Review Article

2021 updates to the World Health Organization classification of adult-type and pediatric-type diffuse gliomas: a clinical practice review

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Abstract: In 2021, the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) underwent significant restructuring to incorporate additional molecular diagnostics, several newly recognized tumor types, and new grading schemes for existing tumor types. The 2021 CNS WHO classification further elaborates and integrates histopathologic and molecular diagnostic criteria to improve diagnostic classification. Furthermore, it is the hope that identification of molecular alterations in pediatric and adult tumors facilitates improved prognostic information and development of novel targeted therapies for adults and children with CNS tumors. In one of the largest changes in the new WHO classification, diffuse gliomas are divided into pediatric-type and adult-type gliomas to highlight our expanding knowledge of their different molecular drivers and prognostic associations. Several new pediatric-type diffuse low-grade gliomas are defined including (I) diffuse astrocytoma, *MYB*- or *MYBL1*-altered, (II) polymorphous low-grade neuroepithelial tumor of the young (PLNTY), and (III) diffuse low-grade glioma, MAPK-pathway altered. In addition, several new pediatric-type diffuse high-grade gliomas are recognized including (I) diffuse hemispheric glioma, H3 G34R-mutant (II) diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, and (III) infant-type hemispheric glioma. These new tumor types have associated clinical, genetic and epigenetic features that are distinct from adult-type diffuse gliomas. This review provides an overview of updates in the 2021 CNS WHO classification specific to diffuse gliomas, with a particular focus on the histopathology and molecular findings of the newly described pediatric-type low-grade and high-grade gliomas.

Keywords: World Health Organization (WHO); brain tumors; diffuse gliomas; adult gliomas; pediatric gliomas

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Introduction

The World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) serves as the international standard for the diagnosis of brain and spinal cord tumors. The new fifth edition of the WHO classification was published online in 2021 and made available in print in 2022 (1). The fifth edition incorporates numerous refinements and advances since the publication of the 2016 revised fourth edition, including interim recommendations from the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy-Not Official WHO (cIMPACT-NOW) working groups (2-9).

Major updates in the fifth edition center upon tumor taxonomy, nomenclature and grading (10). Fourteen new tumor types are included, as are new tumor subtypes with particular clinical relevance. Our evolving understanding of the molecular drivers of CNS tumors, together with

increasing accessibility of novel diagnostic techniques such as DNA methylation profiling, facilitated the recognition and characterization of these additional tumor types and subtypes. Integration of molecular diagnostics with histomorphologic features is now required for several tumor types, and a layered reporting structure for diagnoses is recommended to include all relevant histologic and molecular data. In practice, this translates to an initial preliminary histologic diagnosis followed by a final integrated histologic and molecular diagnosis with WHO grade.

In a new approach to the WHO classification, gliomas, glioneuronal tumors and neuronal tumors are grouped into six families. For the first time, diffuse gliomas are divided into 'adult-type diffuse gliomas', 'pediatric-type diffuse low-grade gliomas', and 'pediatric-type high-grade gliomas' that reflect the clinical behavior and molecular differences between tumor types that arise mostly in adults and those occurring mainly in the pediatric or infant population. The remaining three tumor families include circumscribed (non-diffuse) astrocytic gliomas, glioneuronal and neuronal tumors, and ependymal tumors.

As mentioned, diffuse gliomas in particular have undergone significant changes in nomenclature. Adult-type diffuse glioma classification has been significantly streamlined to three main tumor types, in contrast to the 15 different entities in the 2016 WHO classification. This was accomplished in part by changes in assigning tumor grades within tumor types, and eliminating the term 'anaplastic' to indicate a WHO grade 3 astrocytoma or oligodendroglioma. As such, *IDH*-mutant astrocytomas are now graded within tumor type (CNS WHO grade 2, 3 or 4) based on their histologic and molecular features. Furthermore, the increasing application of molecular diagnostics to tumor classification has decreased 'not otherwise specified (NOS)' and 'not elsewhere classified (NEC)' diagnoses that were previously included for each type of diffuse glioma in the 2016 WHO classification. Finally, the histopathologic diagnosis of oligoastrocytoma no longer exists due to the reclassification of the majority of these tumors based on their defined molecular alterations. Several excellent recent reviews highlight the changes to adult-type glioma classification and their clinical relevance (11-13).

Multiple newly recognized pediatric-type diffuse low-grade and high-grade gliomas are defined in the new WHO classification. For some of these tumor types, insufficient prospective outcome data exist to assign a definitive WHO grade. In many cases, the potential efficacy of small molecular inhibitors or other targeted therapies is unknown and may affect future prognostication. This review focuses on general updates in the 2021 edition of the CNS WHO classification specific to diffuse gliomas, with a particular focus on the histopathologic features of the newly recognized pediatric-type low-grade and high-grade tumor types. Examples of several tumor types are presented, including some representing ongoing diagnostic challenges.

Adult-type diffuse gliomas

Astrocytoma, *IDH-mutant*, *CNS WHO grade 2*, *3 or 4*

The tumor type astrocytoma, *IDH*-mutant, encompasses all diffusely infiltrating astrocytomas with *IDH1* or *IDH2* mutations that lack chromosome 1p/19q codeletion. *P53* and *ATRX* mutations are also frequently present. Astrocytoma, *IDH*-mutant, includes CNS WHO grade 2, 3 and 4 tumors and eliminates the prior terminology of anaplastic astrocytoma, *IDH*-mutant, and glioblastoma, *IDH*-mutant. In addition to defined histologic criteria, the updated grading scheme further incorporates *CDKN2A/B* status to better elucidate the expected biologic behavior of these tumors.

Most *IDH*-mutant tumors are supratentorial, however, *IDH*-mutant gliomas do rarely occur in the infratentorial compartment, most often with *IDH1/IDH2* variants other than *IDH1* R132H (14,15). CNS WHO grade 2 and 3 *IDH*-mutant astrocytomas occur most frequently in young adults in their thirties or forties (16,17). CNS WHO grade 4 tumors tend to arise slightly later in the fourth or fifth decade (17). *IDH*-mutant astrocytomas are very rare in the pediatric population (18).

