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A practical approach for assessing high-grade diffuse gliomas and meningiomas

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ABSTRACT

The field of central nervous system (CNS) tumor diagnostics continues to evolve and expand with the emergence and integration of diagnostic, prognostic, and predictive genomic markers. Despite such ever-increasing complexity, it remains within the ability of practicing surgical pathologists to perform an informed assessment of a majority of CNS tumors. In this review, we provide practical guidelines to evaluate the most common primary malignant and benign CNS tumor entities - high-grade diffuse glioma and meningioma.

1. A framework for glioma evaluation

1.1. Introduction

Since the 2021 WHO Classification of Tumors of the Central Nervous System (2021 CNS WHO), molecular signatures have become an integral component of glioma classification [1]. More recently, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy-Not Official WHO (cIMPACT-NOW) Update 9 provides recommendations on the utilization of genome-wide DNA methylation profiling in gliomas [2], and the cIMPACT-NOW Update 11 proposes further adaptations to the diagnostic criteria for diffuse high-grade gliomas [3]. As genomic markers and technologies continue to emerge, it becomes increasingly essential for pathologists to develop a systematic approach to evaluating gliomas. Here, we propose a guideline applicable in a variety of practice settings, which incorporates clinical, morphologic, immunohistochemical (IHC), and appropriate molecular evaluation to support timely, informative, accurate, and resource-efficient glioma diagnosis.

1.2. Clinical history and radiologic findings

Every neuropathologic evaluation should begin with a careful review of clinical history and neuroimaging findings, which provide critical context and guide preliminary diagnostic considerations.

1.3. Morphologic evaluation

Thorough morphologic examination is the cornerstone of guiding diagnostic considerations and selecting appropriate ancillary tests. Evaluation begins at low magnification to assess the overall architecture and regions involved, and proceeds to higher magnification assessment of cytologic details. Correlation of these morphologic findings with pre- and post-operative neuroimaging findings, and with the operative report, helps to determine whether adequate lesional tissue has been sampled, and whether the tissue represents a non-neoplastic or neoplastic process.

- **Patient demographics. Age offers an important diagnostic clue, as tumors that predominantly affect pediatric patients (<14 years) are generally distinct from those that arise more commonly in adults (>40 years).**
- **Radiologic assessment. The anatomic location, extent of involvement, and pre-operative neuroimaging features further guide diagnostic considerations. These include identifying the involved compartment(s), assessing whether the growth pattern is diffuse or circumscribed, presence of regions showing**

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contrast-enhancement, and determining whether the targeted lesion is entirely represented in the sections being evaluated.

- **Relevant oncologic history. Knowledge of the prior glioma diagnosis, genomic evaluation(s), and therapeutic intervention (s) helps with interpreting histological findings in more recently resected material, as well as determining if any additional evaluation is needed. Concurrent review of any available prior pathology slides may also help distinguish residual or recurrent tumor from therapy-associated changes.**

Key considerations include.

- **Cellularity.** If increased, one needs to determine which cell type is predominant. Increased glial cells with astrocytic morphology may indicate reactive gliosis or glioma, while increased inflammatory cells, such as macrophages or lymphocytes, raise non-neoplastic possibilities such as tumefactive demyelination, infarction, or infection. Essential lineage markers are listed in Table 1.
- **Growth pattern and cytology.** Assessing whether the process is infiltrative or expansile. Diffuse gliomas infiltrate and entrap neurons and axons, often showing "secondary structures" like perineuronal and/or perivascular satellitosis, subpial aggregates, and white matter track infiltration, whereas circumscribed gliomas are often well-demarcated and displace brain tissue. Cytological features such as increased nuclear:cytoplasmic (N/C) ratios, nuclear hyperchromasia and irregularity, and pleomorphism support the diagnosis of glioma.
- **Morphologic hallmarks for grading.** Readily identifiable mitotic activity, palisading tumor necrosis, and microvascular proliferation are typically indicative of a high-grade glioma. On the other hand, Rosenthal fibers and eosinophilic granular bodies suggest a low-grade circumscribed glioma.

1.4. Ancillary testing

It is essential to closely communicate with the neurosurgical team regarding adequacy of tissue obtained for anticipated ancillary studies, ideally at the time of intraoperative evaluation. The selection and prioritization of IHC and molecular studies are, at least in part, guided by the quantity of available tissue and the density of neoplastic cells. Ancillary testing should be selected according to the entities being considered and the most resource-efficient panel to evaluate these, rather than relying on a nonspecific, broad "shotgun" approach. A guide to inform test selection for gliomas and glioneuronal tumors is included in Table 1.

