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# The Power of Human Cancer Genetics as Revealed by Low-Grade Gliomas

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## Keywords

low-grade gliomas, pediatric, development, BRAF, IDH, next-generation sequencing, DNA methylation

## Abstract

The human brain contains a vast number of cells and shows extraordinary cellular diversity to facilitate the many cognitive and automatic commands governing our bodily functions. This complexity arises partly from large-scale structural variations in the genome, evolutionary processes to increase brain size, function, and cognition. Not surprisingly given recent technical advances, low-grade gliomas (LGGs), which arise from the glia (the most abundant cell type in the brain), have undergone a recent revolution in their classification and therapy, especially in the pediatric setting. Next-generation sequencing has uncovered previously unappreciated diverse LGG entities, unraveling genetic subgroups and multiple molecular alterations and altered pathways, including many amenable to therapeutic targeting. In this article we review these novel entities, in which oncogenic processes show striking

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age-related neuroanatomical specificity (highlighting their close interplay with development); the opportunities they provide for targeted therapies, some of which are already practiced at the bedside; and the challenges of implementing molecular pathology in the clinic.

## 1. INTRODUCTION

Primary brain tumors are the leading cause of cancer-related death in children, the third leading cause of cancer-related death in young adults aged 20–39 years, and a growing source of disability and societal burden (32, 72). Gliomas are the most frequent malignant brain tumors, representing approximately 80% of these cancers (48, 66). They are classified traditionally by the World Health Organization (WHO) as high-grade glioma (HGG) or low-grade glioma (LGG) tumors. Classification is based mainly on histology and morphology through the use of light microscopy (with traditional nomenclature according to the tumors' presumed cell of origin) and further grading from I to IV using criteria that include nuclear atypia, cellular proliferation, angiogenesis, and presence of necrosis (58). More recently, the latest 2016 WHO classification incorporated a few ancillary studies of specific markers to specify prognostic subgroups in adult LGGs (60). Standard current therapy includes maximal safe surgery and, for incompletely resected gliomas or HGGs, adjuvant therapies in the form of multimodal chemotherapies with or without radiation. Cure remains a rare exception for HGGs. For LGGs, which have a better prognosis and are the subject of this review, the cost of survivorship is often high, as the late effects from both the disease and treatment are devastating. This is especially true for children with LGGs (1, 2, 52, 61). LGGs in adults carry similar problems, but unlike childhood tumors, they are thought to invariably progress to HGGs with time. Improved diagnosis and classification, the development of more effective and less toxic treatments, and their early use upon diagnosis—representing the major aims of targeted therapies and precision medicine—are therefore imperative to improve care (67). Accordingly, the use of next-generation sequencing and DNA methylation arrays in the past few years has led to giant progress in the diagnosis, classification and therapies of LGG, especially in the pediatric setting.

In this article we briefly describe the different LGG entities as classified today and how they differ between adults and children. We highlight the novel, specific molecular entities that have been and are being identified with these genetic and DNA methylation tools in adult and pediatric LGGs. We also highlight the interplay between altered brain development and the genesis of LGGs through the exquisite age and neuroanatomical distribution displayed by these tumors and the molecular drivers they harbor. The diversity of molecular entities and their clinical correlates arguably renders the term LGG and its use with respect to the current WHO classification somewhat obsolete. This is especially true for pediatric LGG, in which almost every glial or neuroglial cell type could give rise to a tumor entity. Finally, we provide a rationale for more genetics-driven classifications that can be layered with classical tumor morphology, and how some of these changes are being implemented at the bedside in clinical trials.

## 2. LOW-GRADE GLIOMAS IN CHILDREN AND ADULTS: SAME DENOMINATION, RADICALLY DISTINCT ENTITIES

Histopathological classification is more readily accessible than genetic tools and has been the gold standard used for brain tumor diagnosis and prognosis. However, there is high intra- and inter-observer variability and suboptimal prediction of clinical outcomes when it is used as the sole method at the bedside (8, 99). In addition, there are exquisite differences in histological entities

