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INVITED REVIEW



Brain Pathology

The 2021 WHO Classification of Tumors of the Central Nervous System: An update on pediatric low-grade gliomas and glioneuronal tumors

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Abstract

The 2021 5th edition of the WHO Classification of Tumors of the Central Nervous System reflects the discovery of genetic alterations underlying many central nervous system (CNS) neoplasms. Insights gained from technologic advances and novel applications in molecular diagnostics, including nextgeneration sequencing and DNA methylation-based profiling, coupled with the recognition of clinicopathologic correlates, have prompted substantial changes to CNS tumor classification; this is particularly true for pediatric low-grade gliomas and glioneuronal tumors (pLGG/GNTs). The 2021 WHO now classifies gliomas, glioneuronal tumors and neuronal tumors into 6 families, three of which encompass pLGG/LGNTs: "Pediatric type diffuse low-grade gliomas," "circumscribed astrocytic gliomas," and "glioneuronal and neuronal tumors." Among these are six newly recognized tumor types: "diffuse astrocytoma, MYB or MYBL1-altered"; "polymorphous low grade neuroepithelial tumor of the young (PLNTY)"; "diffuse low-grade glioma-MAPK altered"; "Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC)"; "myxoid glioneuronal tumor (MGT)"; and "multinodular and vacuolating neuronal tumor (MVNT)." We review these newly recognized entities in the context of general changes to the WHO schema, discuss implications of the new classification for treatment of pLGG/LGNT, and consider strategies for molecular testing and interpretation.

KEYWORDS

2021 CNS WHO, glioneuronal tumors, low-grade gliomas

1 | INTRODUCTION

Low grade gliomas and glioneuronal tumors represent over 30% of pediatric CNS neoplasms, rendering them the most frequently encountered brain tumors in children, though they remain relatively rare [1–3]. While pediatric low-grade gliomas and glioneuronal tumors (pLGG/GNTs) were understudied for years as a consequence, the last two decades have witnessed revolutionizing insights into their genetic drivers, largely on the strength of technologic advances and novel applications in molecular diagnostics [4].

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Under the umbrella of pLGG/GNTs reside many tumor types and subtypes. The histologic diversity of these tumors was detailed over years of traditional microscopic examination and immunohistochemical study. This formed the backbone of CNS tumor classification, but, while some characteristic histologic features proved so readily distinguishable as to define certain tumor types, purely histologic classification was acknowledged as fraught with difficulties. This was particularly true of the pLGG/GNTS, many of which demonstrated overlapping morphologies. As with all brain tumors, furthermore, limited tumor sampling potentially obscured an appreciation of salient features.

Molecular data have surmounted these challenges to some extent, such that several brain tumor entities are now defined by specific molecular alterations. In this respect, the 2021 5th Edition of the WHO Classification of Tumors of the Central Nervous System represents an extension of the changes first introduced by the 2016 4th Edition [5]. Notable changes include the recognition of several new tumor types, with an accepted WHO grade. Furthermore, throughout the 5th edition of the WHO, the overarching presence of methylome profiling is keenly felt: the utility of this methodology is particularly of interest in low grade gliomas and glioneuronal tumors, which have been shown to segregate primarily based on underlying genetic alteration (FGFR1, MYB/ MYBL1, BRAF, or IDH1/2) when subjected to hierarchical clustering analysis of their DNA methylation profiles [6]. For pLGG/GNTs, a significant body of data has emerged to demonstrate that alterations in the MAP kinase pathway are almost universal across histologic entities, although these alterations may take many forms and are not always disease-defining. Therefore, the classification system introduced in the 2021 WHO represents a system in flux, a state well-reflected in the tumors considered pLGG/GNTs: a given genetic alteration may be the defining feature of the tumor or tumor subtype; it may play a supporting role in the diagnosis; it may be shared across several distinct tumors; or it may be among a number of alterations enriched in a single tumor type. There also remain diagnoses for which no alteration need be demonstrated, and those where the alteration is yet to be discovered. The increased complexity as reflected in the 2021 WHO "hybrid taxonomy" reflects our current understanding of the clinical, histologic and molecular features of CNS tumors, and paves the way for further precision in tumor classification and a shift towards increased use of targeted therapeutics.

