A clinical perspective on the 2016 WHO brain tumor classification and routine molecular diagnostics

Martin J. van den Bent, Michael Weller, Patrick Y. Wen, Johan M. Kros, Ken Aldape, and Susan Chang

Department of Neurology and Brain Tumor Center, Erasmus MC Cancer Institute, Rotterdam, the Netherlands (M.J.v.d.B.); Department of Neurology and Brain Tumor Center, University Hospital and University of Zurich, Zurich, Switzerland (M.W.); Center for Neuro-Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts (P.Y.W.); Department of Pathology, Erasmus MC, Rotterdam, the Netherlands (J.M.K.); Department of Pathology, Princess Margaret Hospital, Toronto, Canada (K.A.); Division of Neuro-Oncology, Department of Neurological Surgery, University of California San Francisco, San Francisco, California (S.C.)

Corresponding Author: M J van den Bent, Department Neuro-Oncology and Brain Tumor Center, Erasmus MC Cancer Center, Groene Hilledijk 301, 3075EA Rotterdam, The Netherlands (m.vandenbent@erasmusmc.nl).

Abstract
The 2007 World Health Organization (WHO) classification of brain tumors did not use molecular abnormalities as diagnostic criteria. Studies have shown that genotyping allows a better prognostic classification of diffuse glioma with improved treatment selection. This has resulted in a major revision of the WHO classification, which is now for adult diffuse glioma centered around isocitrate dehydrogenase (IDH) and 1p/19q diagnostics. This revised classification is reviewed with a focus on adult brain tumors, and includes a recommendation of genes of which routine testing is clinically useful. Apart from assessment of IDH mutational status including sequencing of R132H-immunohistochemistry negative cases and testing for 1p/19q, several other markers can be considered for routine testing, including assessment of copy number alterations of chromosome 7 and 10 and of TERT promoter, BRAF, and H3F3A mutations. For “glioblastoma, IDH mutated” the term “astrocytoma grade IV” could be considered. It should be considered to treat IDH wild-type grades II and III diffuse glioma with polysomy of chromosome 7 and loss of 10q as glioblastoma. New developments must be more quickly translated into further revised diagnostic categories. Quality control and rapid integration of molecular findings into the final diagnosis and the communication of the final diagnosis to clinicians require systematic attention.

Key words
glioma | IDH | 1p/19q codeletion | 7+/10LOH | WHO classification

“The Genotype Trumps the Histological Phenotype”

The World Health Organization (WHO) classification of tumors of the CNS is the standard and universally used diagnostic system for the classification of brain tumors. It was originally built on the morphological appearance of tumor cells and their resemblance to normal brain cells, with a grading system based on the outcome of tumors if left untreated. In recent years, however, classical histopathology with a limited incorporation of genetic changes was no longer meeting current clinical needs, as illustrated by:

- The notorious interobserver variation in the classification and grading of in particular grades II and III gliomas
- The demonstration that a molecular correlate of oligoastrocytoma does not exist, consistent with large differences in outcome of anaplastic oligoastrocytoma
- Molecular reclassification of gliomas containing more prognostic information compared with classical histopathology

The common denominator in all these observations is the additional information contained in the molecular profile of histologically similar tumors, allowing a more accurate classification and better prediction of clinical outcome compared with that according to histology alone. This
insight is now reflected in the conceptual change of the 2016 revision of the “WHO Tumours of the Central Nervous System” (Table 1). In an evidence-based manner, key molecular markers such as mutations in the isocitrate dehydrogenase gene (IDH) and 1p/19q status are now central in the description of brain tumors. For clinicians, this revision is timely and reflects the beginning of an era in which molecular diagnostics are integral to the diagnostic classification. This present review focuses on the major changes the WHO 2016 classification brings to the glioma classification of CNS tumors (Table 1), and discusses which genetic alterations are useful for routine assessment and their implementation in the clinic.

