Genomic Biomarker Assessment in Gliomas

Impacts of Molecular Testing on Clinical Practice and Trial Design

Mary-Jane Lim-Fat, MD[a], Lakshmi Nayak, MD[a,1], David M. Meredith, MD, PhD[b,*,1]

KEYWORDS

• Glioma • Biomarker • Clinical trial • Genomic

Key points

- Recently revised classification schema for gliomas issued by the World Health Organization and the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy integrate numerous genomic biomarkers that facilitate more accurate diagnosis and assessment of prognosis, and oncologists increasingly rely on molecular testing results for treatment planning.
- The wealth of genomic data for gliomas has facilitated the development of numerous clinical trials exploring the efficacy of targeted and immunomodulatory agents, necessitating routine molecular testing for these tumors.
- Given the genomic heterogeneity of gliomas and the narrow window of time for treatment planning, turn-around-time and cost-effectiveness of comprehensive biomarker assessment continue to pose major challenges for personalized treatment.

ABSTRACT

Recent discoveries elucidating the genetic underpinnings of glial neoplasms have revealed myriad recurrent alterations that have clinical value by improving accuracy of diagnosis and prognosis. Furthermore, this wealth of genomic information provides the basis for targeted therapies and the subsequent design of biomarker-based clinical trials. This review summarizes the current landscape of clinically relevant molecular alterations in gliomas and describes the role of routine molecular testing in context of treatment planning for standards of care and clinical trials.

OVERVIEW

Concerted efforts to characterize chromosomal abnormalities, genomic mutations, epigenomic alterations, and proteomic changes have provided a deeper knowledge and appreciation of the biology and taxonomy of brain tumors. In 2016, a revision to the World Health Organization (WHO) classification of central nervous system (CNS) tumors highlighted the integration of molecular diagnostics to complement histologic diagnosis and grading.[1] This has now been incorporated in practice guidelines, endorsed by the National Comprehensive Cancer Network (NCCN) and the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW).[2]

[a] Center for Neuro-Oncology, Dana Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215, USA; [b] Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA
[1]Contributed equally.
* Corresponding author.
E-mail address: dmmeredith@bwh.harvard.edu

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Across many glioma types, copy number alterations, mutational events, and gene rearrangements, as well as tumor mutational burden and methylation profiles have been described and can help broaden our understanding of each tumor's distinct natural history, vulnerabilities, response, and progression patterns. Although many of these changes are yet to be incorporated into widespread clinical practice and in some instances require further validation, these unprecedented advances hold great promise in improving early diagnosis, identifying prognostic and predictive patterns, and finding the best treatment for each patient.

In this review, we discuss how the latest updates in genomic signatures promise to revolutionize care for patients with glioma by helping to inform diagnosis, treatment selection, and prognosis prediction.

CURRENT INTEGRATION OF GENOMICS IN CLINICAL PRACTICE: DIAGNOSIS AND PROGNOSIS

The classification of brain tumors over the past decade has undergone a major paradigm shift with the advent of more widespread use of molecular diagnostic techniques. Next-generation sequencing (NGS), in combination with chromosomal arrays and fusion assays, has allowed for more precise regrouping of gliomas of similar biological lineages and behavior, moving toward integrated histologic/molecular diagnoses with more rigorous diagnostic reproducibility.

MOLECULAR DIAGNOSIS IN ADULT DIFFUSE GLIOMAS

New understanding of the molecular changes driving gliomagenesis was at the basis for the 2016 revision to the WHO CNS tumor classification schema and has proven to be of significant prognostic relevance in glioma. Most important in diffuse adult gliomas are isocitrate dehydrogenase (*IDH*) mutations and 1p/19q co-deletion status. *IDH*-mutant diffuse gliomas are further separated into astrocytomas and oligodendrogliomas, depending on whether they possess co-occurring *ATRX* and *TP53* mutations or 1p/19q co-deletion, respectively. Given the mutual exclusivity of these events, the histologic diagnosis of oligoastrocytoma has no molecular equivalent and has thus been abandoned.[1] In addition, a provisional framework allowing for the molecular diagnosis of glioblastoma can now be made in *IDH*-wild-type astrocytic tumors in which an *EGFR* amplification, *TERT* promoter mutation, or chromosome 7 gain or 10 loss is identified, regardless of their histologic grade.[3]

