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TOPIC REVIEW

The role of neuropathology in the management of newly diagnosed glioblastoma: a systematic review and evidence-based clinical practice guideline

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Abstract

Target Population These recommendations apply to adult patients with newly diagnosed or suspected glioblastoma (GBM)

Question For adult patients with newly diagnosed GBM does testing for *Isocitrate Dehydrogenase 1* or *2* (*IDH 1/2*) mutations afford benefit beyond standard histopathology in providing accurate classification and outcome prognostication?

Level III *IDH 1/2* mutational status by immunohistochemistry (IHC) and/or sequencing is suggested for classification and prognostic information.

Level III Non-canonical *IDH 1/2* mutations are very rare in patients aged 55 or older and universal testing of variant mutations by sequence analysis is not suggested for this age range.

Question For adult patients with lower grade infiltrating astrocytomas (WHO grades II and III) can the IDH-wildtype status designation supersede histopathology to predict prognosis and biologic relevance to eventual behavior as a GBM?

Level III The designation of infiltrating astrocytomas (WHO grades II and III) as IDH-wildtype is not suggested as sufficient for a higher grade designation alone.

Level III It is suggested that IDH-wildtype WHO grades II and III astrocytomas be tested for molecular-genetic alterations typical of IDH-wildtype GBM such as *EGFR* amplification, gain of chromosome 7/loss of chromosome 10 and *TERT*-p mutation to substantiate prediction of behavior similar to IDH-wildtype glioblastoma.

Level III It is suggested that a diagnosis of diffuse astrocytic glioma, IDH-wildtype, with molecular features of GBM, WHO grade IV be rendered for infiltrating astrocytomas that lack histologic criteria of GBM but harbors molecular-genetic alterations of IDH-wildtype glioblastoma.

Question For adult patients with newly diagnosed infiltrating glioma arising in the midline does testing for H3-K27M mutations provide information beyond that gained by histopathology for accurate classification and outcome prognostication?

Level III It is suggested that infiltrating gliomas arising in midline anatomic locations be tested for the H3-K27M mutation as they tend to exhibit WHO grade IV behavior even if they lack histologic criteria for glioblastoma.

Keywords Neuropathology · Infiltrating glioma · Glioblastoma · Biomarker · Immunohistochemistry · IDH · H3F3A · K27M · EGFR · TERT promoter

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Rationale

The definitive diagnosis of GBM that is used to guide clinical management is based on the histopathologic examination and ancillary molecular-genetic testing of neurosurgically sampled tissue. Although clinical and neuro-imaging features can be highly suggestive, the gold standard for diagnosis is based on microscopic examination and biomarker testing of tissue samples. Pathologic studies should be performed in a multidisciplinary setting, in conjunction with a patient's clinical history, neurosurgical impression, and neuroradiologic findings in order to generate an internally consistent, integrated diagnosis [1–3]. Neuroradiologic features are of particular importance, because they highlight neuro-anatomic locations, represent the entirety of the disease as compared to the sample evaluated by neuropathologists, generate diagnostic possibilities that are most likely, and may point out discrepancies between clinicoradiologic findings and pathologic assessment [2, 3]. Diagnostic discrepancies can be resolved prior to definitive therapy in the setting of strong lines of communication between clinical and diagnostic disciplines.

Numerous textbooks contain detailed descriptions of the pathologic criteria used for classifying and grading glial neoplasms [4–7]. Infiltrating gliomas comprises a heterogeneous group of diseases along a histologic spectrum with well-described overlapping morphologic features. The discovery of *IDH 1/2* mutations along with other breakthrough molecular studies during the past decades have consistently shown that molecular-genetic alterations better capture the biologic behavior of these tumors. Hence, the recently revised 4th edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) [7] heavily integrates clinically relevant genomic alterations with histologic findings in the classification schemas. We address the contemporary approach to neuropathologic diagnosis of infiltrating gliomas in the molecular era with an emphasis on GBMs and evaluate the current literature relevant to the focused topics of interest regarding newly diagnosed glioblastomas. We evaluated the literature in order to evaluate the strength of evidence that support current methods used in the neuropathologic diagnosis of malignant gliomas. Published data were assigned to class I, II, or III based on the nature of the information in the manuscript as determined by study methodology and results obtained.

Objectives

The objective of this guideline is to assess the rapidly advancing field of diagnostic molecular neuropathology that has evolved since the last guidelines were published in 2008 [8]. Here we analyze the molecular classification of infiltrating gliomas in the post-IDH era and further document the role of biomarker testing for classification and prognostication of newly diagnosed glioblastomas.

Methods

Writing group and question establishment

The evidence-based clinical practice guideline taskforce members and the Joint Tumor Section of the American Association of Neurological Surgeons (AANS) and the Congress of Neurological Surgeons (CNS) have prioritized an update of the guidelines for management of newly diagnosed glioblastoma. A series of writers were identified and screened for conflict of interest. This group in turn agreed on a set of questions addressing the role of neuropathology in the diagnosis of newly diagnosed GBM and conducted a systematic review of the literature relevant to the histopathologic diagnosis of GBM and molecular-genetic and biomarker testing algorithms that has reshaped the classification of infiltrating gliomas.

Literature review plan

We collaborated with a medical librarian to search for articles published from July 1, 2005 to October 31, 2018. The PubMed, EMBASE, and Cochrane Library data bases were searched for articles relevant to the classification of infiltrating gliomas, the molecular-genetic alterations that define them, and emerging biomarkers employed for diagnostic and prognostic purposes. Articles relevant to the pathology of GBMs were given special consideration as delineated by the scope of this publication.

Data collection process

We performed multiple computerized PubMed searches of the National Library of Medicine database of scientific literature published from July 1, 2005 to October 31, 2018. Keywords that were searched included "glioma", "astrocytoma", "glioblastoma", "diagnosis", "pathology", "neuropathology", "immunohistochemistry", "genetics", "genomics", "molecular", "IDH", "biomarker", and "prognosis". We limited our searches to human studies published in the English language. Key words were searched in multiple combinations. Links to "related articles" from highly relevant studies were utilized to broaden the search. Articles were also identified from the reference lists from articles uncovered in initial searches. Reference lists from the most recent editions of standard textbooks of surgical neuropathology were reviewed, including, *Practical Surgical Neuropathology: A Diagnostic Approach* [4], *Greenfield's Neuropathology* [5], *Diagnostic Pathology:*

Neuropathology [6], and *the WHO Classification of Tumours of the Central Nervous System (Revised 4th Edition)* [7].

Study selection and eligibility criteria

Original articles providing information to establish histopathologic, biomarker or genetic diagnostic criteria for newly diagnosed infiltrating gliomas and GBM, were selected for review. In pathology these types of investigations do not usually generate standard descriptive parameters used for classifying data, such as sensitivity, specificity, positive and negative predictive value, accuracy, likelihood ratio of a positive and negative result, and κ.

The citations were screened for the following inclusion and exclusion criteria:

Inclusion criteria

- Published between July 1, 2005 and October 31, 2018
- Published in English
- Human studies only
- Studies including adult patients (age > 18 years) were included in the study
- Studies including patients aged less than 18 years were included if they were considered highly relevant to adult newly diagnosed GBM
- Number of study participants with newly diagnosed tumors ≥ 20.
- Fully published (i.e., not in abstract form) peer-reviewed primary studies

Exclusion criteria

- in vitro studies only
- Animal studies only
- Studies focused on non-infiltrative gliomas or other CNS tumors

Those abstracts that met with the selection criteria mentioned above were retrieved in full text form. The adherence to the selection criteria were confirmed. The information was then used for construction of the evidence tables the text below. Thirty-one references were selected to construct the evidence tables in the text below.

Classification of evidence and recommendation levels

The concept of linking evidence to recommendations has been further formalized by the American Medical Association (AMA) and many specialty societies, including the American Association of Neurological Surgeons (AANS), the Congress of Neurological Surgeons (CNS), and the American Academy of Neurology (AAN). This formalization involves the designation of specific relationships between the strength of evidence and the strength of recommendations to avoid ambiguity. A summary of these categories of evidence can be viewed at https://www.cns.org/guidelines/guideline-development-methodology. Much of the pathology literature addressed below are well designed and studied large retrospectively collected cohorts and often added meaningful outcome data. However, the lack of prospective validation will qualify many of these studies as Class III. Though there must be room for special considerations, Level I recommendations are generally based on Class I evidence, Level II recommendations are based on Class II evidence and Level III recommendations are based on Class III evidence [9].

