Chapter 2
The Role of Molecular Genetics of Glioblastoma in the Clinical Setting

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Introduction

Neuro-oncology clinical practice has evolved at a rapid pace over the last decade, especially in the most recent years. The World Health Organization (WHO) 2016 classification of central nervous system (CNS) tumors became a pivotal point in the diagnosis and management of brain tumors. The incorporation of molecular biomarkers in addition to histology has importantly impacted the clinical management of gliomas, leading to more accurate diagnosis, better prognostication of tumor behavior, and overall survival (OS) and, at the same time, it has opened new horizons in term of therapeutic approaches [1–3].

The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) was announced in December 2016 as a response to the accelerated expansion of advances on novel molecular markers and their clinical implication in the management of CNS tumors, providing regular and timely updates in between WHO CNS tumor classification editions and proposing future changes to future CNS tumor classifications [4, 5]. In April 2020, cIMPACT-NOW published their 6th update and their recommendations will be further discussed [2].

In this chapter, we will review the molecular markers that are relevant in clinical practice for glioblastoma (GBM) along with emerging novel biomarkers with a potential diagnostic or therapeutic role (Table 2.1).

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Table 2.1 Clinically relevant and emergent biomarkers in Glioblastoma

Biomarker	Diagnostic role	Prognostic role	Prospective treatment target
IDH 1/2 mutations	Defining feature of the majority of WHO grade II–IV gliomas.	*IDH* mutations are correlated with better OS in astrocytic tumors WHO grade II–IV	Yes
MGMT hypermethylation status	No diagnostic role	Predict benefit from alkylating chemotherapy in patients with *IDH* wild-type gliomas. Associated with increased incidence of pseudo-progression Key component in treatment decision in elderly population	No
EGFR amplification	Supports GBM diagnosis in *IDH*-wildtype WHO grade II and III astrocytomas	Associated with poor prognosis	Yes
EGFRvIII expression	Supports diagnosis of GBM	May be associated with poor prognosis, controversial	Yes
PTEN deletion	Supports diagnosis of GBM	May be associated with poor prognosis, controversial	Yes
BRAF V600E	Not diagnostic for GBM. Associated with epithelioid variant of GBM, pleomorphic xanthoastrocytoma and ganglioglioma.	Associated with poor prognosis in epithelioid variant of GBM. On specific cases, the use of BRAF and MEK inhibitors can dramatically improve PFS.	Yes
FGFR-TACC gene fusions	No diagnostic role	Not defined	Yes
PDGFRA	Associated with proneural subtype and secondary GBM	Poor prognosis on *IDH*-mutant WHO grade III and III astrocytomas and very poor prognosis on GBM with H3K27M mutation	Yes
CDKN2A/B homozygous deletion	Supports GBM diagnosis in *IDH*-mutant WHO grade II and III astrocytomas	Poor prognosis	No
*TERT*p mutation	Supports GBM diagnosis in IDH-wildtype WHO grade II and III astrocytomas	Poor prognosis	No

Table 2.1 (continued)

Biomarker	Diagnostic role	Prognostic role	Prospective treatment target
Chromosome 7 gains and chromosome 10 losses (+7/−10) *-not reviewed in this chapter*	Supports GBM diagnosis in IDH-wildtype WHO grade II and III astrocytomas	Poor prognosis	No

IDH isocitrate dehydrogenase, *MGMT* O6-methylguanine-DNA methyl- transferase, *EGFR* epidermal growth factor receptor, *PTEN* phosphatase and tensin homolog, *FGFR-TACC* fibroblast growth factor receptor-transforming acidic coiled-coil, *PDGFRA* platelet derived growth factor receptor alpha, *CDKN* cyclin dependent finase inhibitor, *TERT* telomerase reverse transcriptase promoter mutation

Isocitrate Dehydrogenase (*IDH*)

Isocitrate dehydrogenases (IDH1, IDH2, IDH3) are metabolic enzymes that participate in the Krebs cycle by catalyzing the oxidative carboxylation of isocitrate to α-ketoglutarate and carbon dioxide, resulting in the production of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) or nicotinamide adenine dinucleotide hydrogen in the case of IDH3 [6–11].

