key biomarkers that have emerged over the last decade

and how they serve to influence prognosis both positively and negatively. Of note, the influence of many of these biomarkers on disease course is by and large related to individual effects on development and growth of these tumors. Therefore, in addition to the prognostic implications associated with the alterations described herein, these biomarkers are potential therapeutic targets that are actively under investigation.

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