Beyond the World Health Organization classification of central nervous system tumors 2016: what are the new developments for gliomas from a clinician's perspective?

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Purpose of review
The World Health Organization (WHO) classification of central nervous system (CNS) tumors was revised in 2016 to include molecular biomarkers that are important for tumor classification and clinical decision making. Thereafter, the cIMPACT-NOW initiative further refined CNS tumor classification through a series of recommendations likely to shape the upcoming WHO classification 2021.

Recent findings
Mutations in the isocitrate dehydrogenase (IDH) 1 or 2 genes continue to play a major role in glioma classification. Among IDH-mutant gliomas, loss of ATRX expression identifies IDH-mutant astrocytomas without necessity for 1p/19q codeletion testing. The nomenclature for IDH-mutant glioblastoma has been changed to astrocytoma, IDH-mutant, WHO grade 4, with *CDKN2A* homozygous deletion representing a novel molecular marker for these tumors. IDH-wildtype astrocytomas that lack microvascular proliferation or necrosis but exhibit telomerase reverse transcriptase promoter mutation, epidermal growth factor receptor amplification, and/or a +7/−10 genotype are now classified as IDH-wildtype glioblastoma. H3.3 G34-mutant diffuse hemispheric gliomas have been proposed as a new entity separate from IDH-wildtype glioblastoma.

Summary
These changes increase diagnostic accuracy and refine clinical care by changing treatment recommendations, for example for patients with IDH-wildtype astrocytomas showing molecular features of glioblastoma. They also have major implications for clinical trial design.

Keywords
cIMPACT-NOW, epidermal growth factor receptor, glioblastoma, glioma, isocitrate dehydrogenase, telomerase reverse transcriptase

INTRODUCTION

The World Health Organization (WHO) classification of central nervous system (CNS) tumors was revised in 2016 to update several disease concepts of groups of brain tumors based on major advances in understanding their molecular pathogenesis [1]. Yet, already while the 2016 revision was being prepared, it was apparent that future changes would soon be necessary because of further developments and the discovery of novel distinct entities. Accordingly, a *Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy – not officially WHO* (cIMPACT-NOW) was formed as a platform to prepare recommendations on how to address upcoming challenges in CNS tumor classification. Many of these considerations will probably shape the upcoming 5th edition of the WHO classification that is expected for spring 2021. Here, we summarize clinically relevant developments with regard to current updates concerning the role of molecular markers in the classification of diffuse gliomas and

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KEY POINTS

- The term glioblastoma is now restricted to WHO grade 4 gliomas without IDH and H3 mutations.
- A glioblastoma-associated genetic signature defined by *TERT* promoter mutation, *EGFR* amplification, and/or a +7/−10 genotype is sufficient for the diagnosis of glioblastoma, IDH-wildtype, even if an astrocytic glioma lacks the traditional histological features of necrosis and/or microvascular proliferation.
- IDH-mutant astrocytomas are considered as a distinct entity that may correspond to WHO grades 2, 3, or 4, with homozygous *CDKN2A* deletion being a molecular marker of WHO grade 4.
- H3.3 G34-mutant diffuse hemispheric gliomas have been proposed as a novel glioma entity of WHO grade 4 that is distinct from glioblastoma, IDH-wildtype.

how these may impact clinical practice and clinical trial design. Table 1 provides an overview of the diagnostic molecular markers currently used in the classification of diffuse gliomas as recommended in the WHO classification 2016 [1] and/or the consecutive cIMPACT-NOW recommendations [2,3▪].