A specific and accurate tissue diagnosis is central to the initial patient-provider discussion and establishes the plan of care and prognosis discussion. Specifically, with regard to treatment, the approach to high-grade gliomas routinely includes a gross total resection, followed by adjuvant chemoradiation and adjuvant chemotherapy with temozolomide, an alkylating agent.[4] In low-grade gliomas, although practice may vary depending on the patient's age, extent of resection, size of the tumor, and provider preferences, a combination of resection, radiation, and chemotherapy with temozolomide or with a combination of procarbazine, lomustine (CCNU), and vincristine (collectively referred to as PCV) is typically prescribed.[5]

The power of ancillary molecular testing to refine and correct histologic diagnoses (especially in the setting of surgical undersampling) was quickly recognized, leading to the development of consensus guidelines for integrated diagnosis reporting.[6] As a result, many current clinical trials have specific inclusion criteria pertaining to the integrated diagnosis, granting many more patients access to cutting edge therapies. As more and more patients are enrolled into clinical trials requiring biomarker assessment via molecular testing, the importance of timely integrated diagnostic reporting becomes even greater. This process can be limited by a number of factors, including access to surgical expertise, size of tumor sample, and availability of pathologic expertise and molecular testing.

PROGNOSTIC AND PREDICTIVE MARKERS IN GLIOMAS

An important cornerstone of clinical care of patients with brain tumor relies on an understanding of prognosis and predicting tumor response to therapy. This is particularly important in a vulnerable patient population in which, in addition to prolonging survival, maximizing quality of life and limiting potentially harmful treatments is a primary consideration.

As discussed previously, *IDH1/2* mutations have become central to the classification of diffuse gliomas and has helped stratify prognosis across all histologic grades. In *IDH*-mutant Grade III astrocytomas, the median overall survival is approximately 10 years, whereas in *IDH*-mutant Grade IV glioblastoma, the overall survival is approximately twice that of their *IDH*-wild-type counterpart.[7] However, even within the Grade II and Grade III *IDH*-mutant diffuse astrocytomas, somatic copy

number alterations, in particular homozygous deletions of *CDKN2A/B,* also have been found to be associated with a more aggressive course, prompting a novel grading of diffuse gliomas.[8]

Methylation at the promoter region of the O6-methylguanylmethyltransferase (*MGMT*) gene in patients with newly diagnosed glioblastoma has been found to be a strong predictive marker in terms of response to alkylating therapy or radiotherapy. Based on the Stupp trial published in 2005, the median overall survival with concurrent chemoradiation and adjuvant therapy with temozolomide for *MGMT*-methylated patients with glioblastoma was 23.4 months, whereas it was only 12.6 months in the *MGMT*-unmethylated group.[9] With more robust validation of *MGMT* methylation, replacing temozolomide by investigational agents through a clinical trial in unmethylated glioblastoma has become a reasonable option, particularly as the NCCN guidelines now endorse offering clinical trials to both patients with newly diagnosed glioblastoma and those with recurrent eligible glioblastoma. In *MGMT*-methylated newly diagnosed patients, there has also been interest in capitalizing on a combination of 2 alkylating nitrosureas, and a recently published study investigated the combination of lomustine-temozolomide compared with standard of care therapy in the concurrent radiation and adjuvant stage. The combination group had an overall survival of 48.1 months compared with 31.4 in the temozolomide-alone group.[10] However, potential additive myelosuppressive effects may limit this strategy.