The WHO 2021 now classifies gliomas, glioneuronal tumors and neuronal tumors in 6 different families, under which 3 are tumor types consistent with pLGG/ LGNT: (1) Pediatric type diffuse low-grade gliomas, (2) circumscribed astrocytic gliomas and (3) glioneuronal and neuronal tumors. (See Table 1 for a complete list of tumors listed in these families). Moreover, six of the fourteen newly recognized tumor types in the 2021 WHO could be considered pLGG/GNTs. Under the banner of "pediatric type diffuse low-grade gliomas" are three new tumors: "diffuse astrocytoma, *MYB* or *MYBL1*altered"; "polymorphous low grade neuroepithelial tumor of the young (PLNTY)"; and "diffuse low-grade glioma-MAPK altered." Glioneuronal and neuronal tumors remain grouped together, to which three new tumor types have been added, the first of which is provisional: "Diffuse glioneuronal tumor with oligodendrogliomalike features and nuclear clusters (DGONC)"; "myxoid glioneuronal tumor (MGT)"; and "multinodular and vacuolating tumor (MVNT) [7]."

Here we review these newly recognized tumor types in further detail and in context of general changes to the WHO schema. We then discuss implications of the new classification for treatment of pLGG/LGNT and consider strategies for molecular testing and interpretation.

2 | DIFFUSE ASTROCYTOMA, MYB- OR MLBL1 ALTERED

The 2016 edition of the WHO acknowledged the entity of angiocentric glioma, which, in addition to its histopathological features (monomorphous bipolar spindled cells with perivascular growth pattern), is defined by characteristic MYB-QKI gene fusion [6, 8]. The functional consequence of this fusion is loss of the tumor suppressor function of OKI combined with activation of MYB [9-11]. MYB functions as a protooncogene, critical for proliferation and differentiation; alterations of *MYB* have been described in other human malignancies [12-15]. A shared biological consequence of high amplification, or truncation of either the 3' UTR regulatory site or the inhibitory C terminal domain, is the increased expression of MYB itself [12, 13]. Within the same MYB gene family of transcriptional regulators is MYBL1. Though much less studied, MYBL1 shares with MYB similar structure and functions, resulting in their being commonly grouped together despite non-overlapping expression and unique protein interaction profiles [12, 13, 16].

The category of "diffuse astrocytoma, *MYB* or *MYBL1*-altered" encompasses a subset of pLGGs not bearing the characteristic histologic features of angiocentric glioma, but demonstrating recurrent amplifications and structural variants of *MYB* and *MYBL1* [5, 14, 15], including fusions with various gene partners. Although "diffuse astrocytoma, *MYB* or *MYBL1*-altered" falls under the rubric of pediatric diffuse gliomas, there appears to be a related group of diffuse gliomas that occur predominately in adults, the so-called "isomorphic glioma." These tumors are

TABLE 1 Tumors in three families in the 2021 WHO 2021 including pLGG/ LGNTs

L TUMORS		Brain 3 of 1
		Pathology
Pediatric-type diffuse low-grade gliomas	Circumscribed astrocytic gliomas	Glioneuronal and neuronal tumors
Diffuse astrocytoma, MYB- or MYBL1-altered	Pilocytic astrocytoma	Ganglioglioma
Angiocentric glioma	Pleomorphic xanthoastrocytoma	Desmoplastic infantile ganglioglioma / desmoplastic infantile astrocytoma
Polymorphous low-grade neuroepithelial tumor of the young (PLNTY)	Subependymal giant cell astrocytoma	Dysembryoplastic neuroepithelial tumor
Diffuse low-grade glioma, MAPK pathway-altered	Chordoid glioma	Diffuse glioneuronal tumor with oligodendroglioma- like features and nuclear clusters
	High-grade astrocytoma with piloid ^a features	Papillary glioneuronal tumor
	Astroblastoma, MN1-altered ^a	Rosette-forming glioneuronal tumor
		Myxoid glioneuronal tumor
		Diffuse leptomeningeal glioneuronal tumor
		Gangliocytoma
		Multinodular and vacuolating neuronal tumor
		Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)
		Central neurocytoma
		Extraventricular neurocytoma
		Cerebellar liponeurocytoma

Note: Pediatric type diffuse low-grade gliomas, circumscribed astrocytic gliomas, and glioneuronal and neuronal tumors. Newly defined tumors reviewed here are listed in bold.