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**HGG:** high-grade glioma

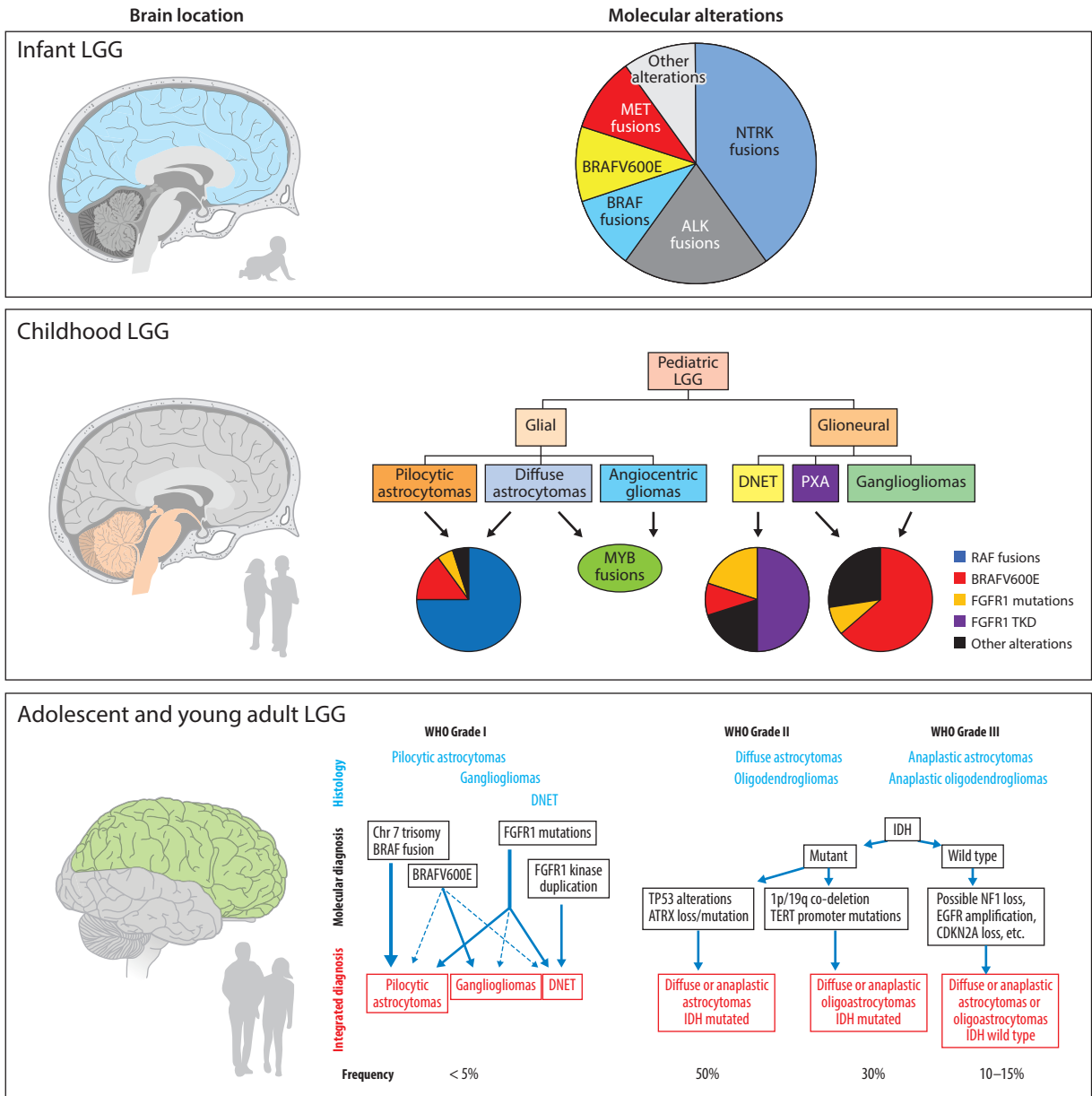
**LGG:** low-grade glioma

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and in molecular alterations within and between adult and pediatric LGGs, and multiple differences in outcome are not captured by standard histology. For example, pediatric LGGs show tumor behavior and clinical outcomes distinct from those of adult LGGs, suggesting they are different diseases that should be identified and treated separately. These differences argue strongly for a more genetics-based classification, which was made critical with recent landmark genomic profiling efforts. Indeed, while these efforts revolutionized our understanding of oncogenic processes, biomarkers, prognostic factors, and therapies in LGGs, they highlighted the discordance between histological and genetic markers and showed that molecular classification captures biological classes of disease more accurately than regular pathology (3, 7, 8, 14, 21, 31, 40, 55, 57, 67, 95, 102, 103). In addition, these large-scale international sequencing efforts on clinically annotated cohorts of LGGs identified novel genetic entities and seminal differences across the life span, ranging from neuroanatomical location and clinical behavior to a vast diversity of specific oncogenic alterations that completely differ between children and adults (**Figure 1**). Consequently, the recent 2016 update to the WHO classification used for the first time molecular/genetic layering to histology and redefined adult LGG subgroups. In contrast, it did not similarly tackle a molecular update for pediatric LGGs, partly because of the daunting amount of distinct molecular entities with overlapping histology in children, as we will show in the sections below (60).

### 3. ADULT LOW-GRADE GLIOMAS ARE MOSTLY DIFFUSE TUMORS DEFINED BY *IDH* MUTATIONS

Most adult brain tumors are HGGs, with LGGs representing 10% of all glioma diagnoses and accounting for barely 3–4% of all brain tumors in this age group. Most LGG cases occur in the cerebral cortex, with a predilection for the frontoparietal cortex (53) (**Figure 1**). The 2007 WHO classification of gliomas had separated entities on the basis of the presumed cell of origin (astrocytic, oligodendroglial, oligo-astroglial) and grade, with grades I and II representing LGGs and grades III and IV representing HGGs (58). The discovery of recurrent, somatic, heterozygous, hot spot mutations in isocitrate-dehydrogenase 1 (*IDH1*) and 2 (*IDH2*) in approximately 80% of adult LGGs was one of the first successes of The Cancer Genome Atlas consortium (70, 102). These mutations affect specific residues on the IDH enzymes, mainly R132 on *IDH1* and R172 on *IDH2*, which are mutually exclusive in tumors and result in the loss of the native function of the wild-type enzyme. IDH mutations are gain-of-function, with the neomorphic enzymes generating oncogenic metabolites through the conversion of alpha-ketoglutarate, the normal enzymatic product, to 2-hydroxyglutarate and the conversion of NADPH to NADP<sup>+</sup> instead of the reduction of NADP to NADPH (11). Further studies showed that *IDH1* *IDH2* mutations have prognostic significance and can be used as biomarkers, especially when coupled to the other genetic events with which they are almost invariably associated, namely 1p/19q co-deletions and *TERT* promoter mutation or their mutually exclusive *ATRX* ( $\alpha$ -thalassemia X-linked intellectual disability syndrome) and *TP53* alterations (7, 14, 38, 57, 95, 102) (**Figure 1**). IDH-mutant LGGs can thus be further divided into the following subgroups: The first consists of *IDH* mutants with 1p/19q co-deletions (~30% of all LGGs), which additionally harbor *CIC*, *FUBP1*, and *NOTCH1* mutations. These are mainly oligodendrogliomas and have the most favorable clinical outcomes. The second consists of *IDH* mutants with mutations in *TP53* (~50% of all LGGs), which are often associated with *ATRX* inactivation (86%). These are mainly astrocytomas and have a somewhat poorer outcome (7, 14, 38, 57, 95, 102). LGGs without an *IDH* mutation (15–20% of all LGGs) have the worse prognosis and often carry the genomic aberrations (*NF1*, *PTEN*, *TERT*, *CDKN2A*) and the aggressive clinical behavior seen in adult primary grade IV astrocytomas (glioblastomas), into which they typically transform within two years of diagnosis. Thus, the newest integrated 2016 WHO classification



**Figure 1**

Genetic alterations in LGGs are age related and show specific neuroanatomical distribution. (*Top*) Infants have mainly hemispheric tumors harboring mostly fusions in development-associated kinases. (*Middle*) LGGs in children aged 3–12 years occur mostly in the posterior fossa and the brain midline, with a large variety of molecular entities that show an absence of 1:1 correlation with specific genetic drivers. (*Bottom*) Adolescent and young adult LGGs are located mainly in the frontoparietal lobes and are predominantly IDH mutant gliomas. Abbreviations: DNET, dysembryoplastic neuroepithelial tumor; IDH, isocitrate-dehydrogenase; LGG, low-grade glioma; PXA, pleomorphic xanthoastrocytoma.