Scientific foundation

The current document is an update to the publication by Brat DJ et al. entitled "Diagnosis of malignant glioma: role of neuropathology" from 2008 [8]. For perspective it is useful to note the results of that publication. Four questions were posed: (1) What are the most appropriate diagnostic criteria for establishing a diagnosis of malignant glioma? (2) What is the best technique for establishing the diagnosis of malignant glioma when a suspicious lesion is identified on CT or MRI and tissue has been sampled? (3) What is the reliability and reproducibility of the diagnosis of a malignant glioma? (4) What is the role of additional testing in the diagnosis of malignant gliomas? In response to them one level I recommendation was made: The diagnosis of malignant glioma should be based on the histopathologic review of tissue. Two level II recommendations were made: (1) Both frozen section and cytopathologic evaluation are recommended for the intraoperative diagnosis of malignant glioma. (2) Consultation from a neuropathologist specialized in brain tumor diagnosis is recommended for problematic cases. Three level III recommendations were formulated: (1) Incorporation of clinical and radiographic information with the final pathologic diagnosis is recommended. (2) The criteria of the WHO classification of brain tumors are internationally recognized and can be utilized for establishing the diagnosis of malignant gliomas. (3) Proliferation studies, such as those based on Ki-67/MIB1 staining, and molecular genetic tests are recommended as adjuvant studies for classification and prognostication of malignant gliomas. This update does not provide information that refutes these. Rather, it provides information that adds to them by utilizing publication data on molecular markers that have led to their selective use in neuropathology practice.

Question For adult patients with newly diagnosed GBM does testing for *Isocitrate Dehydrogenase 1* or *2* (*IDH*

1/2) mutations afford benefit beyond standard histopathology in providing accurate classification and outcome prognostication?

It is now recognized that morphologically similar diffuse astrocytomas have distinct genetic profiles, clinical settings and biologic behavior. IDH-mutant diffuse astrocytomas tend to arise in a younger patient population, are lower grade at the time of patient presentation, progress at a slower rate, and are associated with *TP53* mutations and *ATRX* alterations [7, 10]. The mutational status of *IDH1/2* genes is central to classification of diffuse gliomas, especially in the adult population, since their presence or absence divides these tumors into clinically distinct subsets [10–14]. Parsons et al. [11] and Yan et al. [12] first recognized IDH-mutant infiltrating gliomas as a form of disease that could not be readily recognized on histologic assessment yet had a distinct biologic behavior compared to their IDH-wildtype counterparts. Parsons et al. found that IDH-mutant GBMs occurred in younger patients with a mean age of 33 years vs 53 years in IDH-wildtype GBMs ($p < 0.001$, t test), in nearly all of the secondary GBMs ($p < 0.001$, binominal test) and had better OS of 3.8 years vs 1.1 years in the IDH-wildtype counterpart ($p < 0.001$, log rank test) [11]. Yan et al. also found patients with IDH mutations to be significantly younger than those wildtype for IDH (34 years vs 56 years for patients with anaplastic astrocytoma and 32 years vs 59 years among patients with GBM; $p < 0.001$, Student's t-test). Patients with IDH-mutant GBM had a median OS of 31 months vs 15 months for those that were IDH-wildtype ($p = 0.002$, log-rank test) [12] (See Table 1).

Mutations within the enzymatic sites of *IDH 1/2* result in a enzymatic gain-of-function and the accumulation of the oncometabolite 2-hydroxyglutarate (2-HG), which, in turn, inhibits DNA and protein demethylation resulting in genome-wide DNA hypermethylation that promotes gliomagenesis [15–18]. Gliomas with IDH mutations and global DNA hypermethylation are referred to as CpG island methylator phenotype (G-CIMP) [16, 17, 19–22]. More than 80% of histologic WHO grades II and III diffusely infiltrating astrocytomas and secondary GBMs are IDH-mutant, while only about 5–10% of primary ('de novo') GBMs are [11–13, 23–26]. Adult patients with infiltrating gliomas harboring IDH mutations are significantly younger than those without these mutations; however, IDH mutations are uncommon in patients younger than 18-years-old and very rare in childhood [12, 24, 27–32]. Diffuse gliomas that carry IDH mutations exhibit a slower rate of progression and improved clinical outcomes, compared to IDH-wildtype tumors [12, 31]. The finding of an IDH mutation in a glioma strongly supports the diagnosis of a diffusely infiltrative glioma since they are rarely, if ever, found in other CNS neoplasms [29]. In addition, IDH mutations are also recognized as relatively stable events throughout the course of disease, which further highlights its utility for evaluating residual/recurrent disease in IDH-mutant tumors [15].

IDH1 and *IDH2* mutations result in a substitution for a key arginine at codons R132 and R172, respectively [12, 27, 30]. The most frequent mutation, representing 92.7%, occurs at codon 132 of the *IDH1* gene, and results in the substitution of arginine for histidine (R132H) [27]. Less frequent *IDH1* mutations include R132C (4.2%), R132S (1.5%), R132G (1.4%), and R132L (0.2%) [27]. Residue R172 in exon 4 of the *IDH2* gene is homologous to R132 in the *IDH1* gene, with R172K representing 64.5% of all *IDH2* mutations followed by R172M (19.3%), and R172W (16.2%) [27]. *IDH2* mutations are much less frequent (approximately 3%) than *IDH1* mutations among diffuse gliomas, but are slightly more common in oligodendrogliomas than astrocytomas [27]. Since over 90% of IDH mutations are represented by IDH1 R132H, a monoclonal antibody has been developed to the mutant protein, allowing its use in paraffin-embedded specimens (mIDH1R132H) [33]. The ability of the antibody to detect a small number of cells as mutant makes this method more sensitive than sequencing for identifying R132H-mutant gliomas [34, 35]. However, mutations in *IDH2* and other variant *IDH1* mutations will not be detected using IHC with this antibody, and in the proper clinical setting, it may be necessary to test for other *IDH1* or *IDH2* mutations by sequencing analysis. It has been suggested that sequencing may not be warranted in the setting of a negative R132H immunostain in GBMs arising in patients older than 55 years due to the rarity of non-R132H IDH1 mutations in patients in this age group [36–39]. In a study of cost effectiveness addressing IDH testing in diffuse gliomas, DeWitt and colleagues found a negligible prevalence of noncanonical IDH mutations in GBM patients over the age of 55 years suggesting that there is limited value in screening for variant *IDH1/2* mutations by sequencing in this population and supporting this age-based cutoff to improve test utilization and balance cost effectiveness in clinical practice [38] (See Table 1).

In the setting of a diffuse glioma with an IDH mutation, a diagnosis of an IDH-mutant astrocytoma (WHO grades II-IV), is supported by the presence of a *TP53* mutation and alterations (mutation or deletion) of *α-Thalassemia/Mental Retardation Syndrome X-linked* (*ATRX*), a gene involved in chromatin remodeling pathways and DNA methylation [10, 12, 13, 23, 32, 40–42]. Following the investigation of The Cancer Genome Atlas Research Network (TCGA) on WHO diffuse gliomas, the neuropathologic diagnosis of IDH-mutant diffuse astrocytomas of grade II, III or IV can be established by documenting IDH mutations, *ATRX* alterations and *TP53* mutations [11, 12, 25, 31, 40, 43, 44]. There are a number of ways to achieve this, including focused, targeted or whole genome sequence analysis. In daily surgical