^aNewly defined under the family of "circumscribed astrocytic gliomas," these are high-grade, aggressive tumors and outside the scope of this review, but highlighted here for completeness.

typically well-differentiated, low to moderately cellular glial neoplasms, comprised of astrocytes with small, rounded nuclei and regular chromatin structure, with low proliferative indices. Clinically, they are supratentorially located and associated with seizures [17-19]. Isomorphic gliomas preferentially display alterations of *MYBL1*, rather than *MYB*, including gene fusions, some of which also commonly characterize pediatric MYB/MYBL1-altered gliomas [18]. Despite these similarities, it should be noted that analysis of the methylation profiles of a group of isomorphic diffuse gliomas found these to form a distinct cluster, albeit one closely related to MYB/MYBL1-altered diffuse astrocytomas occurring in children, as well as angiocentric gliomas [18].

The addition of "diffuse astrocytoma, MYB or MYBL1-altered" accomplishes the critical distinction of these indolent diffuse IDH-wild type gliomas from their "adult" counterparts. In the prior edition of the WHO, diffuse gliomas with MYB/MYBL1 alterations could conceivably be simply categorized as diffuse astrocytoma, IDH-wildtype, in the absence of a broad genomic characterization. Inasmuch as the latter category is chiefly composed of tumors that are molecularly high-grade gliomas (i.e. glioblastoma, WHO grade 4), such classification would be grossly erroneous; evidence indicates that MYB/MYBL1 altered diffuse gliomas in both children and adults are generally indolent and usually behave in WHO grade 1 fashion. [18, 20]

3 | POLYMORPHOUS LOW-GRADE NEUROEPITHELIAL TUMORS OF THE YOUNG (PLNTY)

Recently described by Huse et al. in 2017, "polymorphous low grade neuroepithelial tumors of the young (PLNTY)" comprise a group of neoplasms which, while morphologically variable, share histologic characteristics of infiltrative growth, oligodendroglioma-like components, frequent calcification and strong cluster of differentiation 34 (CD34) immunohistochemical expression [21,22]. While prominent oligodendrocyte-like components are regularly encountered in PLNTY, fibrillary, spindled, and pleomorphic astrocytic components may also be present, as well as focal perivascular pseudo-rosetting [21]. Like many other pLGG/LGNT, PLNTYs have been shown to harbor molecular alterations leading to activation of the MAPK pathway, including FGFR gene family alterations. Among these was described a novel, and as yet PLNTY-specific, fusion transcript involving FGFR2 (including the kinase domain) joined with CTNNA3 (to include the entirety of its C-terminal dimerization domain) [22, 23]; the resultant fusion is thought to lead to homodimerization and autophosphorylation of FGFR2 and downstream MAPK/ PI3K/mTOR pathway activation [24-26]. While FGFR2-CTNNA3 appears to be a relatively specific signature of PLNTY, the molecular landscape of PLNTY includes genetic abnormalities involving BRAF and, on occasion, FGFR3. Molecular profiling of PLNTYs has demonstrated that they carry a distinct DNA methylation signature [6, 15], including a potential epigenetic subgroup defined by FGFR2 fusions [27].

PLNTYs mainly afflict children and young adults and are commonly located subcortically within the temporal lobe. PLNTYs share significant histologic overlap with previous reports of "long-term epilepsy associated tumors (LEATs)" and "pediatric oligodendroglioma" [6, 15, 21, 28]. The weight of evidence points to the benign nature of PLNTY [22, 24, 29, 30]. Malignant transformation of PLNTY has been described in a case demonstrating FGFR3-TACC3 fusion associated with additional genomic alterations (TP53, ATRX, PTEN, TEK and RB1) consistent with more aggressive biology [31]. As alterations in BRAF and FGFR3 are not unique to PLNTY and may be encountered in bona fide high-grade entities, tumors displaying morphologic features of PLNTY should not be regarded as benign without considering the larger clinical, molecular, and histopathologic context.