In patients older than 65 years, concurrent fractionated radiation therapy and temozolomide remains the first choice of treatment.[11] Nonetheless, in patients who otherwise have poorer functional status, several studies support the use of temozolomide alone in *MGMT*-methylated patients and conversely the use of radiation therapy alone in *MGMT*-unmethylated patients.[12,13] The significance of *MGMT* methylation in lower-grade gliomas remains less clear, as these tumors retain both *MGMT* alleles (found on chromosome 10q) in contrast to glioblastomas.[14] However, as testing becomes more widely available, correlation with patient outcomes may provide more insight into its predictive or prognostic capability in low-grade gliomas or other tumors.

Adult and pediatric diffuse midline gliomas harboring mutations in histone variants H3.1 (*HIST1H3B*) or H3.3 (*H3F3A*) at the K27 codon have been recognized as a unique entity, as highlighted by the 2016 revision to the WHO classification.[1] *H3K27 M* tumors can occur in both adult and pediatric patients, and the mutation is mutually exclusive with *IDH1/2* mutation and is rarely found to have *MGMT* promoter methylation.[15,16] Because of their behavior, these tumors have been assigned a Grade IV diagnosis, irrespective of their histologic features, given that these tumors are often found in a difficult location, and biopsies are typically preferred. Detection of this mutation has been very helpful in opening clinical trial and therapeutic avenues by supporting the diagnosis of a highly aggressive tumor. In pediatric high-grade gliomas harboring this mutation, the overall survival is approximately 2.3 years shorter,[17] and survival in adults is equivalent to other *IDH*-wild-type gliomas.[16]

HYPERMUTATION IN GLIOMAS

Hypermutated gliomas, whether arising from germline defects in mismatch repair enzymes or acquired secondary to prior treatment with temozolomide, have been the subject of great interest, especially in the era of immunotherapy. Available tumor sequencing panels now have the capability of determining the tumor mutational burden, although the exact cutoff determining "hypermutation" has not been clearly established. In other systemic tumors with microsatellite instability and high mutational burden, such as colorectal cancer, immune checkpoint blockade has been associated with significant and sustained response.[18] However, immunogenicity of CNS tumors, in particular gliomas that acquire hypermutation secondary to temozolomide exposure, has not been well described. Clinical trials investigating PD-1 checkpoint blockade with pembrolizumab in pediatric patients with constitutional mismatch repair deficiency syndrome (ClinicalTrials.gov Identifier: NCT02359565), as well as in adults with recurrent glioblastomas with hypermutator phenotype (ClinicalTrials.gov Identifier: NCT02658279), are currently ongoing to determine the potential benefit of immune checkpoint blockade in these tumors.

TARGETED THERAPIES IN GLIOMAS

Despite our unprecedented understanding of gliomas and their genetic lineage, efforts to find effective therapies have fallen short. This is related to several different challenges, including the redundancy of several oncogenic pathways, lack of CNS penetrance from tested therapies, and intratumoral and intertumoral heterogeneity. Despite these obstacles, targeted therapies, based on individualized tumor genotyping and phenotyping, continue to hold great promise,

aided by a better understanding of drivers of gliomagenesis.

In glioblastoma, several different targets have been identified. *EGFR* amplification occurs in approximately 50% of glioblastoma. Although prior small molecule inhibitors of *EGFRvIII* variant have failed to show significant benefit in clinical trials, a new antibody-drug conjugate targeting amplified *EGFR*, depatuxizumab mafodotin (ABT-414), delivers the active compound monomethyl auristatin F directly into cells harboring mutant *EGFRvIII*, thus circumventing classic EGFR inhibition and cell resistance.[19] A placebo-controlled Phase 2 b/3 study of radiotherapy/temozolomide against radiotherapy/temozolomide in combination with ABT-414 (RTOG 3508- ClinicalTrials.gov Identifier: NCT02573324) has been completed, with promising interim analysis data, although final results are expected in the coming year.