The WHO 2016 Classification: from IDH to Not Otherwise Specified

For practicing neuro-oncologists, the changes in the classification of the diffuse gliomas are the most relevant, as these are by far the most frequent adult primary brain tumors. The above quote “genotype trumps phenotype” is limited to the context of glioma diagnostics and is based on the assessment of IDH mutations and 1p/19q status in diffuse glioma. A tumor with oligodendrogial morphology, showing an IDH mutation but no 1p/19q loss, will be designated astrocytoma, IDH mutated, whereas tumor with features of a glioblastoma but IDH mutated and 1p/19q codeleted will be designated an anaplastic oligodendroglioma (Fig. 1a). For diffuse (anaplastic) astrocytoma and glioblastoma without IDH mutations, the term “IDH wild type” is used (eg, astrocytoma IDH wild type; IDHwt). If molecular testing for IDH status could not be completed or was inconclusive, the term “not otherwise specified” (NOS) is used (eg, resulting in glioblastoma IDH wild type, glioblastoma IDH-mutant, and glioblastoma NOS). Except for childhood oligodendroglioma, the diagnosis of (anaplastic) oligodendroglioma requires demonstration of both an IDH mutation and combined 1p/19q loss: the current WHO classification...

Table 1 The WHO 2016 classification for astrocytoma, oligodendroglioma, and ependymoma and their International Classification of Diseases (ICD10) codes.

<table>
<thead>
<tr>
<th>WHO 2016 classification of astrocytoma, oligodendroglioma, and ependymoma</th>
<th>ICD Code</th>
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<tbody>
<tr>
<td><strong>Diffuse Astrocytoma and Oligodendroglial Tumors</strong></td>
<td></td>
</tr>
<tr>
<td>Diffuse astrocytoma, IDH mutant</td>
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<td>Gliosarcoma</td>
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<tr>
<td>Giant cell glioblastoma</td>
<td>9441/3</td>
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<td>Giant cell glioblastoma</td>
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<td>Diffuse astrocytoma, NOS</td>
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<tr>
<td>Glioblastoma, NOS</td>
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<td>Anaplastic astrocytoma, NOS</td>
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<td>Diffuse midline glioma, H3 K27M mutant</td>
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<tr>
<td>Oligodendroglioma, IDH mutant and 1p/19q codeleted</td>
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<td>Oligodendroglioma, NOS</td>
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<tr>
<td>Anaplastic oligodendroglioma, IDH mutant and 1p/19q codeleted</td>
<td>9451/3</td>
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<tr>
<td>Anaplastic oligodendroglioma, NOS</td>
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<td>Oligoastrocytoma, NOS</td>
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<td><strong>Other astrocytic tumors</strong></td>
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<tr>
<td>Subependymal giant cell astrocytoma</td>
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<tr>
<td>Anaplastic pleomorphic xanthoastrocytoma</td>
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<td><strong>Ependymal tumors</strong></td>
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<td>Subependymoma</td>
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<td>Myxopapillary ependymoma</td>
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<td>Anaplastic ependymoma</td>
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Fig. 1 Glioblastoma diagnostics 2016. (A) T1-weighted MR images of a 40-year-old male with a short history of headache and difficulty walking. At histopathology a glioblastoma was diagnosed, targeted sequencing showed an IDH2 mutation, combined 1p/19q loss, and deletion of chromosome 9 consistent with the diagnosis of anaplastic oligodendroglioma. (B) T1-weighted contrast enhanced MR image of a 50-year-old female who developed over months progressive memory and behavioral complaints. No contrast enhancement was present; at biopsy, histopathology showed a grade II astrocytoma, next generation sequencing failed to show an IDH mutation but instead documented gain of chromosome 7, loss of 10q, and mutations in the EGFR and PTEN gene consistent with a glioblastoma.
Disappearing Glioma Entities

With this emphasis on 1p/19q and IDH, mixed oligoastrocytomas do not exist in the molecular WHO 2016 classification, and what is left are morphological oligoastrocytomas in which the molecular testing was not completed or was inconclusive (NOS). Studies have made clear that mixed oligoastrocytomas are usually either IDH mutated, 1p/19q codeleted, or IDH mutated but with 1p/19q intact; at the molecular level truly mixed tumors do not exist (the rare anecdotal reports do not really contradict that).3,12 Hence, similar to the classification of oligodendroglioma, it is inconsistent that we have anaplastic astrocytoma IDH wild type, but no (anaplastic) oligoastrocytoma IDHwt, as this can potentially be one of the diagnoses rendered (although the text states that in these anaplastic mixed cases a glioblastoma should be considered, no molecular criteria for this have been defined; see below). Another entity that disappeared from the classification is “gliomatosis cerebri.” The prior diagnosis of gliomatosis cerebri was based on the radiological appearance of a diffuse tumor involving more than one lobe without histological specifications. It has long been recognized that this definition was very subjective, with outcome and sensitivity to treatment again reflecting the molecular background.13,14 In the current classification, widely infiltrating glioma are now designated according to their molecular profile. Whether widely infiltrative phenotypes have specific clinical correlations compared with more localized tumors requires further study. For clinicians, its use may continue to be helpful for some cases as initial radiotherapy may be less attractive. At present this radiological diagnosis is, however, quite loosely defined.