4 | DIFFUSE LOW-GRADE GLIOMA, MAPK PATHWAY ALTERED

Similar to the category of diffuse glioma with *MYB* or *MYBL1* alterations, the category of "diffuse low-grade glioma, MAPK pathway-altered" is a generic one, comprised

of bland glial proliferations with minimal atypia, an infiltrating growth pattern and astrocytic, oligodendroglial or mixed phenotypic features similar to other WHO grade II diffuse gliomas. While the precise morphologic features appear to be related to the underlying molecular alteration, mitotic activity is absent or rare, and there is no microvascular proliferation or necrosis [5, 32]. Like other pLGG/LGNTs, tumors in this category can be expected to present mainly in children, but occasionally affect adults, and are commonly associated with epilepsy [7, 20].

Genetic alterations qualifying as "MAPK pathway altered" are similarly varied; commonly encountered are FGFR1 tyrosine kinase domain duplications, hotspot mutations, fusion, and BRAF p.V600E mutations. Genes less frequently altered, but also leading to MAPK pathway activation, include NTRK1/2/3, MET, FGFR2, and MAP2K1 [33]. These alterations must be present in the absence of mutations of IDH1/2 and H3F3A, as well as homozygous deletion of CDKN2A. These alterations may also be encountered in other tumor types defined elsewhere in the WHO, including, but not limited to, extraventricular neurocytoma, pleomorphic xanthoastrocytoma, ganglioglioma, pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor (DNET) and rosette-forming glioneuronal tumor [15, 34–36]. See "Overlapping clinical, histologic, and molecular features in pLGG/LGNT."

As one of the "general categories" in the 2021 WHO, an approach similar to that previously employed for subtyping of medulloblastoma, wherein an optimal integrated diagnosis is rendered by combining a term from a histologically defined list of tumors with one from a genetically defined list of tumors, is recommended in this situation. It is expected that some combinations of histological and molecular abnormalities will be more commonly encountered than others [7, 20]. For example, a bland, minimally atypical glial proliferation, lacking eosinophilic granular bodies, Rosenthal fibers, or high-grade features, bearing a BRAF p.V600E mutation may be subtyped under this category as "diffuse low-grade glioma, BRAF p.V600Emutant [7]." Again, as with "diffuse astrocytoma, MYB- or MYBL1-altered", the creation of this category provides an avenue to distinguish these indolent "pediatric-type" diffuse gliomas from their aggressive "adult" IDH-wild type counterparts [7, 20]. DNA methylation profiling does not unify diffuse low-grade gliomas with MAPK pathway alterations into a distinct cluster, however this analysis may be a useful adjunct to exclude other tumor types [36].

5 | DIFFUSE GLIONEURONAL TUMOR WITH OLIGODENDROGLIOMA-LIKE FEATURES AND NUCLEAR CLUSTERS (DGONC)

Currently a provisional entity, DGONCs were originally identified by genome-wide DNA methylation analysis; a

distinctive methylation profile segregated a group of 31 tumors into a novel cluster, separate from the previously recognized methylation classes of CNS tumors included in the extended Heidelberg cohort [32, 37, 38]. On review, the tumors in this category were noted to share histologic features of oligodendroglioma-like perinuclear halos and clustered tumor cell nuclei. Immunohistochemical studies showed these tumors to display strong MAP2 and synaptophysin expression, while being mostly GFAP-negative [5].