On histopathology, *IDH*-mutant astrocytomas are typically composed of fibrillary glial cells showing variable degrees of nuclear atypia. In CNS WHO grade 2 tumors, the overall cellularity is low to moderate. Tumor cell nuclei are ovoid and mostly monomorphic. Occasionally tumor cell nuclei may be quite round, raising initial diagnostic consideration for an oligodendroglioma. Mitotic activity is usually absent or extremely low in CNS WHO grade 2 tumors (e.g., a single mitosis is identified in a large resection specimen). Increased mitotic activity is diagnostic of a CNS WHO grade 3 tumor, although a threshold for total mitoses has yet to be established and can complicate accurate diagnosis in some cases. CNS WHO grade 3 tumors are often more hypercellular and tumor nuclei may be more atypical. The presence of tumor necrosis and/or

microvascular proliferation is required for histologically defined CNS WHO grade 4 tumors.

On immunohistochemistry, tumor cells are immunoreactive for the transcription factor OLIG2 and show variable immunoreactivity for GFAP. Tumors with an *IDH1* R132H mutation stain positive with the mutation-specific antibody; other *IDH1* and *IDH2* variant astrocytomas are negative. The majority of tumors will show strong expression of p53 and loss of ATRX staining, consistent with underlying *TP53* and *ATRX* mutations. Additional sequencing analysis is required to identify the approximately 10% of cases with an *IDH1* or *IDH2* mutation other than p.R132H to reach the appropriate diagnosis (19). DNA methylation profiling also reliably identifies *IDH*-mutant astrocytomas including low-grade and high-grade *IDH*-mutant astrocytoma subgroups (20).

In addition to the previously described histopathologic criteria, multiple recent studies demonstrate that clinical outcomes are highly associated with *CDKN2A/B* status (21,22). Homozygous deletion of *CDKN2A* or *CDKN2B* in *IDH*-mutant astrocytomas markedly decreases overall survival and is considered diagnostic of a CNS WHO grade 4 tumor in the updated WHO classification, even in the absence of microvascular proliferation or necrosis. Furthermore, there is evidence that *CDKN2A* loss in histologically defined CNS WHO grade 4 tumors is associated with worse clinical outcomes (21,23).

Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, CNS WHO grade 2 or 3

Oligodendroglioma is defined as a diffusely infiltrating glioma with either *IDH1* or *IDH2* mutation and co-deletion of chromosomes 1p and 19q. Oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted, is still designated CNS WHO grade 2 or 3 in the updated WHO classification depending on the presence of various histologic features described below. As previously mentioned, the terminology of anaplastic oligodendroglioma was eliminated in the 2021 CNS WHO classification. There is no oligodendroglioma tumor corresponding to CNS WHO grade 4.

Oligodendrogliomas are typically cortically-based tumors and most often arise in the frontal lobe in adult patients (24). They occur across a wide age spectrum from 20 to 75+ years, with a median age of 41 years for CNS grade 2 tumors and 47 years for CNS grade 3 tumors (25). Oligodendrogliomas are exceptionally rare in children, and most tumors formerly designated 'pediatric oligodendroglioma' are now classified as other pediatric-type diffuse low-grade gliomas with defined molecular features. In addition to the frontal lobe, other locations in the cerebrum include the temporal, parietal or rarely occipital lobe. Very rare case reports describe oligodendrogliomas arising in the posterior fossa, brainstem or demonstrating diffuse involvement of multiple bilateral brain areas in a gliomatosis cerebri pattern (26-28). Recurrent disease is more likely to show leptomeningeal or intraventricular spread (27,29,30).

On routine histopathology, oligodendrogliomas are predominantly composed of monomorphic glial cells with round nuclei that classically show perinuclear clearing imparting the so-called "fried-egg" appearance. Oligodendrogliomas, in particular CNS WHO grade 3 tumors, may also show gemistocytic-type cells with abundant rounded eosinophilic cytoplasm and eccentrically placed nuclei. Scattered tumor microcalcifications are common. CNS WHO grade 2 oligodendrogliomas typically show a branching network of thin-walled capillaries reminiscent of chicken wire. CNS WHO grade 3 tumors often show microvascular proliferation. Mitotic activity and Ki-67 proliferation index are low in CNS WHO grade 2 tumors and increased in CNS WHO grade 3 tumors, but definite thresholds are not established. CNS WHO grade 3 tumors typically show a combination of features that include high cellularity, marked cytologic atypia, necrosis, microvascular proliferation and brisk mitotic activity. Homozygous deletion of *CDKN2A* and/or *CDKN2B* has been reported in a small subset of CNS WHO grade 3 oligodendrogliomas with aggressive behavior and thus may represent a biomarker in cases with borderline grade 3 histologic features (22).

Immunohistochemical stains are very helpful in the diagnostic workup of oligodendrogliomas. Most tumors show immunoreactivity for *IDH1* R132H. In contrast to *IDH*-mutant diffuse astrocytomas, P53 is typically negative and ATRX nuclear staining is retained. Additional sequencing analysis or fluorescence/chromagen-based in situ hybridization assays are required to confirm whole arm loss of chromosome 1p and 19q. Fluorescence in situ hybridization (FISH) testing may occasionally lead to false positive results in cases of partial loss of 1p or 19q. *TERT* promoter mutations are identified in a majority of oligodendrogliomas, but the finding of a *TERT* promoter mutation in an *IDH*-mutant glioma is not diagnostic of oligodendroglioma as it is can be present in some *IDH*-mutant astrocytomas (31-33). DNA methylation profiling reliably classifies oligodendroglioma, *IDH*-mutant and

1p/19q-codeletion (20). Unsupervised clustering analysis can demonstrate distinct methylation clusters that correlate with progression-free survival, suggesting DNA methylation profiling may have predictive value (34).

Glioblastoma, IDH-wildtype, CNS WHO grade 4

Glioblastoma, *IDH*-wildtype, remains the most common malignant adult-type diffuse glioma (24,35). This tumor type occurs most frequently in older adults, but can arise at any age. Tumors may arise anywhere in the CNS, but are most often supratentorial and involve the subcortical white matter and deep gray matter (25). Tumor cells widely infiltrate the CNS parenchyma and can extend to the cortical surface, cross the corpus callosum, infiltrate the brainstem and spinal cord, and appear multifocal with contiguous spread usually along white matter tracts.