Table 1 Role of IDH mutations in newly diagnosed glioblastomas

Author (References)	Description of study	Evidence class*	Conclusions
Shirahata et al. [47]	Retrospective analysis of 211 IDH-mutant astrocytomas (WHO grade II-IV) that included both morphological and genetic information (histological review, image analysis, DNA-methylation studies and copy number profiles) to develop prognostic grading criteria for IDH-mutant astrocytomas. The developed algorithms were validated in three independent cohorts totaling 486 cases	III	Novel algorithms for grading IDH-mutant astrocytomas were developed on the basis of histologic and molecular information. The morphological parameters of strongest negative prognostic value were vascular proliferation ($p < 0.0005$) and necrosis ($p < 0.00005$). High cell density as determined by image analysis was associated with worse OS ($p < 0.002$). A Ki-67 proliferation index above a cutoff of 14.5% was associated with worse OS ($p < 0.006$) but proliferation assessed by mitotic counts was not a strong predictor of outcome. The most relevant molecular parameter for OS was *CDKN2A/B* homozygous deletion ($p < 0.0001$). Those tumors with a high load of CNAs had worse OS ($p < 0.0001$). Three novel algorithms incorporating the presence of necrosis, *CDKN2A/B* homozygous deletion and high load of CNAs were developed, all of which performed better than the current WHO grading criteria
Korshunov et al. [51]	Retrospective analysis of 97 IDH-mutant GBMs (68 'de novo' and 29 'secondary') by genome-wide DNA methylation analysis, copy-number profiling and targeted NGS	III	The incidence of IDH-mutant GBM was estimated at 4%. The median age of all patients with IDH-mutant GBM was 36 years (range 18–57 years). IDH-mutant GBM had distinct methylation profiles that separated them from other infiltrating gliomas, including lower grade IDH-mutant gliomas and IDH-wildtype infiltrating astrocytomas. Most (89 samples, 91%) *IDH1* gene alterations were R132H mutations with the rest representing variant mutations. Homozygous *CDKN2A/B* deletions were associated with worse OS and were identified in 43% of tumors. 'De novo' and 'evolved' IDH-mutant GBM did not differ significantly in terms of their molecular patterns or clinical outcomes
Robinson et al. [39]	Retrospective analysis of 578 infiltrating astrocytomas WHO grades II-IV and the correlation of IHC profile (IDH1 R132H, ATRX and p53) with patient age	III	Eleven patients over the age of 55 years were found to be IDH-mutant by immunohistochemistry. ATRX retention by IHC is possible but rare in IDH-mutant cases, however this can occur at any age. In IDH-mutant tumors, ATRX loss is more common than strong p53 immunoexpression. Regardless of age, IDH1 R132H IHC is recommended in the workup of diffuse gliomas
Cimino et al. [50]	Multidimensional scaling analysis of the TCGA data and independent validation using the German Glioma Network prospective GBM data set to identify distinct molecular subgroups of the 2016 WHO classification of diffuse gliomas	III	IDH-wildtype astrocytomas had the oldest age distribution peak, (56–63 years). The IDH-mutant astrocytoma cluster had the youngest age distribution peak (26–32 years). The IDH-mutant astrocytoma cluster appears highly heterogeneous in terms of CNAs. The IDH-wildtype diffuse gliomas have frequent CNAs including + 7, − 10, and − 9p. IDH-mutant diffuse gliomas clinically behaved better than IDH-wildtype diffuse gliomas across all histologic grades. 2D multidimensional scaling with Oncoscape identified subsets of disease with prognostic significance. The IDH-wildtype WHO grade IV astrocytoma cluster can be further divided into 3 subgroups on the basis of polysomy chromosome 1, polysomy chromosome 19 and CDK4/MDM2 co-amplification with a difference in OS across molecular subtypes ($p = 0.002$, Cox proportional hazards regression). Segregation of the IDH-mutant astrocytoma cluster on the basis of CDK4 amplification, *CDKN2A* deletion, and chromosome 14 gain revealed three molecular subtypes with significant survival differences ($p < 0.001$, Cox proportional hazards regression)

Table 1 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Reuss et al. [45]	Analysis of biomarkers including *ATRX*, IDH and 1p/19q co-deletion in a series of 405 infiltrating gliomas classified by molecular parameters into integrative diagnoses and their correlation to long term follow up in a subset of the NOA-04 trial. The technical standard for ATRX IHC is addressed	III	Loss of ATRX expression by IHC was nearly mutually exclusive with 1p/19q codeletion with only 2 of 167 ATRX-negative tumors being codeleted. Of 141 ATRX-negative tumors, 137 were IDH-mutant. The majority of IDH-wildtype GBMs with loss of ATRX expression carried *H3F3A* mutations. All 1p/19q co-deleted gliomas had IDH mutations. IHC for IDH1 and ATRX followed by 1p/19q analysis and IDH sequencing are part of a powerful algorithm for glioma classification
Liu et al. [92]	Analysis of 140 grade II-IV adult gliomas, 17 grade II-III pediatric astrocytomas, and 34 pilocytic astrocytomas for *ATRX*, *TP53* and IDH mutations by IHC and sequencing. All tumors were *H3F3A*-wildtype	III	*ATRX* alterations significantly overlapped with IDH ($p < 0.0001$) and *TP53* mutations ($p < 0.0001$) and were prevalent in astrocytomas and oligoastrocytomas but not oligodendrogliomas ($p < 0.0001$). *ATRX* alterations frequently occurred in adult diffuse gliomas, and specifically IDH/*TP53*-mutant astrocytomas. None of the pediatric tumors had IDH/*ATRX* mutations. *ATRX* alterations occurred in 26% (10/38) of adult GBMs, significantly overlapping with those that were IDH-mutant ($p < 0.0001$). A strong correlation between *ATRX* mutation and loss of expression by IHC was again confirmed but *ATRX* missense mutations may not lead to loss of expression by IHC
Jiao et al. [93]	Analysis of 363 brain tumors for *ATRX*, *CIC*, *FUBP1* and IDH mutations	III	*ATRX* mutations were associated with IDH mutations and frequently mutated in grade II-III astrocytomas (71%), oligoastrocytomas (68%), secondary GBMs (57%) and were very rare in primary GBMs (4%). Overall, IDH mutations were present in 87 of 88 (99%) adult gliomas with an *ATRX* mutation and most 94% also had *TP53* mutations. *CIC* and *FUBP1* mutations were seen in 46% and 24% of oligodendrogliomas, respectively, and were absent in grade II-III astrocytomas. 98% of those gliomas with a *CIC* or an *FUBP1* mutation had 1p/19q loss. *CIC* and *FUBP1* mutations were mutually exclusive with *ATRX* and *TP53* mutations ($p < 0.001$). Clinically distinct groups of gliomas comprised of IDH/*ATRX* mutation, IDH/*CIC/FUBP1* or neither had median OS of 96, 51 and 13 months, respectively
Hartmann et al. [49]	Analysis of 382 anaplastic astrocytomas and GBMs from the NOA-04 trial and from the prospective translational cohort of the German Glioma Network for *IDH1* mutations and its prognostic significance	III	*IDH1* mutational status is more prognostic of OS than histological criteria among high-grade astrocytomas and differentiates anaplastic astrocytoma and GBMs into clinically meaningful, prognostically distinct subgroups. Presence of an *IDH1* mutation was the most prominent single prognostic factor (RR 2.7; 95% CI1.6–4.5). In terms of OS the range of outcomes from most favorable to poorest was: *IDH1*-mutant anaplastic astrocytoma > *IDH1*-mutant GBM > IDH-wildtype anaplastic astrocytoma > IDH-wildtype GBM ($p < 0.0001$). 60% of anaplastic astrocytoma and 7.2% of GBMs were *IDH1*-mutant. *IDH1* mutations very rarely occur in patients aged over 60 years

Table 1 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Hartmann et al. [27]	Analysis of 1010 diffuse gliomas for type and frequency of IDH mutation	III	716 *IDH1* and 31 *IDH2* mutations were identified. The distribution of *IDH1* mutations was as follows: R132H (92.7%), R132C (4.2%), R132S (1.5%), R132G (1.4%), and R132L (0.2%). Regarding mutations in the *IDH2* gene, R172K represented 64.5% of all *IDH2* mutations followed by R172M (19.3%), and R172W (16.2%). *IDH2* mutations occurred much less frequently (approximately 3%) than *IDH1* mutations, but were more predominant in oligodendrogliomas than astrocytomas. IDH mutations were rare under the age of 18 years and the mean age for IDH-mutant anaplastic gliomas was 43.9 years compared to 50.6 in those that were IDH-wildtype ($p < 0.0001$)
Yan et al. [12]	Analysis of *IDH1* and *IDH2* sequencing in 445 CNS tumors and 494 non-CNS tumors in addition to other alterations involved in gliomagenesis including *TP53*, *PTEN*, *EGFR*, *CDKN2A/B* and 1p/19q co-deletion	III	IDH R132 mutations were not observed in 21 pilocytic astrocytomas, 2 subependymal giant cell astrocytomas, 30 ependymomas, 55 medulloblastomas or any of the 494 non-CNS tumors. IDH mutations were present in 27/30 WHO grade II diffuse astrocytomas, 38/52 WHO grade III anaplastic astrocytomas, 6/123 primary GBM, 11/13 secondary GBM, 43/51 WHO grade II oligodendrogliomas and 34/36 WHO grade III anaplastic oligodendrogliomas. 10/10 oligoastrocytomas grades II and III were also IDH-mutant. Patients with IDH mutations were significantly younger than those wildtype for IDH (34 years vs 56 years for patients with anaplastic astrocytomas and 32 years vs 59 years among patients with GBM; $p < 0.001$, Student's t-test). Patients with IDH-mutant GBM had a median OS of 31 months vs 15 months for those that were IDH-wildtype ($p = 0.002$, log-rank test). IDH-mutant anaplastic astrocytomas had improved outcomes when compared to their IDH-wildtype counterparts (65 months vs 20 months, respectively; $p < 0.001$, log-rank test)
Balss et al. 2008 [30]	Analysis by direct sequencing of the R132 position in the *IDH1* gene in 685 brain tumors	III	221 tumors were positive for somatic *IDH1* mutations with the highest frequencies in diffuse astrocytomas (68%), oligodendrogliomas (69%), oligoastrocytomas (78%) and secondary GBM's (88%). The R132H variant accounted for 92.7% of IDH1 mutations. IDH mutations were absent in cases of SEGA, PXA, gliosarcoma, ependymal tumors, medulloblastomas, schwannomas, meningiomas and pituitary adenomas. Rare cases of pilocytic astrocytomas and giant cell GBMs, pediatric GBMs and PNET were found to be *IDH1*-mutant