Interestingly, some cases in this category demonstrated mitotic activity and elevated proliferative indices [38]. Indeed, the original histopathologic diagnoses of tumors in this novel cluster were varied and included both high and low-grade entities: ependymoma, low grade glioma, DNET, primitive neuroectodermal tumor (a category removed from the 2016 WHO), atypical extraventricular, neurocytoma, glioblastoma, anaplastic oligodendroglioma, and central neurocytoma. From a diagnostic perspective, then, there is little to specifically define DGONC other than its methylation profile. Molecular and cytogenetic analysis has demonstrated that monosomy of chromosome 14 is a highly recurrent copy number alteration in this setting, (present in ~97% of DGONC), yet to date no other defining genetic alterations have been identified. DGONCs, however, seem to lack oncogenic events which are commonly found in other glioneuronal tumors and shared among other pLGG/LGNT, including alterations of BRAF and FGFR genes [37, 39].

While DGONCs occur predominately in young children, cases in adults and elderly patients have been reported. Tumors tend to be localized to the cerebral hemispheres, particularly the temporal lobes. Despite the alarming histologic features of some cases, available data suggest that, in general, DGONCs carries a relatively favorable prognosis: reported 5-year PFS = 79% and 5-year OS = 86% [37]. The full characterization and acceptance of DGONC as a tumor entity awaits analyses of larger cohorts falling into this methylome-defined CNS tumor class.

6 | MYXOID GLIONEURONAL TUMOR (MGT)

The newly recognized myxoid glioneuronal tumor is distinguished by (1) a characteristic location within the septum pellucidum or, less often, corpus callosum and lateral periventricular white matter; (2) recurrent mutations in codon 385 of *PDGFRA*, specifically p. K385L/I [40, 41]. Histologically, MGTs closely resemble DNET and rosette-forming glioneuronal tumors, being composed of oligodendrocyte-like tumor cells in a myxoid background; floating neurons and neurocytic rosettes have also been described. DNA methylation-based profiling suggests a close relationship between MGT and cortically based DNET [41]. MGTs appear to encompass tumors of the septum pellucidum and lateral ventricle

previously described as DNET or rosette forming glioneuronal tumors [42–46].

While alterations in *PDGFRA* are seen in other human cancers, including frequent amplification and mutations in high grade gliomas (glioblastoma), *PDGFRA* codon 385 mutations are thought to be highly specific for MGT [40, 47, 48]. While the precise effects of these mutations are not well understood, they are believed to be oncogenic and are typically present in a genomically quiet background without recurrent co-mutations [40, 47].

Clinically, examples located in the septum pellucidum have been associated with obstructive hydrocephalus. A subset of patients with MGT have been reported to demonstrate local recurrence, progression, and even to present with ventricular dissemination of disease [41, 49]. Nonetheless, although the number of reported cases is small, all patients with MGT described in the literature remained alive after extensive follow-up, including cases followed several (10+) years after diagnosis [41, 49]. Therefore they are thought to be indolent tumors, at the level of other WHO grade I entities [5, 32]. MGT is not strictly a pediatric entity; many affected patients are young, including children, but MGT has also been described in older adults [41, 47].

7 | MULTINODULAR AND VACUOLATING NEURONAL TUMOR (MVNT)

Previously discussed under the category of gangliocytoma in the 2016 update of the WHO, multinodular and vacuolating neuronal tumor (MVNT) now exists as a distinct tumor type in the 2021 WHO [5, 32]. More so than any of the other tumors discussed here under the banner of pLGG/LGNT, MVNT is decidedly not a pediatric tumor as one of the key features distinguishing this lesion from other epilepsy associated neoplasms is an older age at presentation, i.e., adult-onset seizures [50]. Most conventional gangliocytomas occur in young patients [50]. Radiographically, MVNTs are non-contrast enhancing, superficially situated (lying within the cerebral cortex and adjacent white matter), often exhibit mulitnodularity in FLAIR and T2-weighted sequences, commonly involve the temporal lobe, and lack edema or mass effect [50].