now separates LGGs into diffuse and intermediate grade gliomas (grades II and III) and, regardless of lineage (the mixed oligo-astrocytomas are no longer a diagnosis), further classifies them into these three nonoverlapping, prognostically significant subtypes that can be objectively classified by these molecular markers: IDH-mutant 1p/19q deleted, IDH-mutant *TP53/ATRX* altered, and IDH wild-type LGGs (60) (**Figure 1**). These three subtypes of LGGs are diffuse infiltrative neoplasms and highly invasive, rendering complete surgical resection impossible. They often evolve with time to higher-grade tumors, and regardless of initial improved outcome, most IDH-mutant adult LGG patients with *TP53/ATRX* mutations will die within 5–10 years from diagnosis (7, 14, 95). The other rarer LGG entities encountered in adults (less than 5% of LGG) are more frequent in the pediatric setting. They include grade I (pilocytic) astrocytomas, gangliogliomas, dysembryoplastic neuroepithelial tumors (DNETs) or other rarer entities. Despite similar histology, they show striking molecular differences from the respective pediatric LGG entity (**Figure 1**). Indeed, adult pilocytic astrocytomas, which in children are defined by alterations in the mitogen-activated protein kinase (MAPK) pathway (see Section 5), frequently harbor nonrandom aneuploidy with gains of chromosome 5, 6, 7, or 11 and much more rarely show the defining genetic alterations seen in pediatric pilocytic astrocytomas (21, 41). Here also, these LGGs are mainly hemispheric in adults and, in contrast to diffuse LGGs, are more circumscribed and thus often amenable to complete surgical resection with much improved outcomes and clinical behavior.

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**DNET:**  
dysembryoplastic  
neuroepithelial tumor

**PXA:** pleomorphic  
xanthoastrocytoma

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## 4. PEDIATRIC LOW-GRADE GLIOMAS ARE NEURODEVELOPMENTAL DISORDERS

Whereas adult gliomas are characterized by multiple somatic mutations and copy number alterations, pediatric gliomas harbor much lower mutation rates and, in the case of pediatric LGGs, are frequently defined by a single-driver alteration. Pediatric LGGs exhibit a curious clinical course. Those that cannot be resected have a chronic relapsing course of tumor growth during childhood before quiescing on transition to adulthood (1), suggesting potential interplay between tumor cells and the microenvironment of the developing brain. Other striking differences from adult LGGs include neuroanatomical locations and the diverse pathological and molecular entities seen in children (**Figure 1**). Indeed, pilocytic astrocytomas are the most frequent LGGs in children, representing more than 23% of all pediatric brain tumors. In contrast, diffuse astrocytomas are much less frequent and rarely harbor the IDH mutations seen in adults (in children, these are restricted mostly to HGGs in the cortex of adolescents), while oligodendrogliomas are extremely rare and their existence in children is even debated (18, 20, 40, 87, 94, 101, 103). Other entities in pediatric LGGs include gangliogliomas and pleomorphic xanthoastrocytomas (PXAs), which are graded from I to III by the WHO, and other glioneural tumors including DNETs (58, 60); novel entities have been revealed by genetic profiling and DNA methylation arrays as described in Section 5.

### 4.1. Age-Dependent Molecular Alterations and Neuroanatomical Location of LGGs

There is a striking interplay between unique mutations and precise neuroanatomic and age-dependent correlates in pediatric and adult LGGs, which highlights changing selective pressures driving gliomagenesis in different developmental contexts (**Figure 1**). Indeed, in infants (0–3 years) gliomas are located mainly in the cortex; in children aged 3–12 years tumors present in order of frequency in the cerebellum, the optic pathway, and the brainstem (17–19, 36, 40, 48, 103). Midline tumors affecting central nuclei including the thalamus are rare in adults but not uncommon in children aged 8–15 years, and cortical gliomas again become more frequent in

children after 12–15 years of age (**Figure 1**). This age distribution also specifies histological diagnoses and molecular entities. Thus, pilocytic astrocytomas are more frequent in younger children and occur mainly in the posterior fossa, with decreasing frequency from the cerebellum (the most frequent location) to the optic pathway, brainstem, thalami, and spine. Less than 10% of pilocytic astrocytomas occur in the cortex, while this is the privileged location for glioneuronal entities such as DNETs (17–19, 36, 40, 48, 103). Gangliogliomas occur throughout the central nervous system with similar incidence in children and young adults, while PXAs occur mainly in the cortex and arise more frequently after puberty. More than 50% of diffuse astrocytomas occur in the cortex, with the remainder distributed across locations within the posterior fossa and the spine (3, 103).

#### 4.2. Pediatric Low-Grade Glioma Distribution Links with Brain Development

The ordered process of specific neuroanatomical locations and age groups correlating with histology mirrors the successive waves of neurogenesis and gliogenesis during brain development. Indeed, in humans, gliogenesis peaks during late gestation and continues postnatally through the first year of life (80). Myelination is initiated in late gestation and progresses continuously in an ordered pattern from inferior to superior and posterior to anterior brain regions during early childhood and through young adulthood (6, 88). Thus, in children, the absolute numbers of proliferating cells in the developing brain vastly outnumber those in the adult brain, and progenitor cells are programmed to expand, migrate, and differentiate to generate the structures and integrated functions of a developing brain. This provides an opportunity for the clonal expansion of somatic mutations in cell populations during development, while cells in the adult brain are more directed toward homeostatic maintenance and response to damage.

Thus, the innate differences in the role of progenitor cells at different developmental stages likely also confer intrinsically different susceptibilities to transformation and possibly explain why pediatric LGGs are substantially less molecularly complex than adult LGGs. Importantly, aberrant expression of key developmental pathways during inappropriate developmental contexts represents a potential path to pediatric gliomagenesis and pediatric LGG. These tumors represent one of the most genetically quiet human cancers and are most frequently characterized by single-driver alterations that activate the MAPK pathway (36, 37, 40, 103). The MAPK pathway has been broadly implicated in neurogenesis and, in particular, lineage commitment and differentiation of neural precursor cells toward the neuronal or glial lineages (56). Indeed, MEK (the downstream target of BRAF, which is frequently rearranged or mutated in pediatric LGGs) is essential in the regulation of both gliogenesis and neurogenesis during development, while fibroblast growth factor, which signals through its receptor, FGFR (also recurrently mutated or rearranged in pediatric LGGs), to activate MAPK signaling, is also implicit in neurogenesis (28). The precise timing of expression of key mediators of neurogenesis and gliogenesis during specific developmental windows is likely to tightly regulate cell fate commitments during neurodevelopment. Aberrant regulation of the pathway, induced by most pediatric LGG-associated driver alterations, may result in inappropriate activation of cells and lineages, potentially driving cells toward gliomagenesis.