Targeted therapies for *BRAF* alterations have now been well-established in malignant melanoma. Although only approximately 1% to 2% of adult glioblastomas harbor a $BRAF^{v600E}$ mutation,[20] the accessibility of new inhibiting agents offers an attractive treatment option for this subgroup of patients. An open-label multicenter phase 2 basket study in patients with $BRAF^{v600E}$ rare cancers of several histologic types (ClinicalTrials.gov Identifier: NCT02034110) contained a subgroup of patients with high-grade glioma who received a combination of dabrafenib (*BRAF*-inhibitor) and trametinib (*MEK*-inhibitor). This study is currently ongoing, although interim individual patient data analysis has indicated sustained response in some participants. Of note, targeted therapy with *BRAF* inhibitors also has been used in other nonglial CNS tumors. In craniopharyngiomas, NGS has helped differentiate between papillary craniopharyngiomas, which contain the $BRAF^{v600E}$ mutation, and adamantinomatous craniopharyngiomas, which contain the exon 3 activating *CTNNB1* mutations.[21] Targeted therapy with *BRAF* inhibitors for papillary craniopharyngiomas is being investigated and has shown optimistic results in many cases.[22]

Multikinase inhibitors, such as regorafenib, which targets vascular endothelial growth factor receptor, angiopoietin 2, platelet-derived growth factor receptor, and fibroblast growth factor receptor, also have been investigated recently in glioblastoma, having had a longer track record in other systemic cancers. The REGOMA (Regorafenib in Relapsed Glioblastoma) trial enrolled patients with recurrent glioblastoma and randomized salvage therapy with lomustine against regorafenib. Median overall survival in the regorafenib group was 7.4 versus 5.6 months in the lomustine-treated group.[23] Clinical trials using the CNS penetrant dopamine receptor D2 antagonist (DRD2) ONC201 (ClinicalTrials.gov Identifier: NCT02525692) are currently ongoing after a phase 2 study showed some antitumoral activity in a patient with recurrent *H3K27M*-mutant glioblastoma. Other targeted therapies are currently being investigated, including panobinostat, a general histone deacetylase inhibitor with in vitro efficacy against *H3K27M*-mutant diffuse pontine gliomas.[24] Other targets currently being investigated in gliomas include CDK4/6 (abemaciclib, ribociclib, palbociclib), *NTRK* fusion (larotrectinib, entrectinib), and *MDM2* amplification (AMG232).

IDH1 and IDH2 play a crucial role in converting isocitrate to α-ketoglutarate. Mutations in *IDH1* or *IDH2* lead to an overproduction of 2-hydroxyglutarate (2-HG), which has been revealed to be an oncometabolite in several tumor types and a compelling therapeutic target.[25] A number of *IDH1* (eg, AG120, IDH305, AGI5198), *IDH2* (AGI6780, AG221), and combined inhibitors (AG-881) are therefore in various stages of clinical testing. More broadly, this enzymatic pathway offers several other potential targets, including glutaminase inhibitors and the potential to combine *IDH*-inhibitors with vaccines or checkpoint inhibitors due to effective immune-modulation by 2-HG in glioma.[26] The latter strategy is being explored in a currently active study of PD-1 inhibition in recurrent or progressive *IDH*-mutant gliomas (ClinicalTrials.gov Identifier: NCT03557359).

USE OF BIOMARKERS IN CLINICAL TRIALS AND RESPONSE ASSESSMENT

The current landscape of clinical trials in gliomas does not represent an effective means to test out potential treatment targets, as the median time to completion of clinical trials in glioblastoma is 3 to 4 years.[27] Newer and innovative clinical trial designs aim to address this issue, a prominent example of which is the Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT) trial (ClinicalTrials.gov Identifier: NCT02977780), a Bayesian adaptive platform trial for patients with newly diagnosed glioblastoma with a 2-phase approach.[28] In this design, randomization at diagnosis occurs to multiple experimental arms or one control arm, and patient subtypes, including biomarkers, are identified, after which adaptive randomization occurs based on accumulating trial results. Based on interim results, arms can be dropped while new arms can be added over time. On a global scale, GBM AGILE (Glioblastoma adaptive, global, innovative learning environment)

aims to use a similar platform for patients with recurrent glioblastoma with accrual set to begin in the coming year.[29]