Table 1 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Parsons et al. [11]	Genomic analysis of 22 GBMs by sequencing 20,661 protein coding genes, determination of CNAs by high-density oligonucleotide array and NGS	III	Alterations in critical genes of the p53 pathway (*TP53*, *MDM2*, and *MDM4*), the RB1 pathway (*RB1*, *CDK4*, and *CDKN2A*), and the PI3K/PTEN pathway were identified in 64%, 68% and 50% of tumors, respectively. In all but one case only a single member of each pathway was mutated ($p < 0.05$). Five of the 22 GBMs carried an IDH1 R132H mutation and further testing of additional GBMs showed recurrent mutations in the active site of *IDH1* in 12% of GBM patients (18/149 in total). IDH-mutant GBMs occurred in younger patients with a mean age of 33 years vs 53 years in IDH-wildtype GBMs ($p < 0.001$, t test), in nearly all of the secondary GBMs ($p < 0.001$, binominal test) and had better OS of 3.8 years vs 1.1 years in the IDH-wildtype counterpart ($p < 0.001$, log rank test)

CNA copy number alteration, *GBM* glioblastoma, *IDH* isocitrate dehydrogenase, *IHC* immunohistochemistry, *NGS*, next-generation sequencing, *OS*, overall survival, *PFS*, progression free survival, *WHO* World Health Organization

neuropathology practice, IHC for IDH-1 R132H, ATRX and p53 is also reliable and cost-effective in the routine workup of infiltrating gliomas and provides definitive results to guide diagnosis in the majority of cases. The finding of immunoreactivity for IDH-1 R132H in neoplastic cells confirms an *IDH1* mutation, and strong nuclear staining of p53 correlates with *TP53* mutations in this setting. The loss of nuclear ATRX expression in neoplastic cells, with its maintained expression in non-neoplastic cells such as endothelial cells or normal glia, is associated with *ATRX* deletion or mutation. This combination of immunohistochemical staining is diagnostic of an IDH-mutant diffuse astrocytoma and current histologic grading criteria can then be applied while keeping in mind that the prognostic effect of mitotic index has been shown to be dependent on IDH mutational status [45–47]. Grading based on the presence of mitotic figures is not a strong predictor of outcomes among WHO grade II and III IDH-mutant astrocytomas and criteria that better discriminate clinical behavior are greatly needed [46, 48, 49]. Thus far, the evidence suggests that the application of current WHO grading criteria to distinguish grade II and III IDH-mutant astrocytomas does not separate patients based on clinical parameters or outcome.

With the current recognition of IDH-mutant and IDH-wildtype diffuse astrocytomas as distinct disease entities, optimal grading criteria will need to be re-evaluated within each of these molecularly-defined categories. Prognostic markers for use in grading the IDH-mutant astrocytomas, WHO grades II- IV are emerging but will need to be better defined in order to optimally stratify risk for this population. Cimino et al. [50] found that, IDH-mutant astrocytomas could be separated into prognostically significant subclasses on the basis of CNAs that included *CDK4* amplification, *CDKN2A* deletion and chromosome 14 loss. Korshunov et al. [51] also found an association between homozygous *CDKN2A/B* deletion with decreased survival. Furthermore, Shirahata et al. [47] recently studied a cohort of IDH-mutant infiltrating astrocytomas (WHO grades II-IV) and confirmed the strong negative prognostic value of vascular proliferation ($p < 0.0005$) and necrosis ($p < 0.00005$) but also showing that *CDKN2A/B* homozygous deletion had prognostic power superior to microvascular proliferation and necrosis. (See Table 1) Thus, genetic alterations are being recognized as consistent and reproducible markers of clinical behavior and will likely enter grading schemas for diffuse gliomas in the near future.

Synthesis

The field of diagnostic surgical neuropathology has transitioned from a purely histologic discipline to one that is integrative with the routine incorporation of molecular-genetic biomarkers. *IDH 1/2* mutations are an early event

in gliomagenesis that defines the majority of lower grade infiltrating astrocytomas (WHO grades II and III), and secondary GBMs. Testing for *IDH 1/2* mutations are recommended in the work up of infiltrating gliomas for accurate classification as delineated by the new WHO classification. *ATRX* alterations and *TP53* mutations further define IDH-mutant infiltrating astrocytomas of all WHO grades. In one algorithm for infiltrating gliomas, a panel of IDH R132H, p53 and ATRX IHC are performed first, with additional testing if necessary. If IDH R132H IHC is negative in a lower grade diffuse glioma, sequencing of *IDH1* and *IDH2* genes should follow to rule out variant mutations. Testing for *IDH 1/2* mutations by IHC or sequencing is also recommended in GBMs, particularly those arising in younger adults but sequencing may not be necessary in patients aged 55 and older given the rarity of non-canonical IDH mutations. The presence of an *IDH 1/2* mutation has been consistently correlated with slower disease progression and improved overall survival when compared to their wildtype counterparts.

Question For adult patients with lower grade infiltrating astrocytomas (WHO grades II and III) can the IDH-wildtype status designation supersede histopathology to predict prognosis and biologic relevance to eventual behavior as a GBM?

IDH-wildtype astrocytomas arise in an older patient population with the large majority presenting at a high grade (mostly GBM) and with a clinically aggressive behavior. These are often associated with genetic alterations typical of IDH-wildtype GBM including *TERT* promoter (*TERT-p*) mutations and characteristic copy number alterations (CNAs) such as gain of chromosome 7, loss of chromosome 10, *EGFR* amplifications, *PTEN* loss, and *CDKN2A* deletions, among others [10, 52]. Glioblastomas are often referred to as "primary" when they present as WHO grade IV disease as the first clinical manifestation, and as "secondary" when they have evolved over time from a lower grade precursor. Primary (or 'de novo') GBMs typically arise in older patients with a rapid onset of clinical symptoms are nearly all IDH-wildtype (approximately 90–95% of cases) and carry a poor prognosis. In contrast, secondary GBMs commonly arise in patients aged less than 45 years, show slower progression and are nearly all IDH-mutant [11, 25, 26]. By the currently employed grading criteria, IDH-wildtype WHO grade II and III infiltrating astrocytomas lack necrosis and microvascular proliferation, and do not fulfill criteria for GBM, yet their molecular-genetic profiles are often strikingly similar to those of IDH-wildtype GBMs and they also display aggressive clinical behavior [10, 22, 53]. Yan et al. [12] reported a median OS of 20 months for patients with IDH-wildtype anaplastic astrocytoma, WHO grade III, while patients with IDH-wildtype GBM, WHO grade IV had a median OS of 15 months. In the TCGA analysis, WHO grades II-III IDH-wildtype infiltrating astrocytomas had a genetic profile similar to primary (IDH-wildtype) GBM and exhibited a median OS of 1.7 years, which was only slightly longer than those of the IDH-wildtype GBMs (OS, 1.1 years) [10] (See Table 2).