Nomenclature

The WHO classification of 2016 had introduced the term of NOS, *not otherwise specified*, to denote that the diagnostic workup for a tumor was incomplete, e.g., important molecular data such as isocitrate dehydrogenase (IDH) mutation status were missing, precluding a more precise diagnosis. It turned out that this terminology left questions open because it could be interpreted to indicate that testing was not performed or that testing was performed, but did not provide conclusive results, or that testing revealed

Table 1. Molecular markers in diffuse gliomas: biological functions, detection methods, and diagnostic significance

Marker	Biological functions	Diagnostic methods	Clinical significance
IDH1 R132 or IDH2 R172 mutation	Gain of function mutation causing gCIMP	Immunohistochemistry for IDH1 R132H followed by sequencing for noncanonical IDH1 or IDH2 mutations	Diagnostic marker for IDH-mutant diffuse gliomas
ATRX mutation / ATRX loss of nuclear expression	Causes alternative lengthening of telomeres	Immunohistochemistry for loss of nuclear ATRX expression or sequencing	Diagnostic marker for IDH-mutant astrocytomas
1p/19q codeletion	Unclear, possibly biallelic inactivation of tumor suppressors on 1p (e.g., *FUBP1*) or 19q (e.g., *CIC*)	PCR-based loss of heterozygosity analysis, FISH, array-based copy number analysis, MLPA	Diagnostic marker for IDH-mutant and 1p/19q-codeleted oligodendrogliomas
H3 K27M mutation	Histone 3 mutation causing epigenetic alterations affecting gene expression	Immunohistochemistry for H3 K27M or sequencing	Diagnostic marker for diffuse midline glioma, H3 K27M-mutant
H3.3 G34 mutation	Histone 3.3 mutation	Immunohistochemistry for H3.3 G34R/V or sequencing of *H3F3A*	Diagnostic marker for diffuse hemispheric gliomas, H3.3 G34-mutant
MGMT promoter methylation	DNA repair	Methylation-specific PCR, pyrosequencing of bisulfite-modified DNA, array-based DNA methylome profiling	Predictive biomarker for benefit from alkylating chemotherapy, in particular in glioblastoma, IDH-wildtype
EGFR amplification	Aberrant receptor tyrosine kinase signaling driving proliferation, invasion, and resistance to apoptosis induction	FISH, quantitative PCR analyses, array-based copy number analysis, MLPA	Diagnostic marker for glioblastoma in IDH-wildtype astrocytic gliomas
TERT promotor mutation	Increase of TERT expression causing stabilization of telomere length to prevent senescence and drive proliferation	Sequencing, droplet digital PCR	Diagnostic marker for glioblastoma in IDH-wildtype astrocytic gliomas; also frequent in IDH-mutant and 1p/19q-codeleted oligodendrogliomas
+7/−10 genotype	increased expression of tumor promoting genes on chromosome and inactivation of tumor suppressor genes on chromosome 10 (e.g. *PTEN*)	FISH; array-based copy number analysis, MLPA	Diagnostic marker for glioblastoma in IDH-wildtype astrocytic gliomas
CDKN2A homozygous deletion	Alteration of the Rb1 and p53 pathways by biallelic inactivation of the *CDKN2A/p14^{ARF}* tumor suppressor gene	FISH; array-based copy number analysis, MLPA	Diagnostic marker for astrocytoma, IDH-mutant, WHO grade 4

ATRX, alpha-thalassemia/mental retardation syndrome, nondeletion type, X-linked; CIC, capicua; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; FUBP1, far upstream binding protein 1; IDH, isocitrate dehydrogenase; MLPA, multiplex ligation-dependent probe amplification; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TERT, telomerase reverse transcriptase.

results that did not match with any of the current tumor entities in the WHO classification. This has now been specified, that is, NOS should be used to indicate that the diagnostic workup was incomplete, and it was recommended that this should be noted in a report including information of why this was the case. In other words, NOS diagnoses apply if no tissue is left for molecular work-up or if there was a deliberate decision not to embark on further diagnostic procedures, for example, because of lack of consequences when a decision for best supportive care for a patient has already been made. Another category, NEC, not elsewhere classified, was introduced to indicate that molecular testing was successfully performed but did not match to histology to allow for a clear assignment to one of the currently defined WHO tumor entities. A NEC diagnosis would also be appropriate when molecular testing revealed additional information, for example a BRAF V600E mutation, that is not part of the required diagnostic workup of a particular tumor, for example, an IDH-wildtype glioblastoma, but still provides potentially relevant information [4]. The future will tell whether the complicated concepts of NOS and NEC in their current form will gain access to standard neuropathology practice.