Mutations in the *IDH1* and *IDH2* genes result in a single amino acid substitution and are considered to be mutually exclusive [8, 11]. The most common mutation of *IDH1* in glioma is found at the arginine codon 132 (R132) with the most frequent substitution is of arginine by histidine (R132H), which occurs in more than 90%. On the *IDH2* gene, the most common mutation is at codon 172 (R172) and (R140) which is analogous for *IDH1* [8, 9]. Mutations in *IDH1* and *IDH2* have been identified in other malignancies such as chondrosarcoma, intrahepatic cholangiocarcinoma, acute myeloid leukemia and myelodysplastic syndromes [10, 12, 13].

Mutations in *IDH* result in the production of the oncometabolite R(−)-2-hydroxyglutarate (2HG). 2HG competitively inhibits α-ketoglutarate-dependent enzymes affecting histone and DNA demethylation, and adaption to hypoxia, leading to abnormalities of epigenetic regulation, genetic instability, T cell differentiation, and tumor immunity. 2HG also impairs cellular differentiation in a variety of cell lineages promoting oncogenic transformation in association with other cancer genes [6, 9, 10, 14, 15].

IDH1 mutations are present in up to 7% of GBM and in over 70% of grade II and grade III gliomas. Mutations have been also identified in the *IDH2* gene in approximately 4–8% of gliomas [13, 15, 16]. *IDH* mutation has been recognized as an early event in gliomagenesis. It has become a fundamental element for diagnosis, treatment decision and prognostication of tumor behavior.

The presence of *IDH1/2* mutation in gliomas has been associated with younger age and better prognosis [11, 17]. The mean age at diagnosis for *IDH*-mutant GBM is 40 years and median overall survival (mOS) 27–31 months. For *IDH*-wildtype GBM the mean age at diagnosis is 64 years and mOS 15–18 months [17–19].

Recent research advances suggest that *IDH*-wildtype and *IDH*-mutant GBM are two separate diseases, with a completely different age of presentation, molecular profile, and overall survival. This has been translated to the clinical research setting where most clinical trial studies designed for newly diagnosed or recurrent GBM are focused on *IDH*-wildtype GBM and exclude *IDH*-mutant tumors. In the recent years, *IDH* mutation has been explored as a potential therapeutic target in glioma [20–22], with clinical trials designed for *IDH*-mutant solid tumors including dedicated arms for *IDH*-mutant gliomas [23, 24].

The third update of cIMPACT-NOW recognized WHO grade II diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of GBM as an equivalent to a WHO grade IV tumor. This new concept applies to lower grade astrocytic tumors by histology, that contain the presence of *TERT* promoter mutation, *EGFR* gene amplification, and/or the combination of gain of entire chromosome 7 and loss of entire chromosome 10 (+7/−10); given that their behavior is similar than classic *IDH*-wildtype GBM [25, 26]. This was further reviewed in the sixth update from cIMPACT-NOW. On an effort to simplify nomenclature, and clinical trial eligibility, it was proposed that *IDH*-wildtype diffuse astrocytic gliomas can be classified as GBM, *IDH*-wildtype WHO grade 4 (now suggesting the use of Arabic numerals) in the presence of one or more of the aforementioned mutations. For *IDH*-mutant astrocytomas with microvascular proliferation or necrosis or *CDKN2/B* homozygous deletion, or any combination of any of these features will be designated astrocytoma, *IDH*-mutant, WHO grade 4 [2].

These guidelines from C-IMPACT-NOW are giving the clinician timely updates based on recent validated findings for more accurate diagnosis and prognostication that may change clinical management in daily practice, allowing the physician to provide a more tailored treatment recommendation and the possibility to offer a clinical trial that better suit the molecular profile for each patient's tumor.