Structural variants that result in aberrant expression of key developmental pathways are also observed in other pediatric LGGs. Single rearrangements involving MYB or the related family member MYBL1 occur across a range of pediatric LGG histological subtypes, including angiocentric gliomas (3) and diffuse astrocytomas (58, 75, 76). In normal development, expression of MYB is restricted to the developing fetal brain and is not detected in postnatal cortical brain (3). However, in MYB-driven pediatric LGGs, structural variants induce aberrant expression of oncogenic MYB through protein truncation, resulting in loss of negative regulatory domains and

changes in epigenetic structure to promote gene transcription (58). Thus, relatively simple genetic alterations have the potential to drive a cell toward oncogenesis through multiple mechanisms.

## 5. MOLECULAR SUBGROUPS OF LOW-GRADE GLIOMAS AND GLIONEURONAL TUMORS

Molecular subgrouping of LGGs or glioneuronal tumors is currently not as mature or well-defined as for some other pediatric brain tumors, such as medulloblastoma (65, 97) or ependymoma (69). Although there are undoubtedly molecular-histological correlates within the LGG spectrum [i.e., genetic alterations enriched in tumors of a certain morphology (75)], there are very few true 1:1 associations in which a particular mutation is pathognomonic of a single entity at the exclusion of any other diagnosis. This is partly due to the somewhat indiscriminate appearance of genetic alterations across a variety of subclasses [e.g., *BRAF* V600E mutations are found in multiple LGGs (85)]. It is also made more complicated by the overlapping and often indistinct histological descriptions of some of the typical members of the LGG spectrum. For example, regions of tumor resembling pilocytic astrocytoma can be encountered in both gangliogliomas and variants of DNETs according to their WHO definitions (59). In some instances a tumor diagnosed as a pilocytic astrocytoma can longitudinally evolve into a ganglioglioma (16), and oligodendroglioma-like areas are also compatible with multiple histological LGG diagnoses. In the authors' opinion, a true pediatric-type oligodendroglioma as a direct counterpart to the adult tumor but lacking *IDH1* mutation is unlikely to exist, with this designation rather reflecting a variety of other entities displaying overlapping morphology. As noted above, pediatric LGGs, harboring an excellent prognosis, must in general be distinguished from lower-grade gliomas (WHO grades II and III) in adults, which almost all harbor *IDH1* mutations and can undergo malignant transformation (59).

From a technical perspective, some of the methods currently in vogue for classifying brain tumors [most notably DNA methylation analysis (8) but also techniques such as transcriptome or mutation/copy number-based analysis (25)] are also less well developed in the glioma space. On the one hand this is reflective of the daunting task of trying to develop a truly pan-glioma classification due to the enormous heterogeneity in both histological and molecular features. On the other hand it is also at least partly due to the difficulties in obtaining pure tumor samples for profiling; both LGGs and HGGs are characterized by substantial immune infiltration into the tumor microenvironment (reviewed in, e.g., 39, 67) and often display a growth pattern that involves invasion into the surrounding parenchyma. Thus, while multiple glioma classes are included in the most comprehensive methylation-based classifier to date (8), there is still work to be done to further refine this objective, molecular based approach to address the full panoply of LGGs.

In the next sections, we examine some of the genotype-phenotype relationships between molecular alterations and histological (and/or biological) tumor classes that have been identified (see also **Table 1**), with a particular focus on important caveats that should be considered where exceptions to the rule can be encountered. For the *RAF* and *FGFR* kinases reported in the next section, multiple alterations can lead invariably to an active kinase. These include fusions, single activating point mutations, tandem kinase duplications, or amino acid insertions in the kinase domain, all of which are gain-of-function.

### 5.1. *RAF* Kinase Alterations

*RAF* kinase alterations are hallmarks of pediatric LGGs. This is especially true for pilocytic astrocytomas in which more than 80% of tumors harbor a fusion or a mutation that leads to baseline

**Table 1 Summary of common genetic alterations observed across low-grade glial/glioneuronal tumors and their primary histological associations**

	<b>(KIAA1549) BRAF fusion</b>	<b>BRAF (V600E) mutation</b>	<b>FGFR1</b>	<b>NF1</b>	<b>MYB/MYBL1</b>	<b>IDH1</b>
Very common (>50%)	PA, DLGNT	GG, (a)PXA, (epGBM)	DNET (mutation, ITD), EVNCYT (fusion), RGNT (mutation)	Germline NF1-associated LGG (defining)	AG	DA, AA, O, AO
Common (>20%)	AAP	DA, DIG, DIA	AAP (mutation), pediatric-type oligodendroglioma <sup>b</sup>	RGNT, AAP	DA	secGBM
Recurrent (~5%+) <sup>a</sup>	NA <sup>a</sup>	PA	PA (mutation, fusion)	NR	NR	pedGBM
Rare <sup>a</sup>	NA <sup>a</sup>	DNET, others	NR	NR	NR	NR
Additional notes	1p loss co-occurs in DLGNT <i>CDKN2A/B</i> and/or <i>ATRX</i> loss co-occur in AAP	Good potential drug target Concurrent <i>CDKN2A/B</i> loss likely indicates a more aggressive clinical course	Co-occurrence with <i>NF1</i> and/or <i>PIK3CA</i> in RGNT <i>CDKN2A/B</i> and <i>ATRX</i> loss commonly co-occur in AAP	PA: combination of one germline plus a somatic second hit AAP and RGNT: can be a monoallelic somatic hit May respond particularly well to MEKi	Very likely that <i>MYB/MYBL1</i> alterations define a unique subset of LGG variants with varying histology but biological similarity	Very rare in children <18 years Co-occurs with <i>TP53/ATRX</i> mutation in astrocytic tumors or 1p19 loss and <i>TERT</i> promoter mutation in oligodendroglial tumors

<sup>a</sup>The authors believe that most reports of this alteration in other histologies likely result from morphological overlap between entities rather than true occurrence in other tumor types. The existence of other very rare yet undefined classes cannot, however, be ruled out.

<sup>b</sup>The authors believe that this term likely encompasses a variety of molecularly distinct classes with oligodendroglial elements, some of which show recurrent *FGFR1* alterations, and does not represent a single direct counterpart to adult oligodendroglioma.