Grading: IDH Mutant Astrocytoma Grade IV versus Glioblastoma?

The revised WHO 2016 classification does not address grading at the molecular level. There are several reasons for this. First, in IDH mutated histological grades II and III tumors, the impact of histological grade on survival may be less compared with the impact of grade in tumors of unknown IDH mutational status.15 Clearly, though, histopathological characteristics do have an impact on outcome on IDH mutated tumors as well, as grade IV IDH mutated glioblastomas tend to have a worse outcome compared with grades II and III tumors. A reanalysis of the dataset of The Cancer Genome Atlas confirmed the relevance of grade in all molecular subtypes of diffuse glioma.16 At present, there are, however, insufficient data on molecular abnormalities within molecularly defined subgroups that allow a robust and reproducible prognostication. Although some lesions indicative of poor prognosis have been identified (eg, loss of heterozygosity [LOH] 9p in 1p/19q codeleted tumors, phosphatidylinositol-3 kinase mutations in IDH mutated 1p/19q intact tumors), they need validation in larger and independent series.16–18 But it appears a missed opportunity that the naming of “glioblastoma, IDH mutant” has not been further addressed. In the WHO 2016 classification,
these tumors continue to be lumped with the variants of glioblastoma, but these tumors are different from a metabolic perspective, occur in younger patients, and have a better outcome compared with IDHwt glioblastoma. To be consistent, a consideration would have been to label these tumors astrocytoma grade IV IDH mutant in order to distinguish them from IDHwt glioblastoma. That would also have put the IDH mutation at the heart of the “astrocytoma” diagnosis, similar to the role of the 1p/19q codeletion in oligodendroglioma. It would reflect the molecular similarities of these tumors, and the gradual and subjective differences among grades II, III, and IV IDH mutated astrocytic tumors.

### The Genetic Identification of Glioblastoma in Histological Grade II and III Lesions: Identifying Glioblastoma that Present as Low Grade Astrocytoma

The absence of IDH mutations confers a worse prognosis in diffuse grades II and III glioma, but much more can be said about these tumors. Indeed, some IDHwt diffuse astrocytoma or oligoastrocytoma/oligodendroglioma without histological features of glioblastoma (necrosis, endothelial proliferation) have genetic lesions typical of glioblastoma: gain of chromosome 7, loss of 10q, and telomerase reverse transcriptase promoter gene (TERTp) mutations.\(^{6,8,19}\) Usually these patients are 50 years or older, and they typically have a poor outcome. Some of these cases may be explained by sampling error obtained of ring enhancing lesions with a necrotic center, but others are observed in sometimes large tumors without any enhancement on MR scanning. Although the WHO classification mentions that in 1p/19q intact anaplastic oligodendroglioma and in anaplastic oligoastrocytoma with gain of 7 and loss of 10 a glioblastoma must be considered, these tumors continue to be diagnosed as astrocytoma, IDH wild type, or oligodendroglioma/oligoastrocytoma (Fig. 1b). The same holds true for entities in which only TERTp mutations are found without an IDH mutation and which usually have a clinical course similar to glioblastoma.\(^{20}\) If it is accepted that “genotype trumps phenotype,” then the WHO classification could consider going beyond the IDHwt diagnoses and make a next classifying step in grades II and III tumors with glioblastoma-like molecular characteristics. Clinicians are already becoming inclined to treat these tumors like glioblastoma—indeed, one approach could be to call them “grade III glioblastoma.” Signature glioblastoma multiforme (GBM) molecular alterations should be codified to define these tumors, as clearly other subsets of IDHwt low-grade gliomas do not have the molecular characteristics of GBM and instead represent other entities on a biological level.