Astrocytoma, isocitrate dehydrogenase-mutant

The cIMPACT-NOW update 2 [5] clarified a diagnostic controversy concerning IDH-mutant astrocytomas, namely, whether 1p/19q codeletion needs to be invariably excluded in these tumors or whether mutation or loss of nuclear expression of alpha-thalassemia/mental retardation syndrome, nondeletion type, X-linked (ATRX), might be sufficient for diagnostic purposes. It was recommended that immunohistochemical demonstration of loss of ATRX expression or molecular detection of *ATRX* mutation in an IDH-mutant gliomas be sufficient for diagnosing an IDH-mutant astrocytoma. On the other hand, IDH-mutant gliomas without ATRX alterations need to tested for 1p/19q codeletion to distinguish IDH-mutant astrocytoma from IDH-mutant and 1p/19q-codeleted oligodendroglioma.

Another topic relates to the nomenclature and grading of IDH-mutant astrocytomas as the clinical relevance of the existing histological criteria for grading has been challenged [6]. Distinguishing WHO grade 2 from WHO grade 3 relies on mitotic activity but a clear cut-off for a mitotic count with meaningful clinical prognostic correlates has yet to be defined [7▪]. Given the doubt on the clinical significance of separating WHO grades 2 and 3, clinical trials such as EORTC 1635 (IWOT) (NCT03763422) include tumors of both grades in the same protocols. cIMPACT-NOW furthermore recommended to change 'anaplastic astrocytoma, IDH-mutant' to 'astrocytoma, IDH-mutant, WHO grade 3, and 'glioblastoma, IDH-mutant' to 'astrocytoma, IDH-mutant, WHO grade 4' [7▪] (Fig. 1). Furthermore, the diagnosis of IDH-mutant astrocytoma, WHO grade 4, no longer depends only on traditional histological criteria, that is, presence of necrosis and/or microvascular proliferation, but