Abbreviations: AA, anaplastic astrocytoma [adult, IDH-mutant]; AAP, anaplastic astrocytoma with piloid features; AG, angiocentric glioma; (AO), (anaplastic) oligodendroglioma (adult, IDH-mutant); (a)PXA, (anaplastic) pleomorphic xanthoastrocytoma; DA, diffuse astrocytoma; DIA, diffuse astrocytoma; DIG, desmoplastic infantile astrocytoma; DLGNT, diffuse leptomeningeal glioneuronal tumor; DNET, dysembryoplastic neuroepithelial tumor; EVNCYT, extraventricular neurocytoma; epGBM, epithelioid glioblastoma; GG, ganglioglioma; ITD, internal tandem duplication of the kinase domain; LGG, low-grade glioma; MEKi, MEK inhibitor; NA, not available; NR, not reported; PA, pilocytic astrocytoma; pedGBM, pediatric glioblastoma; RGNT, rosette-forming glioneuronal tumor; secGBM, secondary glioblastoma (arising from a preexisting grade II/III lesion).



activation of these kinases. The discovery has revolutionized our understanding of their biology and opened the door for targeted therapies in this setting as described below.

**5.1.1. *BRAF* fusions.** An activating fusion involving the kinase domain of *BRAF*, usually with *KIAA1549* as the 5' partner but also with an ever-growing list of alternative variants, is highly indicative of the diagnosis of a pilocytic astrocytoma. Since it was first discovered in 2008 (42, 71), this alteration has remained one of the alterations that more closely approaches a 1:1 link with histology: In the absence of any other suspicious histological, clinical, or molecular findings, a diagnosis of pilocytic astrocytoma is strongly supported by the presence of this fusion. Multiple reports also indicate the fusion occurs less frequently in other groups such as ganglioglioma, DNETs, and diffuse glioma (WHO grade II), but these could also be explained by the histological uncertainties noted in Section 4.1.

There are, however, at least two distinct entities in which a *BRAF* fusion can arise, whereby a clinical course that would be untypical for pilocytic astrocytoma might be encountered. The first entity is the diffuse leptomeningeal glioneuronal tumor, a rare tumor whose histology shows a diffuse growth pattern with leptomeningeal spread (26, 73, 79). Recent reports have confirmed an association between highly frequent 1p deletion (usually without 19q co-deletion) and *BRAF* fusion (or other MAPK pathway alterations) and suggested the possibility of two molecular subgroups differing in their age distribution and survival outcomes (13). Notably, these reports also demonstrate that not all tumors belonging to this molecular class necessarily display either diffuse or leptomeningeal growth, suggesting that this name may need to be revisited (10, 13).

The second distinct entity commonly harboring *BRAF* fusion is the anaplastic astrocytoma with piloid features (AAP), an IDH wild-type diffuse glioma with a much worse prognosis than pilocytic astrocytomas (77). These tumors, in addition to either *BRAF* fusion or *NF1* or *FGFR1* mutation, frequently harbor deletions of the *CDKN2A/B* locus at the 9p21 locus and loss of *ATRX* (77). They arise primarily in older children and in young adults. This differential should therefore be kept in mind when encountering *KIAA1549:BRAF* fusions in the setting of a clearly anaplastic morphology or where additional 9p21 loss is observed.

**5.1.2. *BRAF* (V600E) mutation.** In contrast to *BRAF* fusions, only rarely encountered outside of pilocytic astrocytomas, mutation of *BRAF* (most commonly the hot spot V600E substitution) can be found across a wide variety of low- and high-grade glial and glioneuronal tumors (85). Thus, while it is essential to be able to reliably detect this alteration in terms of the therapeutic possibilities that it raises (47), its diagnostic or prognostic value is much less clear and also presently the subject of some controversy (44, 54). *BRAF* V600E is most commonly encountered in gangliogliomas and PXAs (85), but it is also found less frequently in pilocytic astrocytomas, DNETs, the epithelioid variant of glioblastoma [which may be biologically related to the PXA spectrum (51)], and seemingly almost any other class in the glioma spectrum. In addition to various histological differences, the main molecular feature distinguishing PXAs from gangliogliomas is that 9p21 is frequently deleted in the former (100), which is clearly associated with a negative prognostic impact (54, 62). PXA was also formally split in the 2016 update of the WHO classification into grade II and anaplastic (grade III) variants, primarily on the basis of mitotic activity (59). This can lead to the application of conflicting treatment protocols to low-grade versus high-grade tumors with similar biology (V600E plus 9p21 loss) and heavily overlapping morphology. Whether V600E alone is sufficient as a prognostic marker regardless of other histological or molecular features remains an open question (44, 54), and the authors' opinion is that all 9p21-deleted tumors and molecular PXAs should be handled on the assumption that they will show an aggressive clinical course. The finding of this mutation should therefore be treated with care according to its

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**AAP:** anaplastic astrocytoma with piloid features

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diagnostic/prognostic relevance, but presently its value in terms of targeted therapy is arguably clearer than that for any other LGG-associated alteration.

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**RGNT:**

rosette-forming  
glioneuronal tumor

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**5.1.3. Other RAF kinase alterations in pediatric LGGs.** *BRAF* V600D-activating mutations were identified in desmoplastic infantile astrocytomas or desmoplastic infantile gliomas, which are rare massive cystic lesions usually found in the cerebral hemispheres of infants (9, 30). Moreover, insertions in the kinase domain ranging from intrakinase duplication to addition of several amino acids leading to kinase activation similar to the *BRAF* V600E/D point mutations have been identified in gangliogliomas and pilocytic astrocytomas (46, 103). *RAF1* fusions have also been identified in less than 5% of pilocytic astrocytomas and similarly lead to activation of the MAPK pathway (40, 103).

## 5.2. *FGFR1* Alterations

One of the most striking new findings to emerge in the LGG field as a result of widespread application of next-generation sequencing was the role of *FGFR1* alterations across a spectrum of LGGs (40, 103). These alterations can appear as a point mutation at one of two hot spot positions (N546, K656), as an internal tandem duplication of the kinase domain (ITD), or as a fusion (most commonly with *TACC1*).

The *FGFR1:TACC1* fusion was first discovered in glioblastoma (92) but has also been found in various low-grade histologies (75, 103). It is also a highly frequent event in molecularly defined extraventricular neurocytomas (90), which sometimes show morphological overlap with glial and glioneuronal tumors.

The internal tandem duplication of the *FGFR1* kinase domain (*FGFR1*-ITD) is a novel finding in LGGs (40, 103). Given the varied breakpoints of the duplication, and that it is too small an alteration to be detected by standard copy number analysis, this is the hardest of the three activating mechanisms to detect. Even when using next-generation sequencing, investigators must take care when analyzing such events, as they can often be overlooked with standard procedures. This activating variant seems to be most common in DNET and rarer in other histologies (75). The true incidence, however, may be masked by the difficulties in detection.