### Ependymoma

New studies on large multicenter datasets on ependymoma have yielded an enormous amount of new biological knowledge.\(^{21,22}\) These have resulted in proposal for an ependymoma classification in 9 subgroups, or 6 if subependymomas are left out. In supratentorial ependymoma, fusion genes involving RELA (v-rel avian reticuloendotheliosis viral oncogene homolog A; occurring in up to 88% of childhood supratentorial ependymoma) and Yes-associated protein 1 (10%) have been identified which are absent in ependymoma posterior fossa (EPN-PF).\(^{21}\) RELA fusion ependymoma is now part of the WHO 2016 classification, but not the Yes-associated protein 1 fusion ependymoma. Using methylation arrays in EPN-PF, 2 completely different subtypes can be distinguished: EPN-PFA (high risk for progression, median age at diagnosis 3 y but occurring in 11% of adults) and EPN-PFB (low risk tumors, good prognosis, occurring in 45% of ependymoma patients between 10 and 17 y and in most patients over 18 y). Importantly, classification using methylation arrays has more prognostic and diagnostic significance compared with classical histopathology. In fact, the currently available data from analysis with methylation arrays suggest a clinically relevant distinction between tumors that postoperatively need further radiotherapy because of poor prognosis (EPN-PFA) and those with a more favorable prognosis (EPN-PFB) which after extensive resection allow a conservative approach; whereas histopathology does not allow this distinction.\(^{22}\) This is another area where clinical knowledge already deviates from the WHO 2016 diagnostic classification, and with therapeutic implications. This example further emphasizes the need to continue the refining of molecular diagnostics and their incorporation into the WHO classification, as well as the need to consider nonmutational diagnostics (ie, epigenetics) as clinically relevant classifiers.

### The 7-Year Cycle of WHO: Beyond the Realm of Pathology

Indeed, the WHO classification of brain tumors is a moving target: as time goes by, novel molecular entities will be defined (and with the observations on 7+/LOH10q tumors, at the time of the WHO 2016 publication the field has moved already).\(^{22}\) The mission of the WHO “blue book” series is to provide a description of neoplastic entities that balances the need for a universally applicable system of classification while at the same time allowing for changes that are warranted based on the evidence from current research. In so doing, it acknowledges that while appropriate molecular markers can be critical to classify tumors appropriately, they are not always universally available, and in such cases, allowance must be made to ensure and promote, to the extent possible, an accurate classification that can be widely applied. That automatically implies, though, that this diagnostic standard may not reflect the advance of medical care. As long as these new developments have only limited clinical correlates (in terms of either prognosis or treatment options), this will not be a major issue, but once these findings have clinical implications, that perception will rapidly change and friction arises with the clinicians using the diagnostic system for day-to-day treatment decisions. Another consequence of the rapid genetic developments is that revisions are required more frequently. To that end, the recent iteration of the WHO classification was termed an “update,” in accordance with the
queue in the WHO blue book series. It is estimated that in several years the time will be appropriate for the formal revision, but facts occurring “on the ground” may dictate otherwise and require earlier revisions. In addition, it is axiomatic that this diagnostic classification requires more diverse multidisciplinary input, including that of molecular biologists, clinicians, and radiologists, who represent the “end-users” of the classification. A “worst case scenario” is an “exit” variant in the field of neuro-oncology: clinicians defining their own classifications.

Which Genes Should Be Routinely Assessed?

For glial tumors, the emphasis in the WHO 2016 classification is on IDH and 1p/19q. Other frequently mutated genes have, however, been identified in glioma, like CIC, FUBP, and ATRX, many of which appear to be subclonal. Others are clearly clonal, like TERTp and TP53. They currently serve no role in the WHO 2016 classification but they may have some significance, especially if more advanced diagnostic platforms are used that routinely assess a wider spectrum of abnormalities. Incorporating these in routine diagnostics may help to better understand the overall picture and increase the overall reliability of a molecular diagnosis even if they are not essential for any diagnosis. In contrast, other rarer mutations in BRAF and histone genes (H3F3A, HIST1H3B) indeed identify tumors with specific clinical characteristics. Of these, H3F3A K27M mutations have been included in the new WHO classification, with the designation of a new entity, the “diffuse midline glioma, H3 K27M mutant.” This raises the question as to which should be routinely assessed, which are optional but nice to have, and which are without clinical relevance.

1p/19q Codeletion

This is now part of standard diagnostics. Loss of 1p/19q was first identified in 1994 as the most characteristic genetic lesion in oligodendroglioma, associated with chemotherapy response in 1998, and subsequently assumed to be both prognostic for survival and predictive for benefit from the addition of procarbazine/lomustine/vincristine chemotherapy to radiotherapy. This combination is occasionally identified in newly diagnosed oligodendrogliomas in patients beyond 65 years of age.