Since many IDH-wildtype astrocytic gliomas of histologic grade II and III (no necrosis or microvascular proliferation) are known to behave clinically aggressively, many studies have been performed in order to identify biomarkers that could reliably be used to predict WHO grade IV behavior, similar to IDH-wildtype GBM [47, 54, 55]. In a recent study of 160 IDH-wildtype histologic grades II and III astrocytomas, Reuss et al. [53] found that 78% were molecular equivalents to conventional IDH-wildtype GBM, with similar outcomes and frequencies of *TERT*-p mutations, 7p gain/10q loss, *EGFR* amplification or combined 10q/13q/14q co-deletion. Wijnenga et al. [56] studied the clinical heterogeneity of 74 IDH-wildtype diffuse lower grade gliomas and found the prognosis of those with hallmark genetic signatures that included + 7/− 10q and *TERT*-p mutations to be similar to IDH-wildtype glioblastomas. In a study of 212 diffuse astrocytomas examined for IDH mutations, DNA methylation status and copy number profiles, Hasselblatt and colleagues identified 25 IDH-wildtype tumors (12%) that were histologically low grade and were non-enhancing on neuroimaging. Of these, 17 were either classified as GBMs by methylation profiling or showed genetic characteristic of GBMs when they were unclassified by methylome analysis [57] (See Table 2). Given the clinical and genomic similarities, WHO grades II-III IDH-wildtype astrocytomas could represent undersampled or incipient GBMs at an early stage and which have not yet developed microvascular proliferation or necrosis [10, 42, 45, 53, 58]. Since documenting the absence of an IDH mutation alone is insufficient for a higher grade designation, the evidence now supports performing further molecular testing of histologically low grade infiltrating IDH-wildtype astrocytomas to identify those with GBM-like genetic alterations and likelihood for aggressive behavior as well as those that lack such alterations and may exhibit better prognosis [56, 57, 59–61].

Hallmark genetic alterations of IDH-wildtype GBMs include *TERT*-p mutation, gain of chromosome 7, loss of chromosome 10, *CDKN2A* deletion, mutation or deletion of *PTEN*, and amplifications of proto-oncogenes, including *EGFR*. These are highly relevant to the diagnosis, since the absence of IDH mutations (IDH-wildtype designation) by sequence analysis and IHC is shared by numerous other tumor entities. A positive finding of one of these hallmark genetic alterations supports the diagnosis of an IDH-wildtype malignant glioma, especially in the setting of an infiltrative growth pattern. Up to 90% of IDH-wildtype GBMs have *TERT*-p mutations, making it the most frequent mutation in this tumor type [52, 61–67]. The

Table 2 Significance of the IDH-wildtype designation in histologic WHO II and III astrocytomas

Author (References)	Description of study	Evidence class*	Conclusions
Stichel et al. [61]	Retrospective analysis of three large cohorts of brain tumors for the distribution of *EGFR* amplification, + 7/− 10, and *TERT*-p mutation in brain tumors by copy number profile, DNA methylation profiling and NGS testing and their correlation with prognosis	III	*EGFR* amplification and the + 7/− 10 signature are very strong surrogate markers for the diagnosis of GBM in IDH-wildtype diffuse astrocytic tumor. The combination of any two of *EGFR* amplification, + 7/− 10 and *TERT*-p mutation is highly specific for IDH-wildtype GBM and the combination of all three alterations is frequent and exclusively seen in IDH-wildtype GBM. Three possible CNAs of chromosomes 7 and 10 that are associated with poor prognosis similar to IDH-wildtype GBM are + 7/− 10, + 7q/− 10, and + 7/− 10q. The sensitivity and specificity of any combination of *EGFR* amplification, + 7/− 10, and *TERT*-p mutation for IDH-wildtype GBM is 58.3% and 99.4%, respectively. The assessment of all three parameters is recommended for upgrading an IDH-wildtype astrocytoma to IDH-wildtype GBM
Hasselblatt et al. [57]	Retrospective analysis of 212 diffuse astrocytomas (WHO grade II) for *IDH1*/2 mutations by immunohistochemistry and sequencing analysis, DNA methylation status, copy number profiles and NGS for 130 genes recurrently altered in brain tumors	III	25/212 tumors did not harbor *IDH 1/2* mutations and of these 10 were classified as IDH-wildtype GBM by methylation analysis and an additional 7 cases showed genetic alterations of IDH-wildtype GBM (+ 7/− 10 signature). Ten of these seventeen cases showed early malignant progression. These 17 patients were significantly older than the whole group (median age 57 years vs 44 years for the whole cohort). The remaining 8 cases stratified with other methylation classes. The data finds no supportive evidence for the existence of diffuse astrocytoma, IDH-wildtype (WHO grade II), as most cases will show a genotype equivalent to IDH-wildtype GBM and may represent an early stage of the disease
Wijnenga et al. [56]	Analysis of 74 IDH-wildtype histologically classified diffuse low-grade gliomas by NGS and copy number profiles and the correlation of molecular signatures such as + 7/− 10q and *TERT*-p mutations with OS	III	Stratification of these IDH-wildtype diffuse low-grade gliomas revealed molecularly heterogeneous tumors. 39 tumors (52.7%) showed a + 7/− 10q signature characteristic of IDH-wildtype GBM and all of these but one tumor harbored a *TERT*-p mutation. *BRAF* and *H3F3A* mutations were identified in 4.1% of the cases. Better outcomes were noted in tumors that did not harbor *H3F3A* or *TERT*-p mutations or the + 7/− 10q CNA. The data highlights the heterogeneity of histologically classified diffuse low grade gliomas and suggest testing for *IDH1/2* mutations and 1p/19q codeletion is insufficient to predict poor outcomes in an IDH-wildtype diffuse low grade glioma and that testing for genetic alterations such as *TERT*-p mutations and copy number profiles are useful to inform on prognosis and guide treatment decisions

Table 2 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Aibaidula et al. [59]	Retrospective analysis of 166 IDH-wildtype WHO grade II and III gliomas for *EGFR* and *MYB* amplifications, and *H3F3A*, *TERT*-p and *BRAF* mutations	III	IDH-wildtype lower grade gliomas (WHO grade II and III) do not have uniformly poor prognosis and testing for additional genetic alterations is encouraged. *EGFR* amplification, and mutations of *BRAF* and *H3F3A* mutations were observed in 13.8%, 6.9%, and 9.5% of patients, respectively, and were mutually exclusive. *TERT*-p mutations were present in 26.8% of cases. Favorable outcome were seen in younger patients, oligodendroglial phenotype, and grade II histology. Independent adverse prognostic values of older age, incomplete resection, grade III histology, *EGFR* amplification, and *H3F3A* mutation were confirmed by multivariable analysis. Molecularly-defined high grade tumors (harboring *EGFR* amplification or mutations in *H3F3A* or *TERT*-p) had median OS of 1.23 years while those lacking all three genetic alterations had median OS of 7.63 years. Lower-grade gliomas with *MYB* amplification had the best OS
Pekmezci et al. [94]	Analysis of 1206 infiltrative gliomas WHO grades II-IV derived from the UCSF Adult Glioma Study, the Mayo Clinic and TCGA with known status for IDH, 1p/19q co-deletion, *ATRX*, and *TERT* and their assignment to one of five molecular categories reflective of the 2016 WHO classification	III	The group of IDH-wildtype diffuse gliomas that included 42 WHO grade II and 112 WHO grade III tumors was enriched for *TERT*-p mutations only (60% of the cases). *TERT*-p mutant tumors occurred in significantly older patients at diagnosis when compared to the wildtype counterpart (median ages 56 and 37 years, respectively, $p < 0.001$). Among the lower grade IDH-wildtype astrocytoma group, the *TERT*-wildtype group had significantly better OS than the *TERT*-mutant group (HR: 0.48, 95% CI 0.27–0.87), $p = 0.02$)
Ceccarelli et al. [41]	Large scale multidimensional analysis of 1122 adult diffuse gliomas (TCGA low grade glioma and GBM cohorts of 516 and 606 cases, respectively) for whole genome, exome, targeted and RNA-sequencing, somatic mutations, copy number variations, gene and protein expression and epigenetic signatures and their utility in molecular classification and mechanisms of progression in gliomas. The findings were validated with retrospectively collected datasets	III	75 significantly mutated genes were identified in the cohort, 45 of which had not been previously associated with gliomas. *TERT*-p mutations were nearly mutually exclusive with *ATRX* mutations and strongly associated with *TERT* upregulation ($p < 0.0001$). Nearly all IDH-wildtype cases with + 7/− 10 harbored *TERT*-p mutations, suggesting that *TERT*-p mutations may precede these characteristic chromosomal CNAs. Unsupervised clustering identified 6 clinically-relevant methylation groups and 4 RNA expression groups associated to IDH mutation status as the primary driver of methylome and transcriptome clustering and the major determinant of the molecular underpinning in gliomagenesis. A newly identified G-CIMP-low IDH-mutant glioma enriched for cell cycle gene abnormalities was associated with worse survival than G-CIMP-high and IDH-mutant 1p/19q co-deleted groups ($p = 3.9 \times 10^{-10}$, log-rank test). A novel subgroup of IDH-wildtype adult diffuse glioma sharing genetic and DNA methylation features with pediatric pilocytic astrocytomas and better clinical outcomes was identified