The point mutations at N546 and K656 have also been detected in various LGG histologies but again seem to be more frequent in DNETs (75, 78, 103). One exception is the rosette-forming glioneuronal tumor (RGNT), which also shows a high frequency of *FGFR1* point mutations (27). Strikingly, however, RGNTs often show co-occurrence of two or even three mutations involving *FGFR1*, *NF1*, and *PIK3CA* (see, e.g., 15, 84, 89), of which the last two are not seen in DNETs. Also, of note is the high occurrence of *FGFR1* mutations in AAPs, an important consideration when encountering this mutation in the context of atypical histology. The detection and interpretation of the consequence of the point mutations at the protein level, particularly around the K656 position, can be complicated because several cases display multiple base changes affecting nearby amino acids around this locus. The mechanism or consequences of this, however, remain unclear. Finally, a subset of LGGs, including both DNETs (78) and pilocytic astrocytomas [in the setting of encephalocraniocutaneous lipomatosis (98)], also seems to arise in the context of germline alterations of *FGFR1*, although the overall incidence of such constellations is currently not known.

## 5.3. *NF1*

Germline alterations in *NF1* account for approximately 10–15% of pilocytic astrocytomas and occur in 15–20% of individuals with neurofibromatosis type 1 (NF1), in whom these tumors

preferentially arise in the optic pathway (e.g., reviewed in 12). However, HGGs can also arise in the context of NF1. *NF1* alterations (both germline and somatic) are also a common finding in AAPs (similar to *KIAA1549:BRAF* and *FGFR1*), again indicating a need for caution when an *NF1* mutation is seen outside of typical pilocytic astrocytoma-like histology (77). Indeed, there is some evidence that pilocytic astrocytomas arising in the context of NF1 may occasionally progress to a malignant AAP, for example, through the acquisition of a 9p21 deletion, and thereby become largely refractory (see 77; D.T.W. Jones, P. Bandopadhyay & N. Jabado, personal observations). Whether the use of radiotherapy during treatment is connected to a higher propensity for malignant progression in this setting remains an open question.

#### 5.4. Other MAPK Pathway Changes

The full spectrum of genetic alterations leading to MAPK activation extends to also cover alterations in *KRAS* (mutation), neurotrophic receptor tyrosine kinase 1/2/3 (*NTRK1/2/3*) (fusions), and *PTPN11* (mutation) (40, 43, 103). These are all essentially too rare for any firm conclusion to be drawn about possible histological specificity of their occurrence. The fact that they have to date been found primarily in pilocytic astrocytomas could also be because pilocytic astrocytoma itself is by far the most common LGG histology in children. In addition to somatic *PTPN11* mutations, which have been reported to often co-occur with *FGFR1* mutations in pilocytic astrocytomas (40), there have also been case reports of pilocytic astrocytomas arising in the context of germline *PTPN11* mutation (i.e., Noonan syndrome) (24, 83, 86).

The NTRK family gene fusions are another class of alterations that can be seen in HGG as well as various LGG histologies; thus, care must be taken when interpreting their finding in a diagnostic setting. These fusions arise mainly in infants and are usually located in the cortex; however, the expanding use of RNAseq is uncovering these molecular alterations in other brain locations and in older children and even young adults. Regardless, these fusions make an excellent therapeutic target and therefore should be screened for in a diagnostic neuropathology setting wherever possible (Figure 1).

#### 5.5. *MYB/MYBL1* Alterations

The *MYB/MYBL1* alterations most commonly found in angiocentric gliomas and diffuse glioma (WHO grade II) are another striking finding of the next-generation sequencing era (76, 103). These were notable for being one of the few alterations in the LGG landscape that do not have an obvious direct mechanism through RAS/RAF signaling. Indeed, the exact downstream functional consequences of these alterations, most commonly *MYB/MYBL1* 3'-truncating fusions but also sometimes amplification (96), remain to be fully elucidated. The most commonly observed variant, *MYB:QKI*, likely acts via a tripartite mechanism of MYB protein structural activation, MYB overexpression, and QKI loss-of-function (3). Further commonalities in downstream signaling with other *MYB/MYBL1* variants, and possible avenues for therapeutic intervention, are currently being investigated.

The precise link between these genetic alterations and angiocentric/diffuse glioma histologies is also not yet fully resolved. The authors consider it likely that *MYB/MYBL1* alterations define a family of molecularly related gliomas with consistent underlying biology but a varied morphological spectrum that includes features of angiocentric gliomas and diffuse astrocytomas (among others). This may therefore represent one of the groups for which a timely move toward a molecularly defined classification would make considerable sense.

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**DMG:** diffuse midline glioma

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## 5.6. H3K27M Mutation

On a strict interpretation, the finding of a H3K27M mutation in a diffuse glial tumor currently means that it should be classified as a diffuse midline glioma, K27M-mutant (DMG-K27), which by definition is WHO grade IV (59). An ever-growing number of published reports and anecdotal findings, however, suggest that there are true histological low-grade (i.e., WHO grade I) tumors that can also harbor this mutation (40, 63, 68, 82, 103). Strikingly, the K27M mutation was often accompanied by a co-occurring canonical MAPK alteration, most typically *BRAF* V600E but also *KRAS* or *FGFR1* mutations, not frequently seen in DMG-K27M. Otherwise, the low-grade tumors tend to be genomically quiet, without the *TP53* or *ATRX* mutations observed in their high-grade counterparts. Thus, although a K27M mutation undoubtedly remains a strong indicator, the finding of a K27M mutation alone does not always indicate a de novo grade IV lesion. Unfortunately, however, many of the low-grade K27M tumors with available long-term follow-up have displayed a late malignant progression (up to 6–7 years, or even longer, after initial diagnosis) and subsequent dismal prognosis (82, 103). Thus, this mutation should be regarded as a harbinger of a likely fatal course, even if the trajectory is not always that of, say, a typical DMG.

## 5.7. *PRKCA*

As with *FGFR1*, where point mutations and gene fusions seem to be more closely associated with tumors of differing histological appearance, the *PRKCA* gene (protein kinase C alpha) can also be activated by distinct genetic mechanisms. Chordoid gliomas of the third ventricle are defined by a single hot spot mutation in the kinase domain of *PRKCA*, D463H (29). This mutation leads to elevated signaling through ERK, which can be blocked by MEK inhibition.