IDH Mutations

Assessing IDH mutations is now also part of standard diagnostics. Two types of IDH mutations are observed in glioma: in the IDH1 and in the IDH2 gene. All mutations in IDH1 and IDH2 are somatic, missense, and heterozygous and affect codon 132 (IDH1) or codon 172 (IDH2). IDH mutations are mutually exclusive; 90% of all IDH mutations concern the IDH1 R132H mutation. Studies have shown that IDH mutations are early events in gliomagenesis, and remain present at the time of tumor progression. About 5%–10% of glioblastoma show IDH mutations, in particular in patients below 50 years of age. In pediatric glioma, IDH mutations are rare but have been described in patients as young as 12 years. IDH mutated tumors have an improved outcome compared with non-IDH mutated tumors of similar histopathological grade. IDH mutations cause an altered enzyme substrate affinity, leading to increased levels of 2-hydroxyglutarate and lower levels of α-ketoglutarate. One of the metabolic alterations that this induces is the development of a global methylation of cytosine-phosphate-guanine islands, including the MGMT gene promoter. This may explain some of the chemotherapy sensitivity of IDH mutated tumors; another explanation is that some of the chemotherapy resistance mechanisms are depending on α-ketoglutarate. It has been suggested that IDH mutations can be used to identify patients that will...
benefit from adding chemotherapy to radiotherapy; other studies, however, did not confirm this and identified MGMT promoter methylation as the best predictive factor, which is usually present in IDH mutant tumors.41,42

Tumor Protein 53

TP53 mutations are predominantly observed in exon 4–8, and occur in 95% of IDH mutated tumors without 1p/19q codeletion. They do, however, also occur in other glial tumors, including glioblastoma, in 1p/19q codeleted tumors (although less frequently), in medulloblastoma, and in pediatric glioma. Therefore, they lack diagnostic specificity and in glial tumors are not associated with treatment outcome. There is currently no role for routine testing; if diagnosed they may support the diagnosis of several entities.

Alpha-Thalassemia Syndrome Gene

Mutations in the alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene occur in 70% of IDH mutated gliomas without 1p/19q codeletion, the astrocytic type of glioma. They are mutually exclusive with TERTp mutations. There are no hot spot regions for ATRX mutations, and they can be subclonal with different ATRX mutations in different parts of the tumor and with different ATRX mutations at first diagnosis versus recurrent tumors. If present, they suggest an IDH mutated TP53 mutated astrocytoma. ATRX mutations also occur in H3 mutated tumors. ATRX mutations can be assessed by immunohistochemistry (IHC) and by sequencing. Loss of ATRX IHC staining in mutated tumors can be a rapid method to detect ATRX mutations, and it has been suggested that it may obviate the need for 1p/19q testing.9 While some neuropathologists use ATRX IHC as a criterion to select which gliomas are to be tested for 1p/19q status, further experience is needed to test whether it can substitute for a 1p/19q test, but for now, the WHO 2016 classification explicitly does not accept positive staining for ATRX in IDH mutated tumors as an alternative to diagnose 1p/19q codeleted IDH mutated oligodendroglioma.

Telomerase Reverse Transcriptase Promoter Mutations

Somatic hot spot mutations in TERTp occur in IDHwt glioblastoma and in 1p/19q codeleted IDH mutated oligodendroglioma. As a consequence, simply assessing both TERTp and IDH mutational status already results in a very powerful prognostic glioma classification.22,23 In some tumors, only TERTp mutations are found, without other typical glioma alterations; these patients tend to have a poor outcome. TERTp mutations are mutually exclusive with ATRX mutations. Interestingly, patients with grades II and III IDHwt tumors but without a TERTp mutation appear to have a better prognosis compared with patients with TERTp mutations. Typically, these studies have been lacking the assessment of chromosome 7 and 10q, which most likely would have identified a glioblastoma-like chromosomal loss pattern in many of the IDHwt/TERTp mutated tumors. More clinical outcome data on these tumors are urgently needed. Assessment of TERTp mutational status can be useful for IDHwt diffuse glioma; they are, however, not specific for glioma and, for example, occur also in medulloblastoma.

The Gain of 7 and Loss of 10q Genotype

The combination of tri/polysomy of chromosome 7 and LOH of 10q is a characteristic combination found in many glioblastomas and probably represents an early event in these tumors.16,44 Usually TERTp mutations are present, and in 40%–50% of cases EGFR amplification, usually with EGFR mutations, including EGFRvIII mutations in 20%. Many IDHwt astrocytomas and anaplastic astrocytomas (especially in patients >45 y) show this 7+/10q− pattern and typically have a clinically aggressive course (Fig. 1b).2,45 Testing for this combination in patients over 45–50 years of age with grade II or III IDHwt tumors may give positive indications for a poor prognosis. The WHO classification strongly suggests that the diagnosis of glioblastoma should be considered in 7+/10q− anaplastic oligodendroglioma and anaplastic oligoastrocytoma, but these abnormalities do not qualify for the diagnosis of glioblastoma in the current classification. The available clinical data support that despite these being histologically grade II or III tumors, they should be treated as glioblastoma, and many clinicians with routine access to diagnostics of 7 and 10q do so.