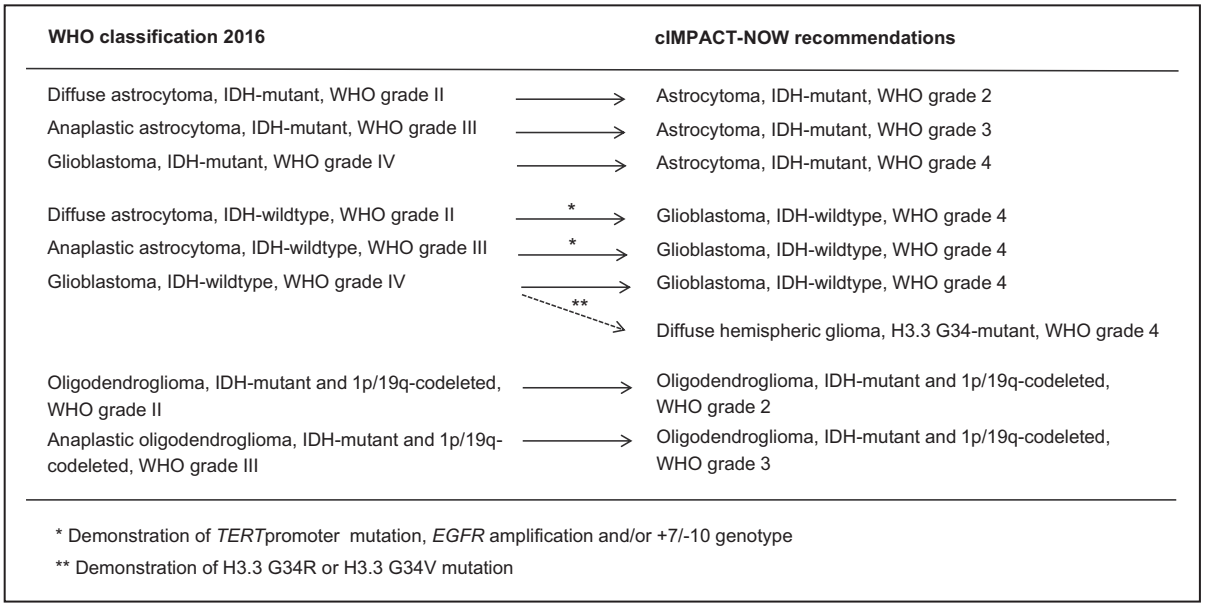

FIGURE 1. Summary of changes in the classification of diffuse gliomas from the WHO classification 2016 to the cIMPACT-NOW recommendations.

may also be based on a molecular marker, namely homozygous deletion of the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) [7▪]. Homozygous *CDKN2A* deletion has been clearly linked to inferior outcome of IDH-mutant astrocytoma patients [8▪], and hence is sufficient to assign a WHO grade 4 irrespective of the presence of necrosis and/or microvascular proliferation [7▪].

Oligodendroglioma, isocitrate dehydrogenase-mutant, and 1p/19q-codeleted

No new considerations regarding the classification of oligodendroglial tumors have emerged since 2016. The distinction between WHO grade 2 and 3 in IDH-mutant and 1p/19q-codeleted oligodendroglial tumors is based on histological criteria, in particular brisk mitotic activity and microvascular proliferation with or without necrosis. However, the clinical significance of this histology-based grading remains controversial and current trials such as CODEL (NCT00887146) include tumors of either grade. Similar to IDH-mutant astrocytomas, homozygous deletion of *CDKN2A* has been linked to malignancy and shorter survival in oligodendroglioma patients [9], but this alteration is rare, detected in less than 10% of WHO grade 3 tumors.

Glioblastoma, isocitrate dehydrogenase-wild type

Already the WHO classification 2016 had noted that histologic glioblastomas without versus with IDH mutations represented rather different diseases with regard to molecular genetics and clinical behavior. Two major changes regarding the classification of IDH-wildtype astrocytic gliomas have been proposed by cIMPACT-NOW. The first proposal addresses the subgroups of IDH-wildtype astrocytic gliomas that histologically correspond to WHO grades 2 and 3. These tumors are biologically heterogeneous and associated with variable outcome, although disease course is commonly unfavorable in patients aged 50 years or more. Several studies have indicated that three molecular markers, telomerase reverse transcriptase (*TERT*) promoter mutation, epidermal growth factor receptor (*EGFR*) amplification, and a +7/−10 genotype defined as gain of chromosome 7 and loss of chromosome 10 may signify an inferior outcome of IDH-wildtype astrocytoma patients that resembles the course of patients with IDH-wildtype glioblastoma [10,11▪]. Hence, detection of one or more of these genetic markers is sufficient to assign a WHO grade 4 to an IDH-wildtype astrocytoma, even when glioblastoma-typical histological features, that is, necrosis and/or microvascular proliferation are absent [11▪]. Instead of the originally proposed term 'diffuse astrocytoma, IDH-wildtype, with molecular features of glioblastoma, WHO grade 4' [11▪], these tumors may now be classified as frank 'glioblastoma, IDH-wildtype, WHO grade 4' [3▪] (Fig. 1). On the other hand, IDH-wildtype histologically lower grade diffuse gliomas lacking glioblastoma-associated genetic markers should be further evaluated. Especially in children and young adults, these tumors may correspond to pediatric-type diffuse gliomas characterized by *BRAF*, fibroblast growth factor receptor (*FGFR) 1* or *MYB/MYBL* aberrations and generally show an indolent clinical behavior and do not require aggressive treatment [12].