Glioblastoma is histologically defined as a high-grade, diffusely infiltrating astrocytoma with microvascular proliferation and/or necrosis. Glioblastoma, *IDH*-wildtype, is by definition both *IDH*-wildtype and H3-wildtype. Tumors with these mutations are classified as astrocytoma, *IDH*-mutant, or as H3 K27-altered or H3 G34-mutant gliomas, respectively. Furthermore, the updated CNS WHO classification also specifies that *IDH*-wildtype glioblastoma may be genetically defined by the presence of *TERT* promoter mutation, *EGFR* amplification, and/or the combination of whole chromosome 7 gain with whole chromosome 10 loss (+7/–10) even in cases without microvascular proliferation or necrosis (1,4,36). An integrated diagnosis of 'diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma, CNS WHO grade 4' is recommended to incorporate all relevant data in cases with discordant histologic and molecular features (4).

On histopathology, *IDH*-wildtype glioblastomas are hypercellular, diffusely infiltrative astrocytic tumors composed of cells with variable degrees of differentiation and nuclear atypia. Tumor cells widely infiltrate the parenchyma and often exhibit secondary structuring with subpial, perivascular and perineuronal aggregates of tumor cells. Glioblastomas can have markedly heterogeneous morphology even within the same tumor. Anaplastic tumor cells appear small, round and hyperchromatic. Gemistocytic tumor cells have abundant glassy eosinophilic cytoplasm and eccentrically placed irregular nuclei. Tumors cells may be spindled or epithelioid in shape and have granular, lipidized or minimal cytoplasm. Multinucleated giant tumor cells may be focal or widespread. Nodules of primitive cells showing neuronal differentiation may be present. Tumor cells are arranged in sheets, nests, or fascicles. Certain morphologic patterns are considered distinct subtypes of glioblastoma including giant cell glioblastoma, gliosarcoma, and epithelioid glioblastoma (1).

Mitotic activity is present in all glioblastomas, and often markedly increased. Microvascular proliferation and either pseudopalisading or geographic areas of tumor necrosis are very frequent. At least one of these two features must be present for a histologic diagnosis of glioblastoma.

IDH-wildtype glioblastomas have a distinct DNA methylation profile that is considered sufficient for diagnosis, and may be very helpful in cases that are diagnostically challenging (20). Molecular subgroups of *IDH*-wildtype glioblastoma can also be distinguished by their methylome profile, include RTK1, RTK2 and mesenchymal subtypes in adult patients (20,37).

Pediatric-type diffuse low-grade gliomas

Diffuse astrocytoma, MYB- or MYBL1-altered, CNS WHO grade 1

Diffuse astrocytoma, *MYB*- or *MYBL1*-altered, is a newly defined tumor type in the updated CNS WHO classification (1). These are low-grade tumors frequently diagnosed in the setting of medically refractory epilepsy since childhood and thus included among the group of long-term (low-grade) epilepsy associated tumors (LEATs) (38-40). This tumor type commonly involves cortical and subcortical regions of cerebral cortex and may arise in any lobe (38,41). On magnetic resonance imaging (MRI), the typical findings are a well-delineated, occasionally infiltrative-appearing, non-enhancing T1-hypointense, T2-fluid attenuated inversion recovery (FLAIR)-hyperintense lesion without restricted diffusion (40,41). Diffuse astrocytoma, *MYB*- or *MYBL1*-altered, is a CNS WHO grade 1 tumor. Long-term outcomes following surgical resection are good from both oncologic and seizure-control perspectives (40,41).

On histomorphology, tumors range from minimally to moderately hypercellular and show diffuse infiltration by a population of monomorphic cells with ovoid to elongated nuclei (*Figure 1*) (41,42). This characteristically bland morphology is reflected in previous nomenclature of isomorphic diffuse glioma (40). Mitotic activity is absent or very low and corresponds to a low Ki-67 proliferation index (40). Microvascular proliferation or necrosis are not

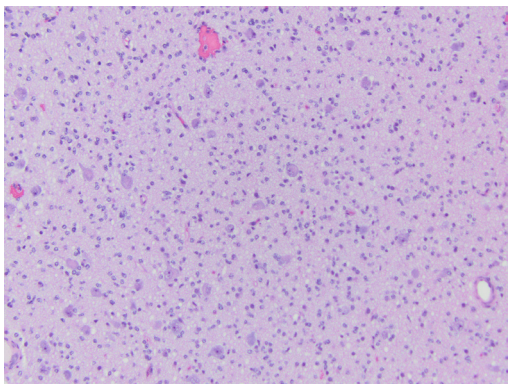

Figure 1 Diffuse astrocytoma, *MYBL1*-altered, in a pediatric patient. H&E stained section passes through a moderately hypercellular, diffusely infiltrating glial neoplasm. Tumor cells are relatively monomorphic with ovoid-to-elongated nuclei, scant cytoplasm, arranged in a fibrillary background. Scattered parenchymal neurons are present, highlighting the infiltrative nature of the tumor. Mitotic activity is absent. A *MYBL1-KHDRBS3* gene fusion was identified in this tumor, and the methylation profile matched with high confidence to diffuse astrocytoma, *MYB*- or *MYBL1*-altered. Image taken at ×20. H&E, hematoxylin and eosin.

present. Tumor cells are immunoreactive for GFAP and may show negative immunostaining for Olig2, although this does not appear to be universal (40,41).

Molecular diagnostic testing that demonstrates fusion between the *MYB* or *MYBL1* genes and a partner gene confirms the diagnosis (6,40-43). A DNA methylation profile matching to diffuse astrocytoma, *MYB*- or *MYBL1*-altered further supports the diagnosis (20,41). Diffuse astrocytoma with *MYB*- or *MYBL1*-fusion and angiocentric glioma with *MYB-QKI* fusion share many morphologic, genetic, and epigenetic similarities, but are considered separate tumor types in the current WHO classification (1). In older adolescents and young adults, it is important to distinguish diffuse astrocytoma, *MYB*- or *MYBL1*-altered, from *IDH*-mutant and *IDH*-wildtype diffuse gliomas with similar histomorphologic features but more aggressive behavior.

Angiocentric glioma, CNS WHO grade 1

Angiocentric glioma is classified as pediatric-type diffuse glioma in the updated CNS WHO classification. Angiocentric gliomas are rare tumors typically affecting children and younger adults with epilepsy and are considered another LEAT (44,45). They may occur in the cerebral cortex or brainstem and on imaging characteristically show a well-delineated, non-enhancing, T2-FLAIR hyperintense lesion occasionally with a stalk-like connection to the lateral ventricle (41,46-51). On histopathology, angiocentric gliomas are moderately cellular, diffusely infiltrating tumors composed of monomorphic spindle cells with elongated nuclei at least focally arranged in single or multiple layers around blood vessels, often in a radial pattern (45). Mitotic activity is infrequent and the Ki-67 proliferation index is low, usually under 5% (1). Microvascular proliferation and necrosis are not features of this tumor type. On immunohistochemistry, the tumor cells are usually diffusely positive for GFAP but often negative for Olig2 (45,51,52). A characteristic finding is epithelial membrane antigen (EMA) immunostaining in a dot-like or ring-like pattern in the cytoplasm of tumor cells (52). Angiocentric gliomas do not show immunoreactivity for neuronal markers.