Epidermal Growth Factor Receptor Amplification and Mutations

EGFR amplification occurs in 40%–50% of all glioblastoma and is usually associated with epidermal growth factor receptor (EGFR) mutations and trisomy/polysomy of chromosome 7.46 Most EGFR amplified tumors also show EGFR mutations affecting the extracellular domain of the receptor, the most frequent being the EGFR variant III (EGFRvIII) mutation. There is currently no drug that specifically or effectively exploits any of these mutations, although several trials on novel agents are ongoing. As a consequence, from both a therapeutic and a diagnostic aspect, the routine assessing of EGFR amplification or EGFR mutations is currently not useful. The presence of EGFR amplification is indeed highly specific: if found, it is diagnostic at the molecular level of a glioblastoma but lacks sensitivity: assays for EGFR amplification will be negative in 50% of the glioblastoma cases. Outcome of EGFR amplified or EGFRvIII mutated tumors is not different from other glioblastoma.47 Currently, for glioblastoma diagnostics, assessing both chromosome 7 and 10q or TERTp mutations is more informative than assessing EGFR amplification status.

Phosphatase and Tensin Homolog

Phosphatase and tensin homolog (PTEN) mutations occur in 20%–30% of glioblastoma and are as a rule accompanied
by LOH10q. When both are present, this results in biallelic PTEN inactivation. They may also occur at low frequency in other gliomas with unclear clinical significance, and in other tumors (medulloblastoma). Thus, it has low diagnostic value and no therapeutic consequences. Routine assessment of PTEN mutations is clinically not indicated.

**BRAF-KIAA Fusion Genes and BRAF Mutations in Glial Tumors**

Abnormalities in the proto-oncogene B-Raf gene (**BRAF**) are characteristic of several subgroups of gliomas. Pilocytic astrocytoma (PA) in the fossa posterior typically have a tandem duplication at 7q34 resulting in a transforming fusion gene between **KIAA1549** and **BRAF** (**BRAF** duplication or **BRAF-KIAA1549** fusion gene), but not the **BRAFv600** mutation. **BRAF-KIAA** fusion genes are also frequent in non-neurofibromatosis type 1 (NF1) optic nerve glioma (73%).** BRAF-KIAA**1549** fusions are age specific, rare in PA patients over 40 years of age (7%). **BRAFv600** mutations are mutually exclusive with the **BRAF-KIAA1549** fusion gene; these are observed in 33% of non-posterior fossa PA. They are also relatively common in pleomorphic xanthoastrocytoma (PXA; 43%–66%), anaplastic PXA (65%), and ganglioglioma (18%–43%), especially if located in the brainstem.49-52; they are rare in adult glioma (glioblastoma: 2%, adult low-grade glioma: 0–3%).49 They are also frequent in the proposed novel (but rare) WHO entity of epithelioid glioblastoma, although their distinction from anaplastic PXA is unclear.53 A study on pediatric diencephalic low-grade glioma reported frequent **BRAFv600** mutated non-PA in this region, with imaging characteristics of vivid enhancement and multiloculated or multinodular appearance and/or infiltrative growth on T2-weighted images (Fig. 2).54 A Canadian series observed **BRAF** fusion positivity in unilateral thalamic low-grade tumors.55 Since **BRAF**-mutated tumors may be treated with targeted agents aiming at the **BRAFv600** mutations, either alone or in combination with a pathway inhibitor of MERK (mitogen-activated extracellular signal-regulated protein kinase), the finding of this abnormality may have therapeutic implications. Responses to these agents have been described, and this appears to be a very promising avenue of research.56 **BRAF** mutations and the **BRAF-KIAA** fusion have not been incorporated into the current diagnostic classification; the diagnosis of PA remains a morphological definition. Routine testing must be considered in relevant cases. Future research should focus on establishing to what extent these tumors share the same background, and to what extent other abnormalities in the **RAS/RAF** pathway may have a similar phenotypic effect. More rare genetic lesions in PA include **NF1**, **KRAS**, and **RAS** mutations and **FGFR1** and other **BRAF** fusions.57