Histologically, MVNTs consist of a combination of well-defined and coalescing nodules of neuronal tumor cells, with frequent vacuolar change of cytoplasm and matrix [50]. Immunohistochemical stains for the neuronal markers HuC/HuD demonstrate widespread nuclear, and at far less intensity, cytoplasmic expression in tumor cells, although synaptophysin labelling is variable and other neuronal markers, including neurofilament and chromogranin, are typically not expressed [49–51]. The neoplastic neurons are generally mid-sized, but may be small or, occasionally, ganglion cell-like. Other features

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commonly associated with gangliocytoma and other glioneuronal tumors, including eosinophilic granular bodies, microcalcifications, and an associated inflammatory infiltrate, are notably absent in MVNT [52].

CD34-labeling ramified neural elements, microhamartia and cortical dysplasia have been noted in the surrounding cortex in several cases of MVNT [52]. These findings initially raised the possibility that MVNT represent ted a developmental (dysplastic) or hamartomatous, rather than neoplastic, process [52–54]. This seemed unlikely given the predilection for adult patients, and subsequent molecular analysis has confirmed that, similar to other pLGG/ LGNTs, MVNTs display solitary alterations leading to activation of the Ras-Raf-MAP kinase signaling pathway. Alterations include small indels and hotspot mutations in MAP2K1 and non-canonical BRAF mutations (i.e., other than BRAF p.V600E), while more common MAPK pathway drivers were notably absent [52, 55, 56]. MVNT may rarely be associated with high grade glioma, particularly in the setting of homozygous deletion of CDKN2A/B [54].

8 | DISCUSSION

8.1 | Overlapping clinical, histologic, and molecular features in pLGG/LGNT

While we have highlighted their characteristic genetic alterations (summarized in Table 2), as well as distinguishing clinico-radiographic, and histologic aspects, in general, LGG/LGNTs share overlapping morphologic features (see Figure 1). These include oligodendrocytelike cells, myxoid change, dysmorphic neuronal components, and an association with ramified CD34-expressing neural elements. Examples may not always display signature characteristics and cardinal features (such as nodularity in MVNT) may not be readily apparent in biopsy material.

Many tumors described under the pLGG/LGNT rubric have also been described as LEATS, and share a presentation in young patients, indolent behavior, and a tendency to occur in the neocortex with a predilection for the temporal lobes [57]. Also shared are a frequent association with cortical disorganization/dysplasia, microhamartia, and aberrant, ramified CD34-expressing neural elements [52–54, 57, 58].

Many of the tumors considered pLGG/LGTs further share a common underlying biology in that they are primarily driven by MAPK/ERK pathway (also referred to as RAS/RAF/MEK/ERK pathway) activation, albeit as a result of a variety of driver alterations. The widespread involvement of dysregulated MAPK signaling attests its importance as an overarching oncogenic mechanism, while the histologic diversity of pLGG/LGNTs suggests that the ultimate tumor phenotype may be dependent on the specific biological context and cell of origin. Inasmuch as the tumor methylome reflects (at least in part) cell of origin, DNA methylation-based classification may offer particular utility in distinguishing members of this broad family.

2021 WHO Classification of Tumors of the CNS Tumor Type	Characteristic genetic feature(s)
Diffuse astrocytoma, MYB- or MYBL1-altered	MYB and MYBL1 amplifications or fusions
Polymorphous low-grade neuroepithelial tumor of the young (PLNTY)	MAPK pathway alterations (including <i>FGFR2</i> fusions)
Diffuse low-grade glioma, MAPK pathway-altered	MAPK pathway alterations (including BRAF p.V600E and FGFR1 alterations)
Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC)	methylation profile; frequent monosomy 14
Myxoid glioneuronal tumor	PDGFRA p. K385L/I
Multinodular and vacuolating neuronal tumor (MVNT)	MAPK pathway alterations (commonly <i>MAP2K1</i> and non-canonical <i>BRAF</i> mutations)

TABLE 2Summary of characteristicgenetic features of six newly definedpLGG/GNTs in the 2021 WHO