Endpoints and response assessment in brain tumors in the context of clinical trials pose a particular challenge in the context of intertumoral heterogeneity, as well as temporal and spatial intratumoral heterogeneity. RNA sequencing provides posttranscriptional data reflecting more accurate changes in tumor cell biology and also promises to offer more insight into individual tumor response and mechanism of resistance. However, this is currently time intensive and costly and has not been fully implemented in the clinical world. Posttreatment or midtreatment data on tumor samples is also limited by the fact that tissue analysis would require invasive sampling. Although neoadjuvant trials, in which the investigative agent is administered before tissue sampling and analysis have been a paradigm shift, temporal sampling across the disease and treatment spans capturing new mutations is not always feasible.

Sampling and sequencing of cell-free circulating tumor DNA (ctDNA) in plasma or blood has been considered as a possible mechanism to circumvent the need of multiple repeat craniotomies for tissue analysis. In particular, sequencing of ctDNA from cerebrospinal fluid (CSF) has yielded longitudinal data on brain tumors, paralleling the changes in tumor burden, and characterizing biomarkers more accurately than in plasma ctDNA.[30] The genomic landscape of gliomas was further characterized in 42 of a cohort of 85 patients with glioma, in which tumor-derived DNA was detected in CSF.[31] Concordance between the CSF ctDNA genomic profile and that of the tumor biopsy was demonstrated, and alterations reflecting temporal evolution in the tumor DNA was also captured in CSF. CSF ctDNA may therefore represent a less invasive method to assess for new targetable mutations throughout the evolution of brain tumors.

PRACTICAL CONSIDERATIONS AND COST-EFFECTIVENESS

As larger validated reference datasets emerge for specific tumor types, curated and relevant NGS assays have become available for gliomas. These platforms can be institution dependent (SNaPshot, OncoPanel, or GlioSeq) or commercially available (FoundationOne CDx) and can contain several hundreds of selected genes. Individual analysis of gliomas for mutations that are potentially targetable has been integrated in several workflows and is particularly crucial for enrollment in clinical trials that may have specific mutations as inclusion criteria. It is also increasingly helping to shape clinical practice with the increased availability of "off-label" drug repurposing for targetable mutations.

The cost of sequencing with newer NGS panels can nonetheless be daunting, and extensive molecular genotyping in the absence of clear targetable mutations and effective therapies may not represent a cost-effective strategy. New studies using cost-modeling analyses are therefore helping clarify financially effective testing algorithms. For example, an algorithm to identify *EGFR*-amplified, *IDH*-wild-type lower-grade diffuse gliomas was developed with the understanding that accurate diagnosis in this particular population has important clinical ramifications but does not necessarily entail extensive and costly sequencing.[32] As understanding of relevant targets and drivers of oncogenesis becomes clearer, cost-effectiveness analyses may help identify other combined strategies that can best help patients on an individual level.

Another concern is whether molecular testing is feasible in the timeline of real-world best clinical practice. Although some groups have been able to integrate a workflow of 5 days from tissue biopsy to finalized report of a 130 NGS gene panel,[33] in most institutions, this can take several weeks and presents a challenge in glioblastoma where a treatment plan needs to be formulated within 2 to 4 weeks from the date of surgery. In addition, with the increase in number of trials for newly diagnosed glioblastomas, molecular information, including *MGMT* status, is typically needed within 2 weeks after surgery at initial consultation with the neuro-oncologist before radiation planning.

As cost-effective and efficient NGS technology becomes more widely available, a vast amount of molecular data will need to be analyzed, correlated, and validated to create a more comprehensive repository of relevant changes and their impact on patients. Managing and sharing these datasets to be able to deduct meaningful conclusions will pose several challenges from an infrastructure and cost standpoint. A collaborative effort from scientists, clinicians, and software engineers will be required to translate big data into precision medicine to ultimately help patients who need better treatments.

DISCLOSURE

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