**Histone H3F3A and HIST1H3B Mutations**

The WHO 2016 classification has accepted the “diffuse midline glioma, H3 K27M mutant” as a diagnostic entity, occurring predominantly in childhood and adolescent brain tumor patients. The mutation is part of a larger family of histone mutations with similar clinical presentation. Pediatric and young adult glioma frequently show mutations in genes encoding H3 variants, which through histone modification alter gene expression.58 Driver mutations occur in the **H3F3A** gene (positions K27 and G34) encoding the histone H3.3 genes, and in the **HIST1H3B** histone H3.1 gene (K27 position). K27-mutated tumors typically arise in the brainstem and midline structures, such as the thalamus and cerebellum, mostly in children and young adults. Thus, diffuse intrinsic pontine glioma frequently harbor K27M mutations in histone H3.3 genes as well as in H3.1 genes.59 Childhood and young adult supratentorial glioma may show mutations in histone H3.3, with K27M mutations occurring in midline tumors. In contrast, pG34R/V histone H3.3 mutations are restricted to pediatric and young adult high-grade gliomas of the cerebral cortex and are almost invariably associated with **ATRX** and **TP53** mutations.59,60 K27 mutations are associated with a poor outcome; G34 mutations appear to have better survival. Intrinsic pontine glioma harboring a K27M mutation in H3.3 are less responsive to radiotherapy, with earlier relapses and more metastatic recurrences than those in H3.1.60 Although the K27M mutation was frequently observed in adult brainstem and thalamic gliomas, this mutation tended to be associated with a poorer prognosis in brainstem gliomas but not in thalamic gliomas.61 The presence of the **H3F3A** K27M mutation is associated with mutations in **TP53**.59,62 An antibody against the K27M allele may prove useful to facilitate detection of this mutation.63 The role of other mutations (eg, **ACVR1**) in diffuse intrinsic pontine glioma remains to be elucidated. Testing for **H3F3A** mutations is insightful in pediatric and young adult cases with midline tumors.

### Challenges: Platforms and Tests to Be Used

The revised WHO 2016 criteria do not make recommendations how to assess molecular alterations, which is wise in view of the rapidly changing landscape of molecular diagnostics and testing platforms. With next-generation sequencing techniques becoming rapidly more affordable, next-generation sequencing panels tailored for glioma diagnostics are increasingly being used for routine diagnostics, including assessment of copy number alterations (CNAs).64 Although there is clearly an advantage of the assessment of more than only **IDH** mutation and 1p/19q status, the routine use of screening for the 50 most frequent cancer genes or whole exome in glioma is without clinically proven benefit. Outside the identification of molecular glioblastoma with WHO grade II or III histology, no proven therapeutic decisions can be taken based on these profiles, with **BRAF** mutant tumors as the most promising exception, as they allow patients to be selected for clinical trials.56 Previous studies have shown the clinical usefulness of gene expression analysis and genome-wide methylation analysis. In particular, the latter approach has been shown to be very informative, allowing the classification of tumors without knowledge of specific mutations. This classification system is based on the assumption that the methylation pattern of a tumor is the consequence of both the lineage of the cell the tumor arises from and...
tumor-specific DNA characteristics. For brain tumors, a relevant aspect here is that the analysis of methylation status simultaneously allows the assessment of MGMT status, which may well be the single most powerful determinant of benefit from alkylating agent chemotherapy.\textsuperscript{\text{45,46}}