FIGURE 1 Newly defined pLGG/GNT entities in the 2021 WHO often demonstrate overlapping histologic features with other glial and glioneuronal tumors: Myxoid/mucinous component in a diffuse astrocytoma, *MYB*-altered (bearing MYB-QKI fusion, A), a diffuse low-grade glioma, *FGFR1*-mutant, (*FGFR1* p.N546K mutant, B), and myxoid glioneuronal tumor (septal location, *PDGFRA* p.K385L mutant, C). Oligodendroglioma-like cells in the same diffuse low-grade glioma, *FGFR1*-mutant, (*FGFR1* p.N546K mutant, D) and a PLNTY with microcalcification (bearing FGFR2-CTNNA3 fusion, E and F). A tumor showing histologic features of DGONC, but lacking monosomy 14, did not match to a known methylation class, and was instead found to be *NTRK2*-fusion positive (G). Vacuolization of cells and nodular growth pattern in an MVNT, (*MAP2K1*-mutant, H, I). Scale bars in A, C, D, E, G and H = 100 µm; Scale bars in B, F, I = 200 µm

8.2 | First, do no harm: clinical implications of molecular characterization of pLGG/LGNT

The 2021 WHO generally avoids recommendation of specific assays for molecular characterization of brain tumors (with some exceptions). Indeed, the number of molecular events that potentially require query is large and a "gold standard" approach has not been accepted; instead, it is generally held that the testing strategy should be chosen dependent on the specific diagnosis in question: ancillary testing should be conducted while bearing in mind diagnostic and therapeutic objectives, tissue stewardship, and financial costs. Common testing strategies for molecular profiling of pLGG/LGNT have been extensively reviewed elsewhere [4], and several tier-based approaches have been proposed [4, 59]. While specific practice recommendations are outside the scope of this review, it is to be hoped that the changes to the 2021 WHO will further underscore the importance of molecular diagnostics and will encourage an era of collaboration and increased access to expert neuropathologists, academic medical centers and laboratories with

the ability to provide the required testing. As in the prior edition of the WHO, the option to use the designation "not otherwise specified (NOS)" remains available for use in cases where diagnostic information (including molecular features) necessary to assign a specific WHO diagnosis is not available. A designation of "NOS" is intended to indicate that a full molecular workup has not been undertaken or was not successful [5, 32]

Beyond assessment for a particular disease-defining alteration, broad molecular characterization excludes the presence of alterations associated with other tumor types and allows for a deeper query of underlying biology. Broad approaches yielding mutation, fusion and copy number data may further disclose alterations more typically associated with high grade tumor biology (deletions of *CDKN2A/2B*, amplifications of receptor tyrosine kinases etc.) that may portend an aggressive disease course [31, 60, 61]. It is important to bear in mind, however, that for pLGG/LGNTs with low cellularity, some techniques, including methylome profiling, may reach the limit of sensitivity of the assay. Negative or inconclusive results should be interpreted with caution [37].

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The expansion of molecular criteria for classification of pLGG/LGNTs paves the way for more precision and greater confidence in therapeutic decision-making. For most pLGG/LGNTs, the consistent benefit of surgery, in particular gross total resection, has been demonstrated with regard to progression-free and overall survival, and this may prove curative [62–66]. Because pLGG/LGNTs can be expected to follow an indolent course, the need for front-line chemotherapeutics or adjuvant therapy for residual or recurrent tumor can be guided by the full clinical context; a "wait and watch" approach may be considered, wherein additional intervention awaits the emergence of incontrovertible clinical or radiographic progression [67, 68]. Definitive classification of tumors as low-grade prevents over-treatment, with avoidance of toxicity and long-term side-effects being of paramount importance in the pediatric population, particularly for interventions with unclear outcome benefits [62, 63, 69, 70].

We can now appreciate that the detection of a given molecular alteration may not be sufficient to precisely classify a particular tumor. This is particularly true of pLGG/LGNTs, many of which share MAPK-pathway activating alterations. It is worth noting that even alterations which may not be disease-defining may still be therapy-relevant. For example, *BRAF* p.V600E has been identified across a number of glial and glioneuronal tumors, both low and high grade. The roster of driver alterations that may serve as treatment targets is likely to expand through the results of ongoing clinical trials. In turn, future trials will be shaped by the restructured classification system for CNS tumors and further refinements to come.

CONFLICTS OF INTEREST None.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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