DNA methylome profiling is increasingly used as a tool to improve brain tumor classification and to aid in the differential diagnosis in tumor groups where diagnosis remains challenging if based on histology alone [13]. For instance, histologically defined glioblastomas of the cerebellum were reassigned by DNA methylation profiling to various different disease entities, including the new entity of anaplastic astrocytoma with piloid features [14,15▪], suggesting that some patients with presumed cerebellar glioblastoma may have been overtreated.

O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation remains the single molecular biomarker that is strongly associated with response to alkylating agent chemotherapy in patients with IDH-wildtype glioblastoma [16]. *MGMT* promoter methylation is most commonly assessed by methylation-specific PCR or by pyrosequencing, and challenges in defining the grey zone between *MGMT* promoter-unmethylated and *MGMT* promoter-methylated tumors remain [17▪]. The *MGMT* status is not a diagnostic marker, but its clinical significance is probably limited to IDH-wildtype gliomas and in particular to tumors that exhibit loss of chromosome 10, that is, glioblastomas. This is because the *MGMT* gene is located on chromosome 10 and loss of one copy combined with transcriptional silencing of the second allele by methylation of the promoter region may confer inability to repair DNA lesions induced by alkylating agent chemotherapy and thus benefit from temozolomide for patients. The *MGMT* status may guide treatment decisions, for example, between radiotherapy or chemotherapy without or with radiochemotherapy in elderly patients with glioblastoma and for or against alkylating agent chemotherapy in recurrent glioblastoma [16].

H3.3 G34-mutant diffuse hemispheric glioma

These tumors are characterized by missense mutations in codon 34 of the histone H3.3 protein encoded by the *H3F3A* gene [18,19]. In the WHO classification of 2016, these tumors were considered as IDH-wildtype glioblastoma. However, they show distinct biological and clinical features, including distinct DNA methylome and mutation profiles, and predominant manifestation in the cerebral hemispheres of adolescents and young adults [13,20]. Hence, cIMPACT-NOW recently proposed to separate these H3.3 G34-mutant diffuse hemispheric gliomas, WHO grade 4 as a distinct entity from IDH-wildtype glioblastoma [3▪] (Fig. 1). H3.3 G34-mutant gliomas demonstrate *MGMT* promoter methylation more often than IDH-wildtype glioblastomas and the treatment with radiotherapy with concomitant and maintenance temozolomide should probably be continued.

Diffuse midline glioma, H3 K27M-mutant

The histone H3 K27M mutation had been identified as a characteristic feature of diffuse gliomas located in midline structures like brainstem, pons, thalamus, and spinal cord [18]. However, the H3 K27M mutation was later on also detected in rare cases of nonmidline gliomas and ependymomas, which prompted cIMPACT-NOW to sharpen the diagnostic criteria by stating that H3 K27M-mutant diffuse midline gliomas must be infiltrative gliomas that are located in midline structures and must carry the H3 K27M mutation [5]. These tumors mostly lack *MGMT* promoter methylation and the value of any therapeutic measure beyond surgery as feasible followed by involved field radiotherapy remains unclear, hence novel approaches are urgently needed [21].

CONCLUSION

The upcoming WHO classification 2021 will definitively continue to separate the categories of IDH-mutant and IDH-wildtype diffuse gliomas in adults to reflect their inherently different biology and clinical outcome. While the traditional term 'glioblastoma' is now restricted to IDH-wildtype astrocytic gliomas, it has become more inclusive in that histologically lower grade astrocytomas lacking IDH and histone 3 mutations are nowadays diagnosed as IDH-wildtype glioblastoma when they exhibit *TERT* promoter mutation, *EGFR* amplification and/or a +7/−10 genotype. These improvements of glioma classification have implications both for clinical practice and for clinical trial design because clinical trials may now focus on either IDH-mutant or IDH-wildtype gliomas, but within these groups may be more inclusive.

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