Papillary glioneuronal tumor, by contrast, is almost always associated with a fusion between *SLC44A1* (or rarely other 5' partners) and the *PRKCA* kinase domain (5, 34). Whether this is also sensitive to MEK inhibitors has not yet been formally proven but seems likely.

## 5.8. Other Rarer Alterations

Finally, we briefly consider other very rare molecular alterations that have recently been suggested to potentially define (or at least be enriched in) novel histological LGG subtypes. For example, fusions of *FGFR2* or *FGFR3* (rather than the more commonly altered *FGFR1*) were a recurrent alteration in a new tumor type, pleomorphic neuroepithelial tumor of the young (35). A second novel tumor type is myxoid glioneuronal tumor of the septum pellucidum/lateral ventricle, which harbors a mutation in the extracellular domain of platelet derived growth factor receptor alpha (*PDGFRA*). This finding marked the first time that this gene had been clearly genetically linked with a low-grade glioneuronal tumor (93).

The number of truly novel entities, and/or more precise molecular definitions overlapping with existing histological classes, is expected to continue to increase for the foreseeable future, as the adoption of comprehensive molecular profiling becomes more widespread (also in a routine clinical setting) and the power to detect new groups increases.

For the reader's benefit we summarized the most common genetic alterations encountered in LGGs in **Table 2**.

## 6. GENOMICS AND CLINICAL OPPORTUNITIES

LGGs represent a greater challenge for targeted therapeutic approaches in adults than in children, largely owing to the presence of more than one genetic driver. However, *IDH* mutations represent potential therapeutic targets. Indeed, *IDH1* inhibitors have shown promise in other cancers and

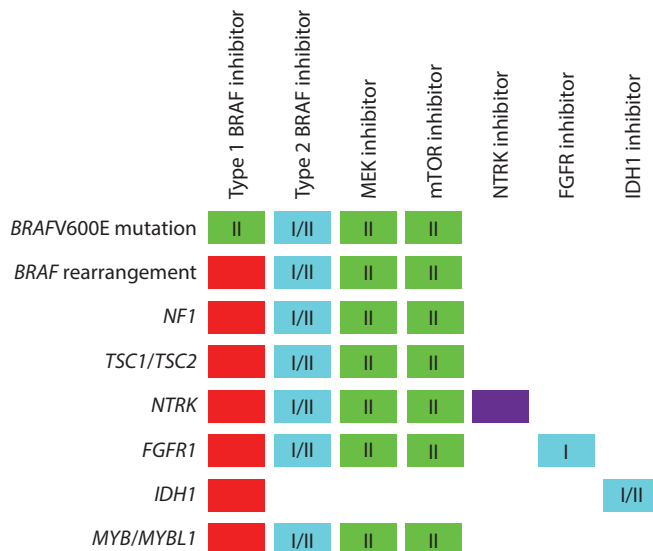
**Table 2 Most frequent genetic alterations identified in gliomas**

Gene	Protein full name and function	Genetic alterations in gliomas
<b>Chromatin and epigenetic regulators</b>		
<i>IDH1</i> and 2	Isocitrate-dehydrogenase 1 and 2, metabolism	Gain of function mutations in gliomas (mostly R132H or C)
<i>H3F3A</i>	Histone 3.3 (H3.3) nucleosome core formation, role in chromatin remodeling	Gain of function mutations in gliomas (K27M and G34R or V)
<i>ATRX</i>	Alpha-thalassemia/X-linked intellectual disability syndrome	Loss of function alterations (mutations/deletions), chromatin remodeler, telomere maintenance
<i>TERT</i>	Telomerase reverse transcriptase, catalytic sub-unit elongating telomeres	Point mutations in gene promoter
<b>Cell cycle regulators</b>		
<i>TP53</i>	P53, cell-cycle regulator	Loss of function alterations (mutations/deletions)
<i>CDKN2A</i>	Cyclin dependent kinase inhibitor 2A/2B, cell-cycle regulator	Loss of function alterations (mutations/deletions)
<b>Growth factors and regulators of the mitogen-activated protein kinases (MAPK) pathway</b>		
<i>BRAF</i>	BRAF, serine threonine kinase, proliferation and development	Gain of function mutations: V600E, V600D, intra-kinase insertions, tandem kinase duplications, gene fusions
<i>NTRK 1/2/3</i>	Neurotrophic tyrosine receptor kinase, CNS development and function	Gain of function gene fusions, mainly NTRK1 in gliomas
<i>FGFR 1/2/3</i>	Fibroblast growth factor receptor, proliferation, and development	Gain of function mutations, tandem kinase duplications, gene fusions
<i>PDGFR</i>	Platelet derived growth factor receptor, proliferation, development	Gain of function mutations, gene amplification, overexpression through enhancer hijacking
<i>MET</i>	Receptor tyrosine kinase, proto-oncogene	Gain of function gene fusions, gene amplification
<i>EGFR</i>	Epidermal growth factor receptor, proto-oncogene	Gain of function gene fusions, amplifications, point mutations
<i>HRAS, KRAS</i>	HRAS, KRAS, small GTP-binding proteins	Gain of function point mutations
<i>NF1</i>	Neurofibromin 1, RAS-GTPase	Loss of function mutations, gene deletions
<i>PTPN11</i>	Protein tyrosine phosphatase non-receptor type 11, inactivates receptors	Loss of function point mutations
<i>MYB/MYBL1</i>	MYB/MYBL1, proto-oncogene transcription factors	Gain of function fusions and other genetic alterations

were recently approved for the treatment of *IDH1*-mutant acute myeloid leukemias (64). Preclinical testing of *IDH1* inhibitors in the in vivo setting have shown efficacy in models of glioma (50, 74), and *IDH1* inhibitors are currently being evaluated in the clinical setting (NCT02073994 and NCT02481154) (**Figure 2**).

In contrast, the presence of single identifiable driver alterations, most of which converge on the MAPK pathway in pediatric LGGs, presents therapeutic optimism for small-molecule inhibition, heralding the way for precision medicine approaches.