Table 2 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Weller et al. [60]	Large-scale genomic, transcriptomic profiling and integrative analysis of 137 grade II and III adult gliomas from the prospective German Glioma Network cohort (61 WHO grade II and 76 WHO grade III gliomas) and the correlation of biomarkers to clinical outcomes	III	Five distinct molecular groups (three IDH-mutants and two IDH-wildtype) were uncovered following genomic profiling. Eight transcriptionally distinct groups resulted from expression profiling (five IDH-mutants and three IDH-wildtype). Integrative analysis revealed three major prognostic groups in which IDH-mutant and 1p/19q codeleted gliomas had the best prognosis and those that were IDH-wildtype had the worst outcomes and often harbored alterations associated with primary IDH-wildtype GBM (chromosome + 7/− 10, gene amplification events, and *TERT*-p mutations). *TERT*-p mutations were more common in gliomas with an oligodendroglial phenotype and a concurrent 1p/19q co-deletion when compared to those that were 1p/19q intact ($p < 0.001$). Gliomas with an IDH mutation and 1p/19q co-deletion had the best outcomes. *MGMT* promoter methylation was associated with a prolonged OS
Olar et al. [46]	Retrospective analysis of 558 WHO grade II-III diffuse gliomas for IDH mutations and the prognostic impact of WHO grading criteria within IDH-mutant and IDH-wildtype subsets	III	Among IDH-mutant tumors, comparison between WHO grades II vs III showed no statistically significant differences (log-rank $p = 0.1739$). Statistically significant survival difference based on WHO grade was seen within the IDH-wildtype subset ($p = 0.0507$). Using a mitotic index cut-off of 4/1000 tumor cells, the mitotic index was significantly associated with outcome in IDH-wildtype tumors (log-rank $p < 0.0001$), but not in IDH-mutant tumors (log-rank $p = 0.5157$), demonstrating a significant statistical interaction ($p < 0.0001$) between IDH mutational status and mitotic index. Patient age was significantly associated with outcome only in the IDH-wildtype subset. The study concludes that grading criteria needs to be optimized within the context of IDH mutations
Reuss et al. [53]	Retrospective analysis of a series of 160 adult IDH-wildtype infiltrating astrocytomas [40 diffuse astrocytomas (WHO grade II), and 120 anaplastic astrocytomas (WHO grade III)] for hallmark molecular alterations (*IDH1/2*, *H3F3A*, and *TERT*-p mutations) methylation profiles and copy number alterations such as *EGFR* amplification and gain of chromosome 7/loss of chromosome 10	III	The majority of the tumors (78%) were found to be molecular equivalents to IDH-wildtype GBMs and 9% were classified as *H3F3A*-mutant GBM (both G34 and K27 variants). *TERT*-p mutations were restricted to tumors restricted to molecular equivalents of GBM. Gain of chromosome 7p and loss of chromosome 10q were characteristic of GBM and in 88% of the cases the whole chromosomes were affected (+7/− 10 signature). The combination of this CNA was not present in any of the H3 K27M-mutant cases. The data demonstrates that IDH-wildtype diffuse and anaplastic astrocytomas can be stratified to other discrete entities at the molecular level. The median survival of the tumors reclassified as IDH-wildtype GBMs on the basis of molecular alterations was 19.4 months. The median survival of the H3 K27M cluster was 16.9 months

Table 2 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
TCGA [10]	Comprehensive and integrative genome-wide analysis of 293 grades II and III adult diffuse gliomas including exome sequencing, DNA copy number, DNA methylation, mRNA expression, microRNA expression and targeted protein expression and their correlation with clinical outcomes	III	Patients with IDH-mutant gliomas were younger than those that were IDH-wildtype; these tumor arose more frequently in the frontal lobes ($p < 0.05$). Three distinct non-overlapping and prognostically significant molecular classes of lower grade infiltrating gliomas were derived from the integration of genome-wide data and unsupervised clustering. These subtypes of gliomas were accurately captured by *IDH1/2* mutation and 1p/19q status. There were no molecular signatures to support the designation of mixed gliomas or oligoastrocytomas. IDH-mutant, 1p/19q intact diffuse gliomas had *TP53* mutations (94%) and *ATRX* inactivation (86%). IDH-mutant, 1p/19q co-deleted diffuse gliomas were enriched for *TERT*-p (96%), *CIC* (62%), *FUBP1* (29%), *NOTCH1* (31%), *PIK3CA* (20%), *PIK3R1* (9%), *ZBTB20* (9%), *ARID1A* (6%) mutations and had the best clinical outcomes. IDH-wildtype infiltrating gliomas that were histologically lower grade had similar molecular-genetic makeup and clinical outcomes as primary GBMs and had more mutations than the IDH-mutant gliomas ($p < 0.001$). Common genes mutated in IDH-wildtype gliomas included *EGFR* (27%), *PTEN* (23%), *NF1* (20%), *TP53* (14%), and *PIK3CA* (9%); the frequency of *TERT*-p mutation was similar to those seen in GBMs (80%). CNAs in lower grade IDH-wildtype gliomas resembled those of primary IDH-wildtype GBMs with +7/− 10 seen in more than 50% of cases, which was absent in IDH-mutant tumors. *EGFR*, *MDM4* and *CDK4* amplifications as well as *CDKN2A* and *RB1* losses were commonly found in the IDH-wildtype gliomas. In terms of clinical outcomes, median survival for IDH-wildtype tumors, IDH-mutant non-codeleted tumors and IDH-mutant codeleted tumors 1.7, 6.3 and 8 years, respectively. Kaplan–Meier analysis show statistically significant differences in OS when lower grade gliomas were molecularly classified on the basis of IDH and 1p/19q status ($p < 0.001$, log-rank test)

Table 2 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Eckel-Passow et al. [52]	Analysis of 1087 gliomas for IDH mutations, *TERT*-p mutations and 1p/19q co-deletion status and their association to clinical characteristics and outcomes	III	Gliomas were classified into five molecular groups on the basis of IDH and *TERT*-p mutations and 1p/19q co-deletion. Among grade II and III gliomas, 29% were 'triple-positive', 5% had *TERT*-p and IDH mutations, 45% had only an IDH mutation, 7% were 'triple-negative', and 10% had only *TERT*-p mutations. Only 5% of grade II and III gliomas had other combinations. Overall, only 2.6% of gliomas could not be assigned to one of these 5 groups, of which the majority (75%) were oligodendrogliomas or 'mixed gliomas' with IDH mutations and 1p/19q co-deletion but were not *TERT*-p mutant. In contrast, the vast majority of grade IV gliomas had only *TERT*-p mutations (74%). The molecular subgroups were independently associated with OS among grade II and III gliomas. Patients with only IDH mutations were significantly younger (37 years) than those that were 'triple-positive' (44 years), gliomas with only *TERT*-p and IDH mutations (46 years) and those that were 'triple-negative' (50 years) [$p < 0.001$]. Regarding survival, *TERT*-p mutation is generally unfavorable in the absence of an IDH mutation, yet favorable in the presence of an IDH mutation and 1p/19q codeletion
Wiestler et al. [21]	Epigenomic DNA methylation analysis of 228 anaplastic gliomas, their comparison to 55 GBMs and its association with tumor classification and outcomes	III	Unsupervised clustering of DNA methylation patterns revealed three distinct groups of clinically relevant anaplastic gliomas that correlated with the *IDH1/2* mutation and 1p/19q status; CIMP-positive (IDH-mutant) tumors were subdivided on the bases of 1p/19q status (CIMP-codeleted vs CIMP-non-codeleted) with the third subtype being CIMP-negative (IDH-wildtype). Loss of ATRX expression by IHC was seen in 80% of CIMP-non-codeleted cases and, conversely, *TERT*-p mutations were present in 88% of CIMP-codeleted anaplastic gliomas. CIMP-positive tumors had high frequency *MGMT* promoter methylation with all CIMP-co-deleted scored as methylated. CIMP-positive tumors had a longer OS than CIMP-negative tumors ($p < 0.001$, log-rank test). CIMP-negative (IDH-wildtype) tumors clustered together with primary GBMs and shared similar frequencies of hallmark CNAs as well as a similar methylation profile ('GBM-like' genotype). Characteristic IDH-wildtype GBM CNAs such as *EGFR* amplification, gain of chromosome 7 and loss of chromosome 10 were enriched in the CIMP-negative tumors and were evenly rare in both CIMP-positive groups

Table 2 (continued)