The majority of angiocentric gliomas are driven by fusion of the *MYB* and *QKI* genes (41,42,50,53,54). Rarely *MYB* shows other fusion partners such as *PCDHGA1* (41). Occasionally angiocentric gliomas may instead have deletions or amplifications at the *MYB* locus on 6q23.3 (55).

Angiocentric glioma is a low-grade diffuse glioma with indolent growth and is designated CNS WHO grade 1. These gliomas share overlapping morphologic, radiologic, genetic and biologic similarities with diffuse glioma with *MYB* alterations. Examples of diffuse glioma with *MYB-QKI* fusion but without a clear angiocentric growth pattern are difficult to classify as either diffuse glioma, *MYB*-altered or angiocentric glioma, especially in biopsy cases with limited sampling (*Figure 2*).

Polymorphous low-grade neuroepithelial tumor of the young (PLNTY), CNS WHO grade 1

PLNTY is a new tumor in the updated CNS WHO classification. PLNTY is a diffusely infiltrating glioma that occurs mostly in children and young adults with epilepsy but has been diagnosed in older patients in their fourth or fifth decade (56-58). PLNTY occur in the cerebral hemispheres and involve cortex and subcortical white matter. On MRI, tumors are typically solid and cystic with T2 hyperintensity and frequent calcification (59,60). Focal contrast enhancement can be present.

On histopathology, tumors show infiltrative and nodular growth, oligodendroglioma-like morphology

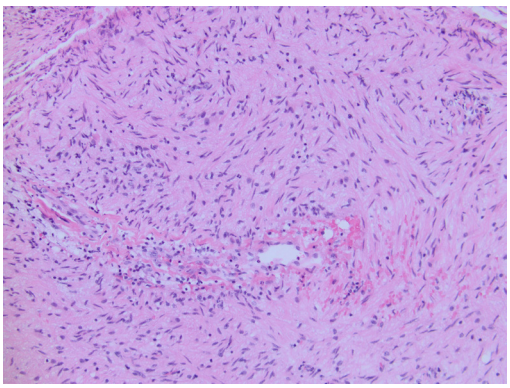

Figure 2 An unusual angiocentric glioma arising at the pontomedullary junction in a pediatric patient. H&E stained section shows a moderately cellular, infiltrative astrocytic neoplasm. The tumor cells have elongated, spindle-shaped nuclei, imparting a pilocytic appearance. A vague angiocentric arrangement of tumor cells is seen around the vessel in the lower left part of the image where many tumor cells are arranged perpendicular to the vessel wall. Mitotic activity was absent. A *MYB-QKI* gene fusion was identified and the methylation profile matched with high confidence to diffuse astrocytoma, *MYB*- or *MYBL1*-altered. Image taken at ×20. H&E, hematoxylin and eosin.

with monomorphic cells with round nuclei and perinuclear clearing, and calcification (*Figure 3*) (56). PLNTY also commonly show astrocytic or pleomorphic morphology, spindle cells, and perivascular rosette-like structures. Occasional mitoses can be encountered. Microvascular proliferation and necrosis are not seen. Foci of neoplastic neuronal cells may be encountered, and it remains to be determined if PLNTY would be best classified as a glioneuronal tumors (58). The tumor cells are immunoreactive for OLIG2, and show variable immunoreactivity for GFAP. Immunostaining for CD34 may be patchy or diffuse, but is generally strongly expressed by both tumor cells and by ramified cells in the surrounding cortex (56). The Ki-67 proliferation index is low.

PLNTY are MAPK pathway driven tumors (58). *BRAF* V600E mutations are commonly identified in tumors from older patients while younger patients often have *FGFR* fusions, most often with *FGFR2* or *FGFR3* (56,58,60). Mutations in *IDH*, *ATRX* and *TP53* are not in keeping with the diagnosis of PLNTY. PLNTY also has a distinct DNA methylation profile with apparent subgroups aligning to *BRAF* V600E mutant and *FGFR* fused tumors (56).

PLNTY are indolent tumors designated CNS WHO grade 1. Very rare cases of malignant transformation have been reported (61). Tumor recurrence is very rare following gross total resection, and seizure freedom is often achieved (56).

Diffuse low-grade glioma, MAPK pathway-altered

Diffuse low-grade glioma, MAPK pathway-altered, is a new tumor type in the updated CNS WHO classification (1). This group of diffusely infiltrating tumors occur most often in children (62). Diffuse low-grade glioma, MAPK pathway-altered tumors share many histomorphologic features of other diffuse low-grade gliomas and thus require confirmation of a MAPK pathway alteration for definitive diagnosis.

On histopathology, MAPK pathway-altered diffuse LGG are typically low to moderately cellular tumors composed of monomorphic cells with either oligodendroglioma-like or astrocytic-type features infiltrating the brain parenchyma (*Figure 4*) (42). Tumors with *FGFR1* alterations typically have oligodendroglioma-like features (42). Mitotic activity is rare. Necrosis and microvascular proliferation are not seen. Tumor cells are typically immunoreactive for GFAP and OLIG2 and negative for neuronal markers. CD34 immunostaining is usually limited to vessels. *IDH*1 and H3K27M immunostains should be performed to help exclude adult-type oligodendroglioma and diffuse midline glioma (DMG).

The most common MAPK pathway alterations are *BRAF* V600E mutation and *FGFR1* alterations, typically either a duplication or mutation in the tyrosine kinase domain (6,62). Other rare MAPK pathway alterations have also been reported (42). DNA methylation profiling of these tumors does not resolve these tumors into a single group and it seems likely that this group will eventually split into several distinct tumor types (20,42).

Diffuse low-grade gliomas with MAPK pathway alterations are low-grade tumors; however a CNS WHO grade has not yet been assigned. Additional long-term follow up data are needed, especially with regard to the various molecular subgroups included in this tumor type.