The first pediatric clinical trials of small-molecule inhibitors focused on inhibitors of mTOR, enrolling children with genetic syndromes such as tuberous sclerosis (TS) and *NF1*, in addition to children with relapsed or refractory sporadic pediatric LGGs. In a randomized phase III clinical trial, treatment of patients with TS-associated subependymal giant cell astrocytomas with the mTOR inhibitor everolimus resulted in reductions of tumor volume of more than 50%, in 35% of all patients treated, by week 24 (22). Patients exhibited sustained responses, and by 96 weeks of treatment, 46% of patients were documented to have tumor reductions of at least 50% (23). Early-phase pediatric clinical trials of everolimus have also been completed in children with



**Figure 2**

Small-molecule inhibitors to target genomic alterations. Red denotes inhibitors that are contraindicated for corresponding alterations, blue denotes inhibitors undergoing evaluation in clinical trials, green denotes inhibitors that are commercially available, and purple denotes inhibitors that are licensed for the indication shown. Roman numerals depict phase of clinical trials specific for gliomas: I, phase I; II, phase II; I/II, phase I with phase II expansion.

*NF1*-associated or sporadic pediatric LGGs (NCT01158651 and NCT00782625), with another currently under way (NCT01734512) (**Figure 2**).

As noted in Section 5, a significant proportion of pediatric LGGs harbor structural rearrangements or point mutations (mostly V600E) of *BRAF*. Type 1 BRAF inhibitors have shown exciting early clinical responses in pediatric LGGs with the *BRAF* V600E point mutation, with single-agent therapy being sufficient to induce tumor responses in some children with refractory or recurrent gliomas (81). These drugs are undergoing early-phase clinical trials, either as single agents, or in combination therapy with MEK inhibitors. However, type I BRAF inhibitors are contraindicated in *BRAF*-rearranged pediatric LGGs, as they result in paradoxical activation of RAF/ERK signaling (91). This is associated with tumor growth as observed in a phase II study evaluating the multikinase inhibitor sorafenib in *BRAF*-rearranged pediatric LGGs, which resulted in early termination of the trial (45). This risk highlights the importance of careful characterization of genetic driver alterations before initiating treatments with small-molecule inhibitors (**Figure 2**).

MEK inhibition has recently emerged as a promising strategy against pediatric LGGs, particularly *NF1*-associated tumors, or those that harbor *BRAF* rearrangements and thus are not suitable for type I BRAF inhibitors. To date, at least four MEK inhibitors, including trametinib (NCT03363217), selumetinib (4), cobimetinib (NCT02639546), and binimetinib or MEK162 (NCT02285439), are undergoing various stages of early-phase clinical testing for children with pediatric LGGs. Phases I and II testing of selumetinib have been completed in children with both *NF1*-associated and sporadic refractory or progressive pediatric LGGs. All children with *NF1* enrolled in the phase I trial had either stable disease or reductions in tumor volume, and overall, 32 of 38 evaluable patients exhibited either stability or reductions in tumor size (4). Given these promising results, efforts are currently under way to evaluate these agents in both the upfront,

newly diagnosed setting and as part of combination treatments. Other case reports show the use of trametinib is also of potential value in multiply relapsed LGGs, especially in the presence of an alteration affecting the MAPK pathway (49). Finally, preclinical studies have shown that type II BRAF inhibitors (which target the inactive conformation of the *BRAF* kinase) effectively inhibit neural stem cell models transduced to express the *KIAA1549:BRAF* fusion without resulting in paradoxical pathway activation and are currently undergoing phase I and II trials for children with recurrent or refractory LGGs (NCT03429803) (Figure 2).

Point mutations and fusions in receptors such as NTRK, FGFR, and less frequently, PDGFRA and epidermal growth factor receptor (EGFR) represent alternate therapeutic targets in subsets of pediatric LGGs. The TRK inhibitor, larotrectinib, was recently approved for the treatment of TRK-altered cancers (33). Small-molecule inhibitors of FGFR are being evaluated in clinical trials. However, these mutations can co-occur with other driver mutations such as *PTPN11*, and the effects of suppressing FGFR in the presence of downstream activating mutations remain to be determined.

## 7. IMPLEMENTING MOLECULAR DIAGNOSIS OF LOW-GRADE GLIOMAS AT THE BEDSIDE

The 2016 WHO classification used for the first time an integrated approach that took into consideration molecular findings to classify LGG entities. This approach focused mainly on adult LGGs, using the clear dichotomy provided by the presence of IDH mutations and specific mutually exclusive associated alterations, which made possible the distinction of three prognostic molecular entities in this setting (see Section 3). The large variety of molecular alterations identified in children and their overlap with multiple histological entities make it somewhat daunting to devise a simple schema. As the cost of next-generation sequencing is drastically decreasing, and the availability of this technology together with custom gene panels and methylation arrays is increasing, it is a matter of time before a new classification is adopted. Indeed, several large centers are already using these techniques to refine diagnosis and provide alternative options in the case of targeted therapies, especially in the case of *NTRK* fusions and *BRAF* mutations, for which clinical responses are often rapid and drastic.

## 8. CONCLUSIONS AND FUTURE DIRECTIONS

This review highlights how LGGs are neurodevelopmental diseases with unique molecular alterations that are neuroanatomical and age specific. The large variety of molecular alterations, which closely follow brain development, identified with genetics-based tools shed light on the pathogenesis of these tumors and have opened increasing therapeutic possibilities, some already at the bedside. Several gaps remain to be tackled if we are to fully improve the outcome of LGGs, especially in the adult setting. Little is known about the influence of background genomic modifiers, which are also important because they dictate how these mutations may impact preneoplastic cells and how nonneoplastic stromal cells respond to an evolving glioma. Recent unpublished data on single-cell sequencing of pilocytic astrocytomas indicate large sets of immune and vascular cells mingled with varying degrees of mature astrocytes in tumors. Also, with new insights coming from comprehensive genetic analyses of LGGs, future availability of accurate small-animal models of these diseases will help researchers design experimental platforms to critically evaluate each contributing factor in isolation, define its responsible mechanism, and translate these findings into improved risk stratification models and treatment strategies for these diseases. *BRAF* fusions, which may be the sole known driver in a given tumor, can nevertheless appear to be present in less than 10% of cells in some tumors, raising the question of what is affecting tumorigenesis in cells

that do not to carry the fusion. Moreover, clinical responses to targeted therapies in *BRAF*-fused pilocytic astrocytomas are much less striking than in *BRAF*-mutant tumors and are highly inconsistent from one patient to another, while *IDH*-mutant gliomas remain intractable. Regardless of the remaining issues to be tackled and the degree of complexity to the entities to be unraveled, the progress made in the last decade is immense and the use of genetic tools in LGGs has drastically improved diagnosis, prognosis, and outcome.

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## Errata

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