Author (References)	Description of study	Evidence class*	Conclusions
Killela et al. [63]	Analysis of 473 adult gliomas for *IDH1/2* and *TERT*-p mutations and their relationship to OS	III	74.2% of GBM's were *TERT*-p mutated in comparison to only 18.2% of grade II-III astrocytomas. In contrast, 78.4% of grade II-III astrocytomas were *IDH1/2*-mutant. *TERT*-p and *IDH1/2* mutations co-occurred in 79% of oligodendrogliomas. An OS of 11.5 months was seen for grade III-IV gliomas carrying a *TERT*-p mutation alone. Those grade III-IV gliomas with only an *IDH1/2* mutation and an astrocytic phenotype had an OS of 57 months. The greatest OS was seen in those gliomas with an oligodendroglial morphology in which there was co-occurrence of *IDH 1/2* and *TERT*-p mutations (125 months in grade III gliomas)

CIMP CpG island methylator phenotype, *CNA* copy number alteration, *GBM* glioblastoma, *IDH* isocitrate dehydrogenase, *MGMT* O6-methylguanine-methyltransferase, *NGS* next-generation sequencing, *OS* overall survival, *TCGA* The Cancer Genome Atlas, *WHO* World Health Organization

chromosomal + 7/− 10 signature has been identified in 59% of IDH-wildtype glioblastomas [61]. Additionally, nearly all IDH-wildtype infiltrating gliomas with the + 7/− 10 CNA harbor *TERT*-p mutations or exhibit upregulated *TERT* expression [41, 56]. Brennan et al. showed that 57% of GBMs had evidence of mutation, rearrangement, altered splicing and/or focal amplification of *EGFR,* reflecting its status as a key oncogenic event [68]. More recently, Stichel and colleagues identified *EGFR* amplification in 36% of IDH-wildtype GBMs and characterized it as the most specific standalone biomarker of the entity with 99.8% specificity [61]. The sensitivity and specificity of any combination of *EGFR* amplification, + 7/− 10, and *TERT*-p mutation for IDH- wildtype GBM has recently been determined to be 58.3% and 99.4%, respectively [61] (See Table 2). Approximately 15–18% of primary, IDH-wildtype GBMs carry *PDGFRA* amplifications while 5–8% harbor *MET* amplifications. Importantly, *EGFR*, *PDGFRA* and *MET* amplifications are not mutually exclusive and may be seen in the same tumor [69]. *MDM2* and *CDK4* gene amplifications occur correspondingly in 5–15% and 14–18% of primary IDH-wildtype glioblastomas [7, 11, 26, 68, 70]. Homozygous *CDKN2A* deletions are present in up to 50% of IDH-wildtype glioblastomas [7, 11, 70–72].

Based on an abundance of supportive evidence, The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy – Not Official WHO (cIMPACT-NOW) [73] recently reviewed the current literature and concluded that the minimal molecular criteria for identifying an IDH-wildtype diffuse astrocytic glioma that would follow an aggressive clinical course similar to that of an IDH-wildtype GBM includes: (1) *EGFR* amplification, or (2) combined whole chromosome 7 gain and whole chromosome 10 loss (+ 7/− 10), or (3) *TERT*-p mutation. If any of these molecular alterations are present in a histologic grade II or III IDH-wildtype diffuse astrocytic neoplasms, the diagnosis of "diffuse astrocytic glioma, IDH-wildtype, with molecular features of GBM, WHO grade IV" is recommended [74]. Additional studies may identify other markers that can be used for grading schemes of the future.

Synthesis

The diagnosis of GBM should be based on the histologic review of neurosurgically sampled material. The diagnosis of malignant gliomas increasingly relies on molecular genetic applications to aid in classification, offer prognostic value, and predict response to therapy. It has become clear that grading criteria that incorporates molecular parameters, especially *IDH 1/2* mutational status, are necessary to better stratify genetically dissimilar disease subsets. IDH-wildtype astrocytomas mostly manifest as primary GBMs but histologically lower grade counterparts with a similar genomic

landscape as that of IDH-wildtype GBM are now recognized. Although the IDH-wildtype designation is insufficient by itself to predict a WHO grade IV behavior, a number of molecular-genetic alterations have been consistently correlated with poor outcomes when identified in lower grade diffusely infiltrative gliomas. These classic genetic alterations include *EGFR* amplification, the combined gain of chromosome 7/loss of chromosome 10, and *TERT*-p mutation. The WHO grades II and III astrocytomas that harbor such alterations show outcomes similar to IDH-wildtype GBM. Upon the confirmation of any of these genetic alterations, a diagnosis of "diffuse astrocytic glioma, IDH-wildtype, with molecular features of GBM, WHO grade IV" can be rendered to inform on its likely poor clinical outcomes and behavior similar to that of IDH-wildtype glioblastomas.

Question For adult patients with newly diagnosed infiltrating glioma arising in the midline does testing for H3-K27M mutations provide information beyond that gained by histopathology for accurate classification and outcome prognostication?

A frequent genetic signature of pediatric high grade gliomas (and a smaller subset that occur in adults) includes mutations in the histone variant H3.3, encoded by the genes *H3F3A* and *H3F3B*, or H3.1 genes (*HIST1H3B* and *HIST1H3C*) [75, 76]. Two specific histone mutations in H3.3 in pediatric GBMs are mutually exclusive with IDH mutations; one is present at amino acid 27 resulting a substitution of lysine for methionine (K27M) and the second at position 34 resulting in a substitution of glycine for either arginine or valine (G34R/V) [77–79]. H3-K27M mutations strongly align with high grade gliomas of the midline, most often presenting in younger children, with the classic location in the pons (i.e. diffuse intrinsic pontine glioma, DIPG) or thalamus. The H3 G34R/V variant is more typical of supratentorial high grade astrocytomas arising in older children, adolescents and young adults and can often exhibit primitive neuroectodermal tumor or embryonal- like histomorphologic features [80–82]. A new H3 G34R antibody has been developed to aid in the detection of such mutation [83]. The clinicopathologic and biologic features of the H3 G34R/V-mutant tumors have not been completely elucidated, hence they are not yet codified in the WHO classification.

The presence of an H3-K27M mutation correlates with malignant behavior and shorter survival regardless of histologic features [77–79, 84, 85] H3-K27M mutations have also been found in midline structures of adults and also carry a dismal prognosis. Studies by Solomon et al. [86], Meyronet et al. [87], and Kleinschmidt-DeMasters et al. [88] have addressed midline H3-K27M-mutant gliomas arising in adults and have documented a wide morphologic spectrum, particularly commenting on cases with lower grade histomorphologies but all reinforcing the strong association of the H3 K27M mutation and midline location. Solomon et al. [86] and Meyronet et al. [87] described that these tumors can arise on other midline structures beyond the thalamus, pons and spinal cord such as the third ventricle, hypothalamus, pineal region and cerebellum and that H3-K27M mutations are mutually exclusive not only with IDH mutations but also with *EGFR* amplifications but could rarely co-occur with *BRAF* V600E and *TERT*-p mutations. When compared to IDH/H3-wildtype tumors, H3-K27M-mutant tumors are commonly unmethylated when tested for MGMT promoter methylation analysis [87]. Meyronet et al. [87], Kleinschmidt-DeMasters et al. [88], and Reuss et al. [53] have reported median survivals of these tumors at 19.7, 8.4, and 16.9 months, respectively, highlighting poor outcomes. (See Tables 2 and 3) The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) has issued a clarification on the entity of diffuse midline glioma, H3 K27 mutant, WHO grade IV and has emphasized that the diagnostic criteria must be that of (1) an infiltrating glioma that (2) arises in a midline location [89]. This entity is reserved for tumors meeting the above stated criteria and should not be applied to other tumors that are H3 K27M –mutant.

TP53 and *ATRX* mutations co-occur with H3.3 mutations, with the highest correlation in G34R/V GBMs and with lower, yet significant, overlap with H3-K27M mutations [26, 78]. H3-K27M mutations involving either the H3.3 or H3.1 histones can be detected by nuclear staining using the commercially available K27M IHC with a sensitivity and specificity of 100% [86, 90, 91]. Loss or reduction of H3K27me3 nuclear staining by IHC are seen in H3-K27M-mutant tumors, however there is subjectivity in its assessment and K27M IHC is considered a superior biomarker to detect such mutations [91]. The antibody can be applied in the proper clinical and radiological setting to establish the now codified entity of diffuse midline glioma, H3 K27 mutant, WHO grade IV [86].

Synthesis

Diffuse Midline Glioma, H3-K27M-mutant, WHO grade IV is a newly codified entity that highlights an aggressive tumor arising in midline locations and their genotype differs from ordinary IDH-wildtype GBM, as documented by the differences in frequency of *EGFR* amplifications, *TERT*-p mutations, and MGMT promoter methylation, for example. This diagnosis only applies to diffuse gliomas that arise in midline locations. The mutation-specific K27M antibody is highly effective in correctly identifying these tumors.