Pediatric-type diffuse high-grade gliomas

DMGs, H3 K27-altered, CNS WHO grade 4

DMG terminology has been updated in the current WHO classification to reflect our growing understanding of the

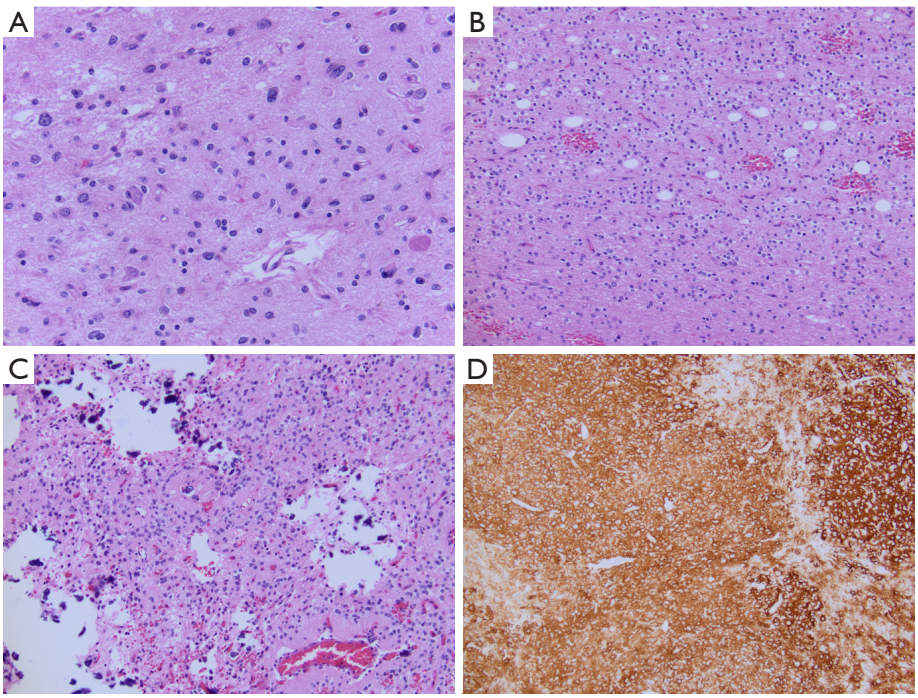

Figure 3 PLNTY in an adolescent patient. Heterogeneous appearance of PLNTY on H&E stain (A-C) with (A) areas of infiltrating fibrillary and pleomorphic astrocytoma, (B) oligodendroglioma-like component, (C) perivascular pseudorosette-like structures (image center) and coarse microcalcifications, and (D) robust CD34 immunostaining. A *FGFR2-CLIP1* gene fusion was identified. Images taken at ×40 (A) and ×20 (B-D). PLNTY, polymorphous low-grade neuroepithelial tumor of the young; H&E, hematoxylin and eosin.

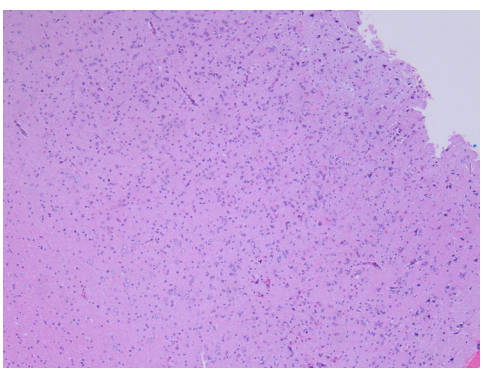

Figure 4 Diffuse low-grade glioma, MAPK pathway altered, in a pediatric patient. H&E stained section demonstrates cortical involvement by a low-grade glial neoplasm composed of uniform cells with ovoid nuclei arranged in a loose, slightly vacuolated background. The tumor cells show infiltrative growth as evidenced by numerous entrapped neurons. Mitotic activity is absent. There is no tumor necrosis or microvascular proliferation. A *KRAS* p.Gln61Lys variant was detected by targeted sequencing. KRAS is an upstream molecule in the RAS/MAPK pathway. Image taken at ×20. MAPK, mitogen-activated protein kinase; H&E, hematoxylin and eosin; KRAS, Kirsten rat sarcoma virus; RAS, rat sarcoma.

pathogenesis of these tumors. DMG with histone H3 K27M mutations were previously classified as DMG, H3 K27-mutant in the 2016 WHO classification. It is now evident that multiple pathogenic alterations involving the histone 3.1, 3.2 and 3.3 genes can be identified in this tumor type. The new terminology of DMG, H3 K27-altered, includes tumors with loss of H3 K27 trimethylation due to p.K27M mutation, overexpression of enhancer of zeste homolog inhibitory protein (EZHIP), or an *EGFR* mutation. H3 K27-altered tumors include diffuse intrinsic pontine gliomas (DIPG) in addition to diffuse gliomas arising in other midline locations. Recognizing that H3K27 alterations have also been identified in other circumscribed or low-grade tumor types, DMG must also meet criteria of being diffusely infiltrative and in a midline anatomic location.

In the pediatric population, DMG most frequently involve the brainstem, principally the pons (DIPG) (63). Tumors also often involve the bilateral thalami, and tumors with this presentation frequently harbor *EGFR* mutations (64). In older patients, either unilateral thalamus or spinal cord are more common sites, although DMG have been reported in most supratentorial and infratentorial midline structures.

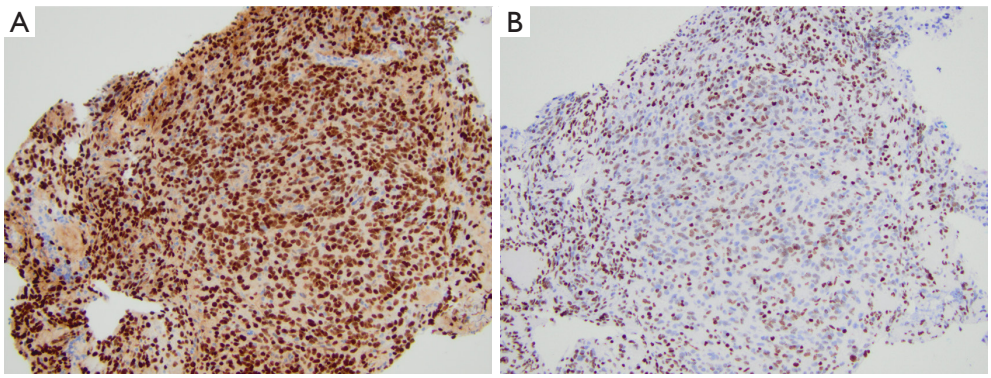

Figure 5 DMG, H3 K27-altered, arising as a bithalamic tumor in a pediatric patient. (A) Tumor cells show strong diffuse mutation-specific immunoreactivity for H3 K27M and (B) loss of H3 K27-trimethylation. Images taken at ×40. DMG, diffuse midline glioma.