O^6-Methylguanine-DNA Methyl- Transferase (*MGMT*)

Despite the fact it was not incorporated to the 2016 WHO classification of CNS tumors, *MGMT* promoter methylation status is one of the most relevant biomarkers used in the management of GBM as its presence predicts benefit from alkylating chemotherapy in patients with glioblastoma [27, 28]. The *MGMT* gene is located on chromosome 10 (10q26). It encodes the repair protein MGMT that reverses the damage created by alkylating agents by repairing damaged guanine nucleotides by transferring the methyl at O^6 site of guanine to its cysteine residues. Epigenetic modification of the cytosine-phosphate-guanine (CpG) island at specific CpG sites

within the *MGMT* promoter silences the gene, causing defective repair of DNA alkylation, promoting gene mutation and cell death [29, 30].

MGMT promoter methylation has been associated with better OS in glioma [28, 31–33]. *MGMT* promoter methylation status has been defined according to the percentage or level of methylation detected. Different testing methods have been studied, however, there is no agreement on the best test modality. Among the testing with the most reliability are methylation specific PCR (qMSP) and pyrosequencing [32, 34, 35]. Thresholds on the level of methylation have been studied in GBM: Unmethylated ≤9%, indeterminate or "gray zone" 10–29% and methylated >30%. Methylation levels above 30% have been correlated with better PFS and OS than below 30% (25.2 vs 15.2 months) [32]. Additional studies demonstrated that patients in the indeterminate methylation status also benefit of radiation and temozolomide therapy reflecting an OS of 10–17 months for truly unmethylated, 15.4–20 months for indeterminate and 19.7–34.1 months in methylated patients [35, 36]. It is important to underline that these studies did not correlate consistently with *IDH* status of the tumor samples studied.

Reliable and consistent assessment of *MGMT* methylation status at the first clinic visit is of utmost importance in the evaluation of patients with GBM due to its role in patient counseling and clinical trials eligibility. *MGMT* status has become relevant in the design of clinical trials for newly diagnosed GBM with some trials excluding patients with *MGMT* promoter hypermethylated tumors, other trials include it as a parameter for randomization.

A special population in which the value of *MGMT* has been particularly important for consideration of treatment decision, is the elderly. Multiple trials have demonstrated that concomitant treatment with temozolomide and hypofractionated radiotherapy increased OS regardless of the *MGMT* promoter methylation status [37–41]. However, for older patients who are not candidates for a combined-modality approach because of poor functional status or significant comorbidity, *MGMT* promoter methylation has a particularly important role. Emerging data support the use of temozolomide chemotherapy as an alternative to radiation therapy, in those patients with *MGMT* methylated tumors. Radiation therapy alone is an effective alternative for patients with *MGMT* unmethylated tumors.

Pseudoprogression is defined as a new or expanding area(s) of contrast enhancement that occur early after the end of radiation therapy, within the first 6 months (typically between 3 and 4 months), in the absence of true tumor growth, and that tends to stabilize or resolve without a change in therapy. In GBM, *MGMT* promoter methylation was associated with a 3.5-fold greater risk of developing pseudoprogression in up to 30% of patients treated with chemoradiation with concomitant temozolomide and has been linked to a better outcome. Pseudoprogression can also occur in unmethylated tumors, but to a lesser frequency. The response assessment in neuro-oncology (RANO) criteria recommended that patients should be excluded from clinical trials for recurrent disease within the first 12 weeks after radiation therapy, unless progression is clearly outside the radiation field or there is histologic documentation of progression [42–46]. In the clinical setting, magnetic resonance

perfusion and spectroscopy and 18-FDG brain PET may aid in the differentiation between pseudoprogression versus progressive disease although histopathological analysis continues to be the gold standard.

Epidermal Growth Factor Receptor (*EGFR*)

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinase (RTK). Its structure includes an N-terminal extracellular domain, a transmembrane domain, an extracellular kinase domain, and a cytoplasmic C-terminal tail containing several phosphorylation sites that serve as signal transduction modules. Binding of one of several ligands to the extracellular ligand-binding domain induces receptor homo-dimerization or hetero-dimerization and results in kinase activation. In normal cells, this leads to DNA synthesis, cell proliferation, migration, and adhesion. *EGFR* mutations lead to production of mitogenic RTKs that inhibit the activity of p53 [47, 48].