Table 3 Role of testing for H3 K27M mutations in midline infiltrating gliomas

Series (References)	Description of study	Evidence class	Conclusions
Kleinschmidt-DeMasters et al. [88]	Retrospective analysis of 28 H3 K27M-mutant midline gliomas confirmed by IHC arising in both adults and children and the correlation between histology and outcomes	III	Among 13 tumors arising in adult patients, the mean and median age was 52 years (range: 28–81 years). The mean and median survival was 9.3 and 8.4 months for adult patients. The authors recognized some variant tumor lower grade histomorphologies and those patients also had adverse outcomes
Meyronet et al. [87]	Retrospective analysis of 21 adult H3 K27M-mutant gliomas confirmed by mutational analysis and their comparison to 135 IDH/H3-wildtype adult diffuse gliomas	III	The median age at diagnosis was 32 years (range: 18–82 years). All tumors were IDH and BRAFV600E wildtype. The authors recognized some variant tumor lower grade histomorphologies. Compared to the IDH/*H3*-wildtype counterparts, these tumors arose at an earlier age (32 vs 64 years, $p < 0.001$), less frequently had MGMT promoter methylation (1/21 vs 52/129, $p = 0.002$) and lacked *EGFR* amplification (0/21 vs 26/128, $p = 0.02$). Only two cases had *TERT*-p mutations. The median survival was 19.6 months. The study reinforces that, as in children, H3 K27M mutations define a distinct subgroup of IDH-wildtype glioma that arises in midline compartments and is associated with a poor prognosis
Solomon et al. [86]	Analysis of a prospective cohort of 73 infiltrating gliomas and 15 pilocytic astrocytomas arising in the midline of children and adults. This study addressed the clinical features of H3-K27M mutant gliomas along with their morphologic variability and results of K27M IHC	III	Using the mutation specific H3-K27M immunostain, 64% of the infiltrating midline gliomas had H3-K27M mutations (47/73). No hemispheric infiltrating gliomas or pilocytic astrocytomas were H3-K27M mutated. Their morphologic spectrum was widely variable and overlapped with other entities. H3-K27M mutations were mutually exclusive with *IDH1* mutations and *EGFR* amplifications, and rarely co-occurred with *BRAF* V600E mutations. H3-K27M mutation was commonly associated with p53 overexpression, ATRX loss and loss of chromosome 10. The use of H3-K27M IHC is recommended for the routine workup of midline gliomas
Bechet et al. [90]	Analysis of 143 high grade astrocytomas, 297 other primary tumors and normal brain for H3K27M and H3K27me3 IHC	III	There was 100% concordance between genotype and immunohistochemical analysis of H3K27M. Both H3.1 and H3.3 K27M mutations are recognized by the mutation specific antibody. The H3K27M antibody is highly sensitive and specific in recognizing the specific mutation and has clinical utility in the workup of midline gliomas

Table 3 (continued)

Series (References)	Description of study	Evidence class	Conclusions
Khuong-Quang et al. [78]	Analysis of 42 DIPG's for H3.3 mutations and its comparisons to *HIST1H3B*, IDH, *ATRX*, and *TP53* mutations by Sanger sequencing, copy number alterations and outcomes	III	30/42 DIPG's (71%) carried the *H3F3A* K27M mutation of the histone H3.3 gene. In this cohort, there were no G34 R/V-H3.3, *IDH1/2* or H3.1 mutations. H3K27M-mutant DIPG's showed specific CNAs including gains and amplifications of *PDGFRA* and *MYC/PVT1*. *TP53* and *ATRX* mutations occurred in 77% and 9% of DIPG's. H3K27M-mutant tumors were strongly and uniformly associated with a worse OS by Kaplan–Meier survival analysis ($p=0.0027$, log rank test). H3K27M-mutant DIPG's had a mean OS of 0.73 years (± 0.48) versus 4.59 years (± 5.55) in wildtype tumors ($p=0.0008$). The study reinforces the location-based predilections for K27M- and G34V-H3.3-mutant GBMs (i.e. midline vs. hemispheric)
Schwartzentruber et al. [77]	Analysis of 48 pediatric GBMs by exome sequencing	III	Somatic mutations in the H3.3-ATRX-DAXX chromatin remodeling pathway occurred in 44% of tumors (21/48) with recurrent mutations in *H3F3A* occurring in 31%. The mutations resulted in amino acid substitutions in two critical positions within the histone tail: K27M and G34R/V variants. *ATRX* and *DAXX* mutations were present in 100% of G34R/V-mutant tumors and 31% of all tumors, overall. In a large cohort of gliomas ($n=784$), *H3F3A* mutations appeared specific to GBMs of children and young adults. K27M mutations had a tendency to occur in younger patients (median 11 years) with a thalamic predilection while G34R/V arose more frequently in the cerebral hemisphere of young adults (median 20 years). *H3F3A/ATRX-DAXX/TP53* mutations were associated with the alternative lengthening of telomeres and there was mutual exclusivity between *H3F3A* and IDH mutations ($p=0.00016$). The study reinforces the location-based predilections for K27M- and G34V-H3.3-mutant GBMs (i.e. midline vs. hemispheric)

DIPG diffuse intrinsic pontine glioma, *GBM* glioblastoma, *IDH* isocitrate dehydrogenase, *IHC* immunohistochemistry, *OS* overall survival

Conclusions

While the pathologic diagnosis of GBM and other infiltrating gliomas still relies heavily on the histopathologic examination of H&E stained slides prepared from tissue received from stereotactic biopsy or neurosurgical resection, the contemporary approach also requires the incorporation of molecular biomarkers for an integrated diagnosis. The diagnosis should be established in a multidisciplinary setting with knowledge of clinical information, neurosurgical impression, radiologic findings and now the integration of definitional molecular-genetic alterations. The determination of *IDH 1/2* mutational status – by IHC and/or sequencing analysis – is used to distinguish IDH-mutant from IDH-wildtype diffuse astrocytic gliomas, since these represent different diseases. In the setting of an IDH mutation, the presence of *TP53* mutations and inactivating *ATRX* alterations provide support for the diagnosis of an IDH-mutant astrocytoma. Grading schemes are currently imperfect for IDH-mutant astrocytomas, but microvascular proliferation and necrosis are sufficient for a grade IV designation, while *CDKN2A* homozygous deletions also predict aggressive clinical behavior. IDH-wildtype infiltrating gliomas are more rapidly progressive than IDH-mutant gliomas and have distinctive genomic alterations. Among IDH-wildtype diffuse astrocytomas of histologic grade II/III, the presence of a + 7/− 10 cytogenetic signature or *EGFR* amplification or TERT-p mutation is sufficient for a grade IV designation. In certain clinico-radiologic contexts specific biomarkers addressing histone H3 or *BRAF* mutations are highly informative.

Key issues for future investigation

Integrative diagnoses that incorporate molecular genetic test results have allowed for more robust and reproducible diagnoses and have improved our abilities to predicting patient outcomes for patients with diffuse gliomas. In the near future, we will need to improve the ability to stratify risk within molecular subsets of diffuse glioma. In particular, we need to identify molecular markers that predict more aggressive clinical behavior in IDH-mutant diffuse gliomas so they can be incorporated into grading schemes. Homozygous deletion of *CDKN2A* is one such marker, but there may well be many more that could be identified. Similarly, for IDH-wildtype diffuse gliomas, there will need to be a comprehensive list of genomic alterations that identify grade IV behavior, independent of histology. *TERT*-p mutations, *EGFR* amplifications and the + 7/− 10 signature have been identified, but others should be validated as well, so that patients can be enrolled in clinical trials for GBM if they fit molecular criteria.

There have been outstanding advances in the area of molecular profiling of brain tumors by whole genome methylation platforms. The definition of clinical and genetic alterations within methylation classes has allowed the identification of numerous new diagnostic entities that are a model for personalized approach to patient care. These approaches are capable of reproducibly identifying diffuse astrocytomas that are IDH-wildtype, IDH-mutant or H3-mutant. There may be a need to combine methylation with other genomic platforms to provide prognostic data (i.e. tumor grade) within each methylation category, especially for IDH-mutant astrocytomas. Lastly, there is much to learn regarding the molecular trajectories of diffuse gliomas once they have been treated. It is now clear that genetic evolution of novel clones occurs in many tumors as they progress and that hypermutation is often present following chemotherapy in substantial subsets. However, we are the infancy of using this data to effectively diagnose and treat patients more specifically based on these findings.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interest.

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