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CORRESPONDENCE

cIMPACT-NOW update 4: diffuse gliomas characterized by *MYB*, *MYBL1*, or *FGFR1* alterations or *BRAF*^{V600E} mutation

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Diffuse gliomas occur at all ages, but their incidence is highest among older adults [7]. They have astrocytic or oligodendroglial morphologies and are represented across WHO grades II–IV. In childhood, they are uncommon, presenting less frequently than the broad range of pediatric circumscribed gliomas, particularly pilocytic astrocytoma, and a few glioneuronal tumors, e.g., ganglioglioma [16].

The genetically defined IDH-mutant diffuse gliomas and the diffuse midline glioma, H3 K27M-mutant were introduced in the 2016 edition of the WHO classification [13]. In that update of the classification, IDH-wt/H3-wt diffuse gliomas are currently assigned to IDH-wt or NOS (not otherwise specified) diagnoses based upon morphology, grade, and IDH status when available. However, this scheme masks a heterogeneity that has implications for outcome and treatment; IDH-wt/H3-wt tumors with the same histologic features can harbor distinct genetic alterations and can demonstrate significantly different clinical outcomes and responses to targeted chemotherapy, all of which might influence the selection of an optimal adjuvant therapy.

IDH-wt/H3-wt diffuse gliomas, arising mainly in middle-aged adults, with WHO grade II/III histologic features and either combined chromosome seven gain and chromosome ten loss, or a *TERT* promoter region mutation, or *EGFR* amplification have a relatively aggressive behavior, with outcomes that are marginally better than those of IDH-wt glioblastomas [2, 4]. In contrast, historic studies of WHO grade II diffuse gliomas from children and adolescents, which we now know must be dominated by IDH-wt/H3-wt tumors with either a *BRAF*^{V600E} mutation, an *FGFR* alteration, or a *MYB* or *MYBL1* rearrangement, describe an indolent clinical behavior and rare anaplastic progression [3, 8, 18, 23, 24]. Patients with these tumors generally have a prolonged disease course and good overall survival, despite suffering significant morbidity during their chronic disease.

In the context of this heterogeneity, WHO grading and the term 'low-grade glioma' have diminished utility; entirely different approaches to the post-operative management of a WHO grade II diffuse glioma from each of these two genetic categories would be appropriate. Therefore, the cIMPACT Steering Committee decided that it would be valuable for our working committee to complement the recommendations of 'Update 3′ by addressing the heterogeneity among IDH-wt/H3-wt diffuse gliomas from the perspective of pediatric practice and focusing on tumors from the latter genetic category [2].

An integrated approach to the diagnosis of 'pediatric-type' diffuse gliomas

Diffuse gliomas with a *BRAF*^{V600E} mutation, *FGFR1* alteration, or a *MYB* or *MYBL1* rearrangement are distinctive tumors defined by a combination of their histologic and

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genetic features. Their diagnosis and classification, therefore, rely upon integrating these features, which should be done by compiling tiers of histologic and molecular information in the diagnostic report, as set out in the 2014 International Society of Neuropathology (ISN)-Haarlem consensus guidelines [12].

Diffuse gliomas with the above specific genetic alterations are uncommon. They present mainly in children and sometimes in adults and are often associated with epilepsy. Morphologically, their architectural and cytologic features are the same as those of other WHO grade II diffuse gliomas, with infiltration of CNS parenchyma a defining feature. Glial differentiation can be astrocytic, oligodendroglial, or a combination of these. In line with the definitions of other WHO grade II diffuse gliomas—diffuse astrocytoma and oligodendroglioma—mitotic activity should be absent or at a low level, and microvascular proliferation and necrosis absent.

The defining genetic alterations for this group of 'pediatric-type' diffuse gliomas are *BRAF*^{V600E} mutation; a *MYB* or *MYBL1* structural variation, including amplification; and *FGFR1* alterations, either an internal tandem duplication (ITD) of the tyrosine kinase domain (TKD) or single nucleotide variants (SNVs) [18, 27]. In a majority of tumors, these alterations are found to be the only potential genetic driver [27]. *MYB* and *MYBL1* alterations occur in somewhat distinct clinicopathologic settings [1, 19, 27], and data suggest that the two principal types of *FGFR1* alteration—TKD duplication and SNV—may also be associated with different clinical settings [18]; in some tumors, *FGFR1* SNVs, but not ITDs, are germline alterations [21]. Other genetic alterations, such as mutations of *KRAS* and fusions of *FGFR2* or *BRAF*, are occasionally found, the consistent feature being activation of the MAPK pathway [10, 27]. These alterations are rare; however, in one study, a *BRAF*^{V600E} mutation, an FGFR alteration, or a rearrangement of *MYB* or *MYBL1* was detected in 84% of IDH-wt/H3-wt diffuse gliomas from a largely pediatric cohort [18]. In the same dataset, DNA methylation profiling revealed a pattern of clustering that was largely based on these three classes of genetic alteration.