Assessment of Molecular Characteristics in Everyday Practice and Pitfalls

Testing for IDH is part of routine diagnostics, but it seems reasonable to limit routine testing to an age range of 15 to 55–60 years, for example, and test beyond that only on clinical indications (eg, in all adult grades II and III glioma, in the presence of oligodendrogial features, in case of a hemispheric astrocytoma in a 13 year old). For the R132H mutation, a very reliable IHC assay is available, but this represents only 90% of all IDH mutations. As a consequence, IHC has at best a 90% sensitivity, implying that in case of IHC negativity this must be followed by sequencing for both IDH1 and IDH2 mutations (Fig. 4). IHC can be used as a first screen, but not as a tool to rule out IDH mutations. Testing for 1p/19q status and IDH mutations should be performed in all patients presenting with possible oligodendrogial tumors. Testing for 1p/9q status should use an assay that allows assessment of loss of the entire 1p and 19q arm. Fluorescence in situ hybridization for 1p using a probe for the 1p36.6 region is less specific, as it may suggest loss in tumors with partial 1p deletion only, limited to the tip of chromosome 1p.\textsuperscript{\text{65,66}} This part can be lost without loss of the rest of chromosome 1p, which in combination with 19q loss has been observed in glioblastoma. If CNAs are considered to be relevant, this should be assessed with other techniques. Both 1p/19q codeletions and IDH mutations are early events in gliomagenesis, and their presence or absence is unlikely to change over time.\textsuperscript{\text{67,68}} Therefore, retesting of 1p/19q and IDH status at the time of a re-resection in tumors with already known status is of limited use, unless a significant clinical change occurred indicating a second tumor. Incorporation of assessment of TERTp mutations into routine diagnostics of gliomas has been suggested.\textsuperscript{20} In the absence of 1p/19q loss, diffuse gliomas with TERTp mutations tend to have a poor outcome reminiscent of glioblastoma.\textsuperscript{7,43} Although some studies on targeted mutation assessment have shown that in some tumors only TERTp mutations were observed, this deserves further clinical study, since in most of these series tumors were not tested for CNA of 7 and 10q.

Reporting

Centers must develop automated workflows that incorporate molecular testing in their routine procedures, including the incorporation of the molecular diagnostics in the final pathology result. It is important for the reporting of the diagnosis to be standardized and made available for capture in the national cancer registry databases, so the incidence of the specific entities based on molecular features can be reported. Since the turnaround time for histopathology is shorter than for molecular diagnostics, ensuring accurate and timely feedback on molecular findings in patients in whom a histopathological diagnosis has already been established is important. It is recommended to routinely include (MR) imaging characteristics in the final diagnostic considerations: MRI should be consistent with the pathological diagnosis, and if not, this should give rise to additional scrutiny. The interpretation of molecular findings depends on the context: if the tumor is unlikely to be a diffuse glioma, the molecular findings may not contribute except when a diagnostic mutation is found with supportive MR and clinical findings.

Clinical Studies

With the new classification, the clinical data from past trials without molecular analysis have become outdated. Since the first results of the trials on anaplastic oligodendroglioma, follow-up trials in the newly diagnosed setting (CODEL, CATNON) but also in recurrent disease (TAVAREC) enrolled patients based on their 1p/19q status because of the difference in prognosis, and many prospective trials reported retrospectively on the molecular status (Table 2). New studies should now distinguish among IDH mutant tumors, IDH mutant and 1p/19q codeleted, and IDH wild type diffuse gliomas. This complicates matters. As an example, the presence of IDH mutations also identifies a more favorable subgroup of glioblastoma which may also hold true at the time of recurrence.\textsuperscript{20} This questions whether these tumors should be enrolled in trials on recurrent glioblastomas and whether they should be routinely tested for. On the other hand, the still modest survival of IDH mutated glioblastoma also argues against enrolling
these tumors in trials aiming at IDH mutated diffuse grades II and III glioma, although the limited difference in outcome between grades II and III IDH mutated tumors provides a rationale for combining these grades.\textsuperscript{15} Today’s changes emphasize that all trials should collect tissue samples as part of the study design. Analysis of existing datasets may help to improve our understanding of the outcome of these subsets of patients.

Quality Control

The interobserver variation in the histopathological classification of glioma is well known, but early experiences with interlaboratory tests on diagnostic molecular assays on the same set of tumors revealed that differences between laboratories may exist as well.\textsuperscript{70,71} Proper quality control is critical now that diagnostics and clinical decisions are based on molecular testing. Laboratories need to certify and validate their testing procedures with appropriate controls. This is not exciting work and requires significant efforts, but is absolutely essential for reliable diagnostics.

Conclusions

The new WHO 2016 classification for brain tumors brings molecular diagnostics to the center of glioma classification. This revised classification will improve treatment selection of brain tumor patients and clinical trial design. This will not be the last revision of this classification, as new molecular insights into brain tumors will further refine the classification of brain tumors. Further refinements already seem indicated, such as in the IDH wild type categories of grades II and III glioma, as these represent in many cases—especially in patients over 50 years of age—glioblastoma-like lesions with 7+10q LOH. Further analysis of TERTp mutational status in non-glioblastoma 1p/19q intact tumors will be needed to better understand the prognostic role of that mutation in diffuse glioma. More responsiveness to the rapidly changing and multidisciplinary field of neuro-oncology will be crucial to maintain a well-accepted WHO classification of brain tumors. For this, a more transparent and multidisciplinary process of change of these pivotal criteria will be needed.

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References