1.4.1. Immunohistochemistry

A logical IHC panel is typically applied to determine the lineage of the cellular components and cells of interest, and to detect common molecular alterations. Neurofilament may help determine the growth pattern, if not readily apparent on H&E, by highlighting the presence or absence of entrapped axons. Lineage markers include GFAP and OLIG2 for glial cells, synaptophysin and chromogranin for a neuronal component, CD68 and CD163 for macrophages and activated microglial cells. If lymphoma is suspected, CD3 and CD20 (or PAX5) can help distinguish between T-cell and B-cell lineages.

Surrogate IHC markers may detect common molecular alterations in gliomas at this early stage of diagnostic evaluation and hence abrogate the need for molecular studies to assess these. However, it is important to keep in mind that these IHC surrogates are specific to the mutation being assessed, and do not exclude the possibility of other mutations involving the gene. For example, while IDH1-R132H positivity indicates the presence of this specific *IDH1* mutation, a negative stain does not exclude other non-canonical *IDH1/IDH2* mutations (such as *IDH1* p.R132C or *IDH2* p.R172X).

Adjunct IHC markers may help determine the likelihood of such

Table 1
Common genetic alterations in glioma, glioneuronal tumors, and neuronal tumors.

Initial morphologic evaluation	Potentially Informative Immunostains	
Lineage, Growth pattern, and Morphologic features distinctive of tumor subtype(s)	GFAP, OLIG2, S100, SOX10 (glial lineage) Synaptophysin, Chromogranin, Neu-N (neuronal lineage) CD68, CD163 (to distinguish microglial activation from diffuse glioma cells, especially in hypocellular samples) Neurofilament (to assess growth pattern. Within cellular regions, numerous axons indicate diffuse growth, while sparse axons indicate circumscribed/expansile growth) CD34 (expression in abnormal neurons/neuropil) Ki-67 (when mitotic activity appears low)	
Tumor type favored by morphology	**Potentially Informative Stains**	**Informative Genomic Tests**
Adult-type diffuse gliomas		
• Astrocytoma, IDH-mutant	**IDH1-R132H+** (common), **ATRX lost**	**SEQ** *(IDH1/2)* **CNA** *(CDKN2A/B* homozygous deletion*)*
• Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	**IDH1-R132H+** (common), **ATRX retained**	**SEQ** *(IDH1/2*, and often *TERTp, CIC, FUBP1)* **CNA/FISH** *(1p/19q-*codeletion*)*
• Glioblastoma, IDH-wildtype	**IDH1-R132H-, ATRX retained** (especially >54 years of age)	**SEQ** *(IDH-wt, H3-wt, TERTp)* **CNA** *(+7/-10, EGFR* amplification)
Pediatric-type diffuse high-grade gliomas		
• Diffuse midline glioma, H3 K27-altered	**H3–K27M+** (common) or EZHIP+, **H3K27-me3 lost**	**SEQ** (*H3-3A/EGFR/ EZHIP)*
• Diffuse hemispheric glioma, H3 G34-mutant	**GFAP+, OLIG2-, ATRX lost, p53 widespread overexpression**	**SEQ** (*H3-3A*)
• Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype	No specific immunophenotype	**SEQ** *(*IDH-wt, H3-wt, *PDGFRA* or *EGFR*) AND **CNA** (*PDGFRA*, *EGFR*, or *MYCN* alteration, NO +7/-10) **OR DNA methylation**
• Infant-type hemispheric glioma	No specific immunophenotype	**SEQ** (RTK fusions, including *ROS1, ALK, MET*)
Pediatric-type diffuse low-grade gliomas		
• Diffuse astrocytoma, MYB- or MYBL1-altered	**GFAP+, OLIG2 -**	**SEQ or FISH** (*MYB* or *MYBL1* alteration)
• Angiocentric glioma	**GFAP+, OLIG2 -**	**SEQ** (*MYB*::*QKI* fusion)
• Polymorphous low-grade neuroepithelial tumor of the young	**CD34** + (parenchymal), **BRAF-V600E+** (common)	**DNA methylation** (supportive)
• Diffuse low-grade glioma, MAPK pathway-altered	**BRAF-V600E** (+ in the *BRAF* p.V600E-mutant subtype)	**SEQ** (MAPK alteration) **CNA** (NO *CDKN2A/B* homozygous deletion) **DNA methylation** (supportive)
Circumscribed astrocytic gliomas		
• Pilocytic astrocytoma	BRAF-V600E+ (uncommon)	**SEQ** (MAPK alteration, most commonly *KIAA1549::BRAF*)
• High-grade astrocytoma with piloid features	BRAF-V600E+ (rare), **ATRX lost, p16/MTAP lost**	**SEQ** (MAPK alteration, *ATRX*) and **CNA** (*CDKN2A/B* homozygous deletion) indicative; **DNA methylation (required)**