The median age at diagnosis is 7–8 years of age for H3.3 p.K27M-mutant DMG, DMG with EZHIP overexpression, and EGFR-mutant DMG. H3.1 or 3.2 p.K27M-mutant tumors are diagnosed in slightly younger children with a median age of approximately 5 years old (1).

On histopathology, DMG are diffusely infiltrative tumors with heterogeneous morphology (65,66). Many DMG are composed of bland monomorphic cells with small, ovoid nuclei. Others resemble other low-grade astrocytomas or oligodendroglioma, or show epithelioid or highly pleomorphic cytology. Rare cases have a primitive neuroepithelial cell (PNET-like) appearance with neuropil-like islands (67). Mitotic figures are often present, and microvascular proliferation and necrosis may be found. It is important to note that the histologic features of DMG may align best with a low-grade CNS WHO grade 2 or 3 glioma, however, all DMG are CNS WHO grade 4 tumors by definition. A layered reporting structure of the integrated pathologic diagnosis is recommended so both histologic and molecular information are represented.

By immunohistochemistry, DMG with H3K27M mutation or EZHIP overexpression are generally positive for OLIG2 and MAP2 and variably positive for GFAP. DMG with *EGFR* mutations may be GFAP positive but lack OLIG2 expression. Neuronal markers are negative in the tumor cells. Mutation-specific antibodies for H3 K27M show strong nuclear expression in the H3 K27-mutant subgroup (*Figure 5A*). Loss of H3 K27me3 immunoreactivity is a highly sensitive marker of tumors with either H3 K27M or H3 K27I mutations or those with EZHIP overexpression (*Figure 5B*). Some laboratories have the ability to stain for EZHIP overexpression using antibodies against EZHIP (Cxorf67). Additionally, many DMG cases show P53 overexpression (~50%) or loss of ATRX expression (~15%) consistent with the presence of *TP53* and *ATRX* mutations, respectively (68,69).

Our current understanding of DMG based on multiple lines of genomic analysis supports the division of DMG into four biologically and clinically distinct subtypes that are recognized in the updated CNS WHO classification (1,68-70). H3.3 p.K27-mutant tumors harbor a somatic mutation resulting in a substitution of lysine (K) to methionine (M), or rarely isoleucine (I), at position 27 of the histone H3 variant. This subgroup is enriched for tumors with *TP53* and less frequently *PPM1D* cooperating or subclonal gene mutations (71). Alternatively, H3.1 and H3.2 p.K27M-mutant tumors are enriched for concomitant PI3K or MAPK pathway alterations. *ACVR1* mutations are strongly associated with H3.1p.K27M tumors (72,73). H3 K27M-mutant DMG may also have gain-of-function mutations or amplifications of *PDGFRA* and *FGFR1*. H3-wildtype DMG with EZHIP overexpression are quite rare and require a combination of immunohistochemical analysis for H3 K27me3 and RNA expression analysis for diagnosis. *EGFR*-mutant DMG most commonly have alterations in exon 20 of the *EGFR* oncogene on chromosome 7p encoding the intracellular tyrosine kinase domain. The *EGFR*-mutant DMG subgroup is also enriched for tumors with p53 cooperating mutations.

Patients with DMG have a poor prognosis independent of anatomic location. A large study by Mackay and colleagues demonstrated a median survival of 13.5 months and a 2-year overall survival of 21.4% for non-DIPG DMG. DIPG cases had a median survival of 10.8 months

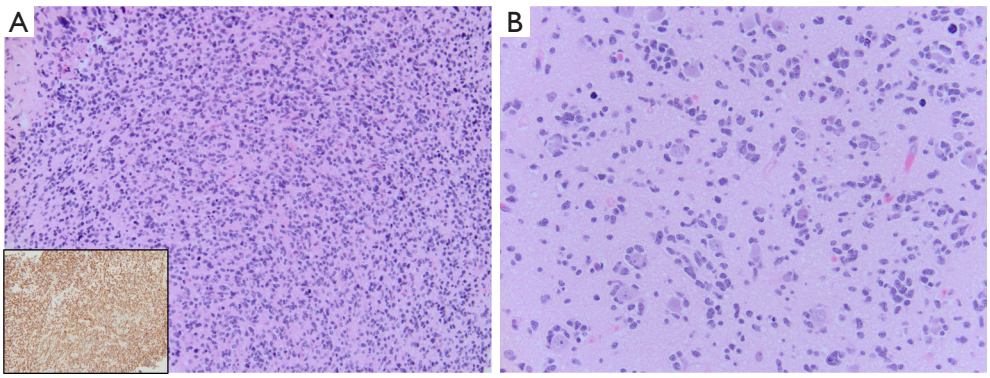

Figure 6 Diffuse hemispheric glioma, H3 G34-mutant, diagnosed in a pediatric patient. (A) H&E stained section passing through a hypercellular, diffusely infiltrating tumor composed of mildly pleomorphic cells with ovoid-to-angulated nuclei. Inset: The tumor cells are strongly immunoreactive for H3 G34R mutation-specific antibody. (B) H&E stained section showing diffuse hemispheric glioma, H3 G34R-mutant, may show conspicuous secondary structuring of tumor cells around neurons and blood vessels. Mitotic activity is high. Images taken at ×20 (A and inset) and ×40 (B). H&E, hematoxylin and eosin.

and a 2-year overall survival of 21% (63). Other studies indicate that DMG with H3.1 or 3.2 p.K27M mutations or with EZHIP overexpression have a slightly increased overall survival compared to patients with H3.3 p.K27M DMG (70,74,75). Patients younger than three or older than 10 years also show somewhat improved clinical outcomes compared to older children (63,75).

Diffuse hemispheric glioma, H3 G34-mutant, CNS WHO grade 4

Diffuse hemispheric glioma, H3 G34-mutant, is a diffusely infiltrative high-grade glioma that arises in the cerebral hemispheres but may spread to include midline structures. By definition, these tumors have a missense mutation in the *H3-3A* (*H3F3A*) gene resulting in a substitution of p.G34R or p.G34V on the histone variant.