On the basis of these data, our committee concluded that the following classification would provide valuable diagnostic and prognostic information and, for some entities, suggest targeted therapies:

- Diffuse glioma, *MYB*-altered;
- Diffuse glioma, *MYBL1*-altered;
- Diffuse glioma, *FGFR1* TKD-duplicated;
- Diffuse glioma, *FGFR1*-mutant;
- Diffuse glioma, *BRAF* V600E-mutant[a];
- Diffuse glioma, other MAPK pathway alteration.

[a]This diagnosis should not be made in the presence of *CDKN2A/B* homozygous deletion.

Each diagnosis depends upon histopathologic and molecular assessments, including confirmation of IDH-wt/H3-wt status, and these assessments provide information to be entered into the tiers of an integrated diagnosis [12].

In the following example, microscopic examination showed the features of a diffuse astrocytoma, and sequencing demonstrated a *MYB–PCDHGA1* fusion gene, alongside confirmation of IDH-wt/H3-wt status. While the tumor could be classified as WHO grade II, following current practice for diffuse gliomas, anecdotal evidence suggests that some of these 'pediatric-type' diffuse gliomas, particularly those classified as *MYB*-altered or *MYBL1*-altered, behave more like WHO grade I tumors. Despite such observations, our committee concluded that, until more outcome data for these specific tumors become available, any recommendation on assigning a WHO grade should be deferred.

Report format for integrated diagnosis—2014 ISN-Haarlem guidelines	
Layer 1	Integrated diagnosis incorporating all tissue-based data
Layer 2	Histologic classification
Layer 3	WHO grade
Layer 4	Molecular data
Report format for diffuse glioma	
Integrated diagnosis	Diffuse glioma, *MYB*-altered
Histologic classification	Diffuse astrocytoma
WHO grade	TBD[a]
Molecular data	IDH-wildtype, H3-wildtype, *MYB–PCDHGA1* fusion gene

[a]To be determined. Further outcome data are needed to assign a WHO grade to the various diffuse gliomas listed above. Anecdotal evidence suggests that some variants might behave as grade I (*MYB*-altered or *MYBL1*-altered), while others (*BRAF* V600E-mutant) have an outcome that might align with grade II

The sixth entity, 'Diffuse glioma, other MAPK pathway alteration', could be used for those rare tumors in which another genetic alteration capable of activating the MAPK pathway is detected. Use of this diagnosis would require a description of the genetic alteration predicted to activate the pathway. For example, an *FGFR2* fusion would be specifically mentioned in the molecular tier of the integrated diagnosis, and reference to its pathogenic function would be included in the 'Comments' field of the report. When there is doubt about a genetic alteration's role in activating the MAPK pathway, directly or indirectly, then this diagnosis should be avoided.

Diagnostic testing for the principal genetic alterations found in such 'pediatric-type' diffuse gliomas does not necessarily require massively parallel (next generation) sequencing methods. Various approaches are available, and some surrogate tests, e.g., immunohistochemical detection

of *BRAF*^{V600E} gene product or interphase fluorescence in situ hybridization for the detection of a *MYB* or *MYBL1* rearrangement, are common in diagnostic laboratories.

In the setting of investigating a potential diagnosis of 'pediatric-type' diffuse glioma and when molecular analysis fails to discover one of the genetic alterations that would classify a diffuse glioma among the listed entities, the default diagnosis would be 'Diffuse astrocytoma' or 'Oligodendroglioma' with the qualifier NEC ('not elsewhere classified'), as recommended in cIMPACT update 1 [14]. This usage should apply even if a tumor is demonstrably IDH-wt/H3-wt, because 'Diffuse astrocytoma, IDH-wildtype', currently a provisional diagnosis in the WHO classification, does not distinguish 'adult-type' and 'pediatric-type' tumors. This uncertainty about the tumor's molecular nature, even accounting for patient age, is better managed using the term NEC than using 'Diffuse astrocytoma, IDH-wildtype' or 'Oligodendroglioma, NOS', which are the only two currently listed alternatives. The default diagnosis based upon histologic features alone would be 'Diffuse astrocytoma, NOS' or 'Oligodendroglioma, NOS'.

Practical aspects of classifying 'pediatric-type' diffuse gliomas

Our committee recognized that there is overlap between the histologic features and genetic alterations of the diffuse gliomas listed above and those of more circumscribed low-grade glial and glioneuronal tumors, many of which present in childhood. Such overlap implies that combined morphologic and genetic assessments will not always facilitate a definitive diagnosis, particularly in small biopsies where the typical histologic features of a tumor might not be present.