(*continued on next page*)

Table 1 (*continued*)

Initial morphologic evaluation	Potentially Informative Immunostains	
• Pleomorphic xanthoastrocytoma	**BRAF-V600E**+ (common), **p16/MTAP lost**	**SEQ** *(BRAF, TERT)* **CNA** *(CDKN2A/B* status)
• Subependymal giant cell astrocytoma	**GFAP**+, **TTF1**+	**SEQ** (*TSC1*, *TSC2*)
• Chordoid glioma	**GFAP**+, **TTF1**+	**SEQ** (*PRKCA* p. D463H)
• Astroblastoma, MN1-altered	**GFAP**+, **OLIG2**+/−, **EMA ±**	**SEQ** (*MN1* alteration)
Glioneuronal and neuronal tumors		
• Ganglioglioma	**Chromogranin**+ (binucleated/dysmorphic neurons), **BRAF-V600E**+ (strong in neuronal component)	**SEQ** (*BRAF* p.V600E or other MAPK alteration)
• Desmoplastic infantile ganglioglioma/ desmoplastic infantile astrocytoma	**Reticulin** (widespread pericellular deposition), **BRAF-V600E**+	**SEQ** (MAPK alteration)
• Dysembryoplastic neuroepithelial tumor	OLIG2+, S100+,. GFAP- (oligodendrocyte-like component), Neurofilament (axons within specific glioneuronal element), NeuN+ ("floating" neurons)	**SEQ±CNA** (*FGFR1* alteration, typically mutation)
• Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters		**CNA** (Monosomy 14) **DNA methylation (required)**
• Papillary glioneuronal tumor	**GFAP**+, **OLIG2**+ (glial component in pseudopapillary structures), **Synaptophysin**+, **Chromogranin±** (neuronal component in interpapillary areas)	**SEQ** *(PRKCA* fusion, often *SLC44A1::PRKCA*)
• Rosette-forming glioneuronal tumor	**Synaptophysin**+ (neurocytic rosettes and perivascular pseudorosettes)	**SEQ** (*FGFR1,* frequently with concurrent *PIK3CA* ± *NF1)*
• Myxoid glioneuronal tumor	**GFAP**+, **OLIG2**+ (glial component), **Synaptophysin**+ (neuronal component)	**SEQ** (*PDGFRA* p. K385L/I dinucleotide mutation)
• Diffuse leptomeningeal glioneuronal tumor	**OLIG2**+, **GFAP ±** (glial component), **Synaptophysin**+, **Chromogranin±, NeuN ±** (neuronal component)	**SEQ** (MAPK alteration, commonly *KIAA1549::BRAF*) **CNA** (1p deletion)
• Multinodular and vacuolating neuronal tumor	OLIG2+, Synaptophysin + (weak), Chromogranin -, NeuN ± (weak)	**SEQ** (MAPK alteration, mostly *MAP2K1, BRAF*, and *FGFR2*)
• Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)	PTEN lost	**SEQ and/or CNA** (*PTEN* mutation or deletion)
• Central neurocytoma	Synaptophysin +, NeuN+	
• Extraventricular neurocytoma	Synaptophysin +, Chromogranin ± (focal), NeuN ± (focal)	**SEQ** (*FGFR1* alteration, often *FGFR1::TACC1*)
Ependymal tumors (GFAP+, OLIG2-, EMA+/− (dot-like or ring-like), Neurofilament: circumscribed growth)		
• Supratentorial ependymoma, ZFTA fusion-positive		**SEQ** (*ZFTA* fusion*)*
• Supratentorial ependymoma, YAP1 fusion-positive		**SEQ** (*YAP1* fusion)
• Posterior fossa group A (PFA) ependymoma	**H3K27-me3 lost, EZHIP**+	**CNA** (few, if any, alterations), **DNA methylation** (supportive)
• Posterior fossa group B (PFB) ependymoma	**H3K27-me3 retained, EZHIP -**	**DNA methylation (required)**
• Posterior fossa mixed subependymoma-ependymoma		**SEQ** (*TERTp*) **and CNA** (chromosome 6 loss) **DNA methylation** (supportive)
• Spinal ependymoma		**DNA methylation** (supportive)
• Myxopapillary ependymoma	**Alcian blue**+ (myxoid material)	