On histopathology, these high-grade gliomas are hypercellular infiltrative tumors. Tumor cells may have a glial or embryonal-type appearance. Tumor cells may be markedly pleomorphic and multinucleated, or be small, hyperchromatic cells that occasionally may form Homer Wright rosettes. Tumors cells often infiltrative the cortex and show secondary structuring around neurons and vessels. Mitotic activity is increased and the Ki-67 proliferation index is usually high. Microvascular proliferation and necrosis are typically present but not required for CNS WHO grade 4 designation. Tumor cells often show loss of ATRX expression and P53 overexpression by immunohistochemistry, which are clues that the tumor may harbor a H3.3 p.G34R/V mutation. MAP2 and FOXG1 are usually positive in the tumor cells (1,37). Mutation-specific antibodies are available in some laboratories to detect both G34R and G34V mutant proteins with high sensitivity (*Figure 6*). Notably, these gliomas are often OLIG2 negative and can be variably positive for GFAP unlike other HGG that typically show OLIG2 and GFAP immunoreactivity.

In addition to the required identification of a *H3-3A* gene missense mutation replacing glycine with arginine or valine at position p.G34, these gliomas have frequent mutations in *ATRX* (95%) and *TP53* (90%) (76). *PDGFRA* amplification is relatively common in tumors with glial morphology while *CCND2* amplification is more frequent in tumors with primitive neuroepithelial morphology (76). Mutations identified in other types of pediatric or young adult high-grade gliomas including H3 K27M, *IDH1/IDH2*, and *BRAF* are absent. Diffuse high-grade glioma, H3 G34-mutant, has a distinct DNA methylation profile that includes both G34R and G34V variants. These gliomas often show MGMT promoter hypermethylation which may be associated with improved outcomes together with the absence of *PDGRFA*, *CCND2* or other less common oncogene amplifications reported in this tumor type (76). Patients often have multiple local tumor recurrences and the overall prognosis of H3 G4-mutant diffuse hemispheric glioma is poor (63,76).

Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, CNS WHO grade 4

Diffuse pediatric-type high-grade gliomas (pHGG), H3-

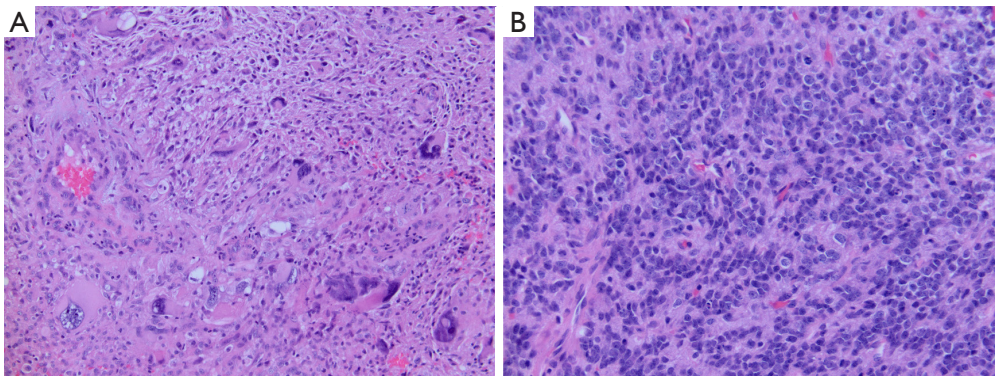

Figure 7 Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype. Examples from two different tumors demonstrating heterogeneous histomorphology within this diagnostic group. (A) H&E section shows a hypercellular, diffusely infiltrating high-grade glioma composed of highly pleomorphic cells including many giant cells. Mitotic activity is high. Microvascular proliferation is present and necrosis is seen in other sections. Mutations in *PTEN*, *RB1* and *TP53* were detected on sequencing analysis, together with a near-haploid somatic state with loss of heterozygosity across most of the genome. No mutations in histone H3 genes or *IDH* genes were identified. (B) H&E section showing a densely cellular tumor composed of moderately pleomorphic cells with ovoid-to-angulated hyperchromatic nuclei and scant cytoplasm arranged in sheets. Numerous mitotic figures and apoptotic cells are present. Microvascular proliferation and necrosis are present in other sections (not shown). Molecular genetic studies showed focal high-level *EGFR* and *MYCN* amplification. No mutations in histone H3 genes or *IDH* genes were identified on sequencing analysis. DNA methylation profile matched to glioblastoma, subclass *MYCN*. Images taken at ×20 (A) and ×40 (B). H&E, hematoxylin and eosin.

wildtype and *IDH*-wildtype, are a group of malignant gliomas primarily occurring in children or young adults that by definition lack alterations in histone H3, *IDH1* and *IDH2* genes. Not unexpectedly, this group of tumors is heterogeneous in terms of clinical, histopathologic, genomic and epigenetic features yet remain distinguishable from adult-type high-grade gliomas.

pHGG, H3-wildtype and *IDH*-wildtype, may arise anywhere in the supratentorial or infratentorial compartments. On histopathology, these tumors often appear malignant with increased mitotic activity. Microvascular proliferation and necrosis may or may not be present, and likely do not have the same prognostic significance as in adult-type high-grade gliomas (77). Tumor cells many have an astrocytic or primitive embryonal-type morphology (*Figure* 7). In a small series of pHGG with *MYCN* amplification, tumors were described as having both diffusely infiltrating and circumscribed nodular tumor areas with frequent leptomeningeal involvement. Tumor cells were arranged in sheets with spindled or epithelioid morphology and prominent nucleoli, and were immunoreactive for neuronal but not glial markers (78). All pHGG, H3-wildtpe and *IDH*-wildtype, should not show immunoreactivity for H3K27M or *IDH1* R132H or loss of immunoreactivity for H3 K27me3 on immunohistochemical stains. *TP53* mutations are common so many tumors will show strong immunohistochemical staining for P53.

Molecular testing is needed to exclude other types of high-grade gliomas, particularly in older adolescents and infants. Differential diagnoses include DMG with H3 K27-alteration, *IDH*-mutant astrocytoma, adult-type glioblastoma with gain of chromosome 7 and loss of 10 and/or *EGFR* amplification, and epithelioid glioblastoma with *BRAF* mutation and *CDKN2A/B* homozygous deletion. In young children, CNS embryonal tumors should be a diagnostic consideration. In infant patients, infant-type hemispheric glioma and desmoplastic infantile ganglioglioma/astrocytoma should be ruled out.