For example, distinguishing a diffuse glioma from a pilocytic astrocytoma, the glial element of a ganglioglioma, or even a pleomorphic xanthoastrocytoma (PXA) could be difficult, particularly if the classic histopathologic features of these tumors are not apparent and because the infiltrative nature of 'pediatric-type' diffuse gliomas can be variable. Similarly, a diffuse glioma with oligodendroglial differentiation appears identical to those regions of dysembryoplastic neuroepithelial tumors (DNTs) that contain oligodendrocyte-like cells dispersed in neuropil and subcortical white matter, rather than arranged in nodules or in a specific glioneuronal element. In these circumstances, reference to the tumor's radiologic features can be helpful, but might not be conclusive.

While molecular information can sometimes direct treatment decisions, it might not help decisive tumor classification, because all of the common genetic alterations in 'pediatric-type' diffuse gliomas are found in other low-grade neuroepithelial tumors. *MYB* fusion genes are present in nearly all angiocentric gliomas [1, 18], and *FGFR1* TKD ITDs or SNVs occur at high frequency in DNTs and rosette-forming glioneuronal tumors [11, 18]. The situation is further complicated by shared morphologic features among those tumors with the same genetic alterations; 'pediatric-type' diffuse gliomas showing oligodendroglial differentiation generally have the same *FGFR1* alterations as DNTs, and *BRAF*^{V600E} mutation, which is common in ganglioglioma and PXA and occurs occasionally in pilocytic astrocytoma, is generally found in the 'pediatric-type' diffuse gliomas with astrocytic differentiation [18].

Despite the challenges presented by overlapping histologic and genetic features, several molecular pointers are available to direct the neuropathologist when these diffuse gliomas enter the differential diagnosis—genetic alterations that are both rare in 'pediatric-type' diffuse gliomas and frequent in morphologically related tumors. When found, these alterations should suggest alternative diagnoses, some of which have a more aggressive biologic behavior than these diffuse gliomas. For example, a *KIAA1549–BRAF* fusion is almost pathognomonic of pilocytic astrocytoma; the rare diffuse leptomeningeal glioneuronal tumor, anaplastic astrocytoma with piloid features, and pilocytic astrocytoma with focal gangliocytic differentiation are the only other neural tumors to exhibit a high frequency of this specific *BRAF* fusion [5, 6, 9, 20, 22]. Therefore, when a *KIAA1549–BRAF* fusion is detected in the setting of a WHO grade I/II astrocytoma with a partially infiltrative architecture and no idiosyncratic morphologic features with which to distinguish pilocytic astrocytoma and diffuse astrocytoma, then a diagnosis of pilocytic astrocytoma would be preferred to 'diffuse glioma, other MAPK pathway alteration'.

Detection of homozygous deletion at the *CDKN2A/B* locus is another molecular marker that should direct the neuropathologist away from a diagnosis of 'pediatric-type' diffuse glioma. It is not a feature of the genetically defined, 'pediatric-type' diffuse gliomas, which are the focus of these recommendations, but is detected at high frequency alongside *BRAF*^{V600E} mutation in PXAs [17, 26]. Among other childhood astrocytic tumors, it is often associated with a poorer than expected outcome and sometimes with high-grade features or anaplastic progression [15]. Other molecular markers that can help to identify high-grade gliomas in small biopsies of astrocytic tumors are mutation of *TP53* or *ATRX*, *TERT* alterations, and amplification of *PDGFRA*, *EGFR*, *MET*, or *MYCN* [25].

The IDH-wt diffuse gliomas that usually present in adults also enter the differential diagnosis and can demonstrate genetic alterations that activate the MAPK pathway, e.g., mutations in *NF1* and *FGFR3*. However, these are exceedingly rare in WHO grade II tumors, and many such 'adult-type' tumors show combined chromosome 7 gain and chromosome 10 loss [4].

Summary

cIMPACT has reviewed the status of WHO grade II IDH-wt/H3-wt diffuse gliomas, focusing on those with a *BRAF*^{V600E} mutation, *FGFR1* alteration, or a *MYB* or *MYBL1* rearrangement, and recommends the use of an integrated diagnosis to combine their histologic and genetic features. Although our cIMPACT committee sees the utility of distinguishing these diffuse gliomas in diagnostic practice, it also acknowledges that the overlap between their morphologic and genetic features and those of other neuroepithelial tumors could occasionally compromise an accurate diagnosis. These other tumors, including pilocytic astrocytoma, PXA, and DNT, could themselves benefit from a classification based upon their combined histologic and genetic features; indeed, it seems likely that such tumors will be the subject of future cIMPACT recommendations on their classification.

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