EGFR is one of the first oncogenes identified in GBM. *EGFR* gene amplification is presented in about 40% of GBM [47, 49–51]. *EGFRvIII* mutation is found in 20% of GBM and it is particularly interesting as it is constitutively active and a potential neoantigen. The presence of *EGFR* amplification supports a GBM diagnosis and differentiates from other gliomas [47, 48, 50, 52–54]. *EGFRvIII* mutation alone is not predictive of outcome, however, the downstream altered molecular pathways associated as a result of its deletion may have a clinical impact [50, 52, 55]. *EGFR* has become one of the hallmark alterations that, if present in *IDH*-wildtype anaplastic astrocytoma, it supports the diagnosis of WHO grade IV astrocytoma [25].

EGFR has been extensively studied as therapeutic target. However, the results of multiple clinical trials evaluating *EGFR* tyrosine kinase inhibitors (TKI) and peptide treatment/vaccine, such as rindopepimut, in recurrent and/or newly diagnosed GBM patients have been disappointing [56, 57]. Clinical trials with newer generation *EGFR* TKI, *EGFRvIII* CAR-T cells alone and in combination with PD-1 inhibitors are currently ongoing and may elucidate the precise role of *EGFR* as a therapeutic target in GBM [50].

Phosphatase and Tensin Homolog (*PTEN*) Deletion

PTEN plays a major role regulating multiple biological functions at the level of the membrane and nucleus. It regulates genomic stability, cellular proliferation, migration and survival, tumor microenvironment among other functions. It has been implicated in multiple malignancies including gliomas. The loss of *PTEN* expression has been associated with glioma cells proliferation. This alteration is present in

approximately 40% of primary GBM, and its relevance in OS has been under debate. However, it is considered an additional biomarker in the diagnosis of GBM [58–60]. Recent clinical trials have focused on the PI3K/Akt pathway, with targeted therapies such as buparlisib, sonolisib, pilaralisib, dactolisib, alone or in combination with mTOR inhibitors, are ongoing or have demonstrated no clinical benefit [61, 62].

BRAF V600E Mutation

The BRAF protein is an intermediary in the RAS-RAF pathway. After a ligand-mediated receptor tyrosine kinase is triggered by extracellular growth factors, it activates *RAS*, which initiates BRAF-mediated activation of MEK and ERK, causing transcription of factors for cell proliferation. The *BRAF* V600E mutation results in constitutive activation of the MEK-ERK pathway and uncontrolled cell division [63, 64]. *BRAF* mutations are drivers of oncogenesis in approximately 6% of human malignancies including melanoma, thyroid, colorectal and non-small cell lung cancer [65]. *BRAF* V600E mutations have been identified in a variety of primary brain tumors such as pleomorphic xanthoastrocytoma (up to 60%) [66, 67] and 47–58% ganglioglioma [68, 69], but they are uncommon in GBM (1–2%), except for the epithelioid variant in which is present in about 56% [65, 70, 71]. Epithelioid GBM is a rare and aggressive variant that is more common in children and young adults. It carries a dismal prognosis of about 6 months OS and frequently has leptomeningeal dissemination [72–74].

Even though *BRAF* mutations are rare in GBM, it is important to consider testing for it, especially in the younger population, as the use of BRAF and MEK inhibitors have shown a dramatic response on imaging and prolonged PFS [65, 74].

FGFR-TACC Gene Fusions

Fibroblast growth factor receptor-transforming acidic coiled-coil (*FGFR-TACC*) gene fusions are present in 3% of GBM. In astrocytes, fusions between *FGFR3* and *TACC3* genes can lead to malignant transformation and GBM progression due to the activation of mitogenic, antiapoptotic and migratory functions. Preliminary data of a Phase 2 trial with infigratinib showed PFS6 of 16% with a mOS of 6.7 months, demonstrating a partial response or stable disease in approximately one-third of patients with recurrent GBM and other glioma subtypes [75]. Futibatinib, another *FGFR* inhibitor, has shown to be well tolerated, however, efficacy results have not been published yet [76].

Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*)

PDGFRA amplification is found in approximately 15–20% of adult GBM, especially in cerebellar variant. *PDGFRA* amplification increases with grade and is associated with a less favorable prognosis in WHO grade II and III *IDH1*-mutant astrocytoma comparable to WHO grade IV [49, 52]. In GBM harboring *H3F3A*-K27M mutation, positive *PDGFRA* expression was linked to even worse prognosis [77]. *PDGFRA* has been studied as a potential target in the treatment for GBM using dasatinib and other multikinase inhibitors alone or in combination with bevacizumab with no significant results [78, 79].

CDKN2A/B Homozygous Deletion

Cyclin Dependent Kinase Inhibitor 2A (*CDKN2A*) encodes Ink4a and Arf proteins, which play an important role in activating Rb and p53, respectively. *CDKN2B* encodes the tumor suppressor p15INK4b. Ink4a and p15INK4b inhibit *CDK4* and *CDK6* and maintain the growth-suppressive function of the Rb gene. When dysregulated, uncontrolled cell growth occurs [52, 80].

The prevalence of homozygous deletion of *CDKN2A/B* has been reported in 22–35% of all gliomas (16–47% *IDH*-mutant GBM and up to 58% of *IDH*-wildtype GBM) [54]. The presence of *CDKN2A* homozygous deletion in LGG and *IDH*-mutant GBM was associated with lower PFS and OS when compared to *CDKN2A* intact tumors [81]. The fifth and sixth updates of C-IMPACT-NOW have incorporated *CDKN2A/B* homozygous deletion as a marker for malignant behavior *IDH*-mutant WHO grade 2 and 3 astrocytomas, upgrading them to WHO grade 4 astrocytoma [2, 82].

These recent changes are quite impactful to daily clinical practice, as the presence of this mutation in astrocytic tumors dramatically changes the prognosis. *CDKN2A/B* deletion should be obtained routinely in the pathological analysis of *IDH*-mutant astrocytoma of any grade [83]. This marker could become a landmark parameter for clinical trial inclusion criteria in the near future [82, 84–86].

Telomerase Reverse Transcriptase Promoter Mutation (*TERTp*)

Telomerase reverse transcriptase (TERT) is a rate-limiting catalytic subunit of telomerase, an RNA-dependent DNA polymerase that lengthens telomeric DNA to maintain shorter telomeres in human cells function to prevent uncontrolled cellular proliferation. *TERT* promoter mutations result in the upregulation of *TERT* transcription, have been identified in over 50 different types of cancer, including several CNS neoplasms [87, 88]. Somatic hot spot mutations in *TERTp* occur in

IDH-wildtype GBM and in *1p/19q* co-deleted *IDH*-mutant oligodendroglioma. *ATRX* mutations are found to be mutually exclusive with *TERT*p mutations in adult GBM [89–91]. *TERT*p mutation has been linked to worse prognosis if found on *IDH*-wildtype astrocytoma WHO grade II or III as their clinical course resembles to the one of a WHO grade IV GBM [25, 90, 92]. Although *TERT* promoter mutation has not become a major pharmacological target for cancer therapy yet, it has significant role in glioma diagnosis and prognosis.

Conclusion

Advances in tumor molecular profiling technologies have allowed molecular characterization of GBM as never before. The addition of molecular biomarkers and histology to the 2016 WHO Classification of CNS Tumors has deeply impacted the clinical management of gliomas providing not only a more accurate diagnosis and prognostication, but also the opportunity to develop innovative clinical trials tailored to the genetic and epigenetic alterations of each tumor. The inclusion of next generation sequence-based assays and other molecular methods in the evaluation of newly diagnosed and recurrent GBM is becoming essential, as it may dramatically impact the diagnosis and management of our patients.

As more discoveries rapidly arise, and the pathogenesis of GBM continues to be better understood, it is likely that more markers will become part of additional classifications of this complex and heterogeneous tumor.

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