The molecular alterations driving tumorigenesis for these pHGG are diverse. In addition to the previously mentioned *TP53* mutations and *MYCN* amplification, these pHGG have frequent *PDGFRA* alterations (mutations or amplification), *ID2*, *NF1* alterations, and *EGFR* amplification (18,63,68,79). pHGG, H3-wildtype and *IDH*-wildtype, comprise a distinct group by DNA methylation profiling with several molecular subgroups (RTK1, RTK2 and MYCN) based on their genetic and epigenetic features (20,80). RTK1 pHGG subtype gliomas have frequent *PDGFRA* amplification and may be associated with prior therapeutic cranial radiation, constitutional

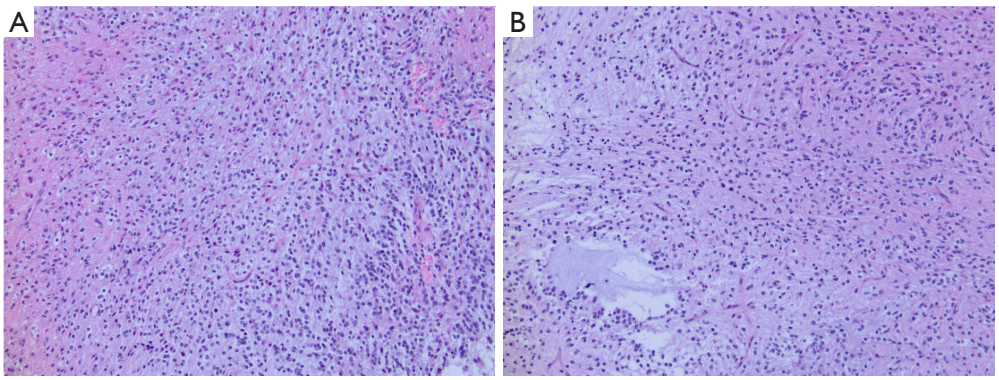

Figure 8 Infant-type hemispheric glioma. H&E sections show a moderately cellular, diffusely infiltrative glial neoplasm. Tumor cells are characterized by round, ovoid or spindled nuclei. In some areas, an oligodendroglioma-like pattern is suggested (A) while other areas show a biphasic pattern (B) with compact fibrillary areas with cells in vague short fascicles (right side) and less cellular areas with a looser background and occasion mucin-filled microcysts (left side). Mitotic activity was identified. This tumor was initially favored to be a pilocytic astrocytoma based on the histopathologic appearance, and later classified as an infant-type hemispheric glioma following identification of *SOX10-NTRK* gene fusion. (A,B) Taken at ×20 magnification. H&E, hematoxylin and eosin.

mismatch repair deficiency or Lynch syndrome (81). RTK2 pHGG subgroup tumors are associated with *EGFR* amplification and *TERT* promoter mutations and appear to have a better prognosis than RTK1 subgroup tumors. MYCN pHGG subgroup tumors include tumors with *MYCN* amplification, detected in about half of tumors, and frequent *ID2* amplification, detected in about 70% of tumors (78). pHGG MYCN subgroup tumors are associated with a very poor prognosis (80).

Infant-type hemispheric glioma

Infant-type hemispheric glioma is a newly defined tumor type in the CNS WHO classification with distinct molecular drivers and clinical behavior as compared to gliomas in older children. Infant-type hemispheric gliomas are comprised of multiple subgroups, most with receptor tyrosine kinase (RTK) fusions involving the *NTRK*, *ROS1*, *ALK* and *MET* genes (82,83).

Infant-type hemispheric gliomas arise in very young children typically under one year of age (82). These gliomas are large masses arising in the cerebral hemispheres, often superficially and occasionally with variably solid and cystic components. Leptomeningeal involvement is common and leptomeningeal dissemination has been reported (83,84). On histopathology, the tumors are hypercellular and often show a sharp border with adjacent brain tissue. Tumor cell morphology is variable and includes astrocytic cells with mild to moderately pleomorphic nuclei and occasionally gemistocytic cells arranged in sheets. Tumor cell nuclei may also be spindled and form fascicles (*Figure 8*). Increased mitotic activity, microvascular proliferation, and pseudopalisading necrosis are common. *ALK*-fusion tumors may show ependymal differentiation or focal ganglion cells (83,84). On immunohistochemistry, tumor cells are immunoreactive for GFAP and negative for neuronal markers. Immunostaining for ALK may be positive in some tumors with *ALK* fusions. NTRK immunohistochemistry is not considered useful to detect *NTRK*-fusion positive tumors as NTRK expression is elevated in background brain tissue.

Molecular testing for gene fusions is recommended in the diagnostic workup of all infant gliomas to confirm the diagnosis and identify potential molecular targets for therapy. DNA methylation profiling reveals a distinct methylome profile for all infant-type hemispheric gliomas and is useful to help exclude other high-grade gliomas with similar or overlapping histomorphology (e.g., desmoplastic infantile ganglioglioma/astrocytoma and ependymoma).

A CNS WHO grade is not yet assigned for infant-type hemispheric gliomas as outcome data are still sparse. At least some fusion-positive subgroups appear to have a better prognosis. Guerreiro Stucklin *et al.* have reported that patients with *ALK* fusion-positive tumors carry the best prognosis, *NTRK*-fused tumors showed an intermediate outcome, and *ROS*-fused tumors were associated with the lowest five-year overall survival (82). Larger studies are needed to confirm these early observations and to assess outcomes following targeted therapy with several promising

small molecular inhibitors of specific tyrosine kinase receptors.

Limitations

This review is limited by its focus on recent changes to diffuse glioma classification and includes relatively few examples of the spectrum of gliomas that arise in children and adults. As our knowledge continues to evolve, tumor classification based on these histologic and molecular features will inevitably become outdated. An overview of the current literature was the goal of this review; however, additional findings discussed in recent publications may have been inadvertently excluded.

Conclusions

The CNS WHO classification continues to evolve and is likely best considered a snapshot-in-time that reflects our current understanding based on the information and tools available. The number of pages in the print versions of the CNS WHO classification nearly doubled in past 20 years, in large part due to the development of molecular diagnostics that have refined our ability to distinguish tumor types and subtypes with clinical significance. At the same time, histopathology and immunohistochemistry remain proven methodologies for CNS tumor classification and importantly guide practical and affordable approaches to further molecular testing. The classification of adult-type and pediatric-type diffuse gliomas will undoubtedly continue to evolve as new knowledge is introduced in future CNS WHO classifications.

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Footnote

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