SEQ: DNA/RNA sequencing (*Caveat: Ensure genes of interest are included in targeted panels).
CNA: Chromosomal copy number analysis, wt: wild-type/non-mutant.

alternative mutations being present. Loss of ATRX expression in an IDH1-R132H-negative diffuse glioma suggests further evaluation for mutations involving IDH and H3 genes, as well as alterations involving MAPK pathway genes, may be warranted. Similarly, loss of H3K27-me3 expression may indicate an H3–K27 M or *EZHIP* mutation. Such adjunct IHC markers can help inform the selection of appropriate molecular tests.

A preliminary diagnosis based upon clinical, radiological, morphologic, and immunohistochemical features may be issued for cases requiring further molecular testing. Such a preliminary diagnosis allows the clinical team to begin planning the next steps in patient care even while a more accurate diagnosis is awaited. For example, a preliminary diagnosis of *"high-grade diffuse glioma"* or *"diffuse glioma, histologically low-grade"* may meet the immediate needs of clinical decision-making during the weeks following surgery while awaiting molecular testing results.

1.4.2. Molecular testing

A variety of molecular tests may be utilized in the evaluation of gliomas, with each technology having unique strengths and limitations. Selection of the most appropriate test(s) should be based on the diagnostic considerations in light of the clinical, radiologic, and histological findings, the size and cellular density of the sampled tissue, and the tissue requirements for reliable positive and negative results for each molecular test.

1.4.2.1. Chromosomal copy number alterations. Copy number analysis is an integral component of glioma diagnosis for several entities. For example, detection of whole arm 1p/19q co-deletion is an essential criterion for diagnosing oligodendroglioma, IDH-mutant and 1p/19q-codeleted. Similarly, identification of *CDKN2A/B* homozygous deletion upgrades astrocytoma, IDH-mutant to WHO grade 4 regardless of histologic features. In a diffuse glioma with non-specific histological features, detection of *EGFR* amplification or combined gain of chromosome 7 and loss of chromosome 10 (+7/-10) in the absence of IDH or H3 mutation would support the diagnosis of glioblastoma, IDH-wildtype.

Fluorescence in situ hybridization (FISH) is a simple yet effective technique to assess specific deletions, insertions, translocation breakpoints, and copy number alterations. FISH has the advantage of a relatively rapid turnaround time and limited tissue requirements. On the other hand, FISH has lower resolution compared to other techniques and is limited to evaluating only the targeted chromosomal regions.

Chromosomal microarray (CMA) offers high-resolution, genome-wide detection of copy number alterations and losses of heterozygosity. However, CMA is not extensively available in all settings, requires a greater quantity of tissue, may not provide reliable results in the presence of artifactual tissue distortion or when neoplastic cell density is low, and cannot detect balanced rearrangements.

1.4.2.2. DNA and RNA sequencing. Detection of key mutations is critical for definitively diagnosing many glioma subtypes. For instance, establishing the *IDH1* and *IDH2* mutation status is essential for the diagnosis of astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant and 1p/19q-codeleted, as well as for glioblastoma, IDH-wildtype. According to WHO 2021, IDH testing is recommended for all diffuse gliomas in adults under 55 years of age and in diffuse glioma with a non-classic histologic appearance. Similarly, detection of *TERT* promoter mutation may support the diagnosis of glioblastoma, IDH-wildtype, and of *H3F3A* (K27 M or G34 R/V) mutation supports the diagnosis of diffuse midline glioma, H3K27-altered, and of diffuse hemispheric glioma, H3G34-mutant.

Similarly, oncogenic fusion transcripts may be diagnostically and/or therapeutically significant. Examples include *KIAA1549::BRAF* in pilocytic astrocytoma, *FGFR1::TACC1* and *FGFR3::TACC3* in diffuse gliomas and glioblastoma, *MYB::QKI* or *MYBL1*-related fusions in angiocentric glioma and diffuse astrocytoma, *MYB/MYBL1*-altered, and *ETV6::NTRK3* in infant-type hemispheric glioma.

Whole-genome sequencing (WGS) interrogates almost the entire genome, including nuclear and mitochondrial DNA, while whole exome sequencing (WES) evaluates the 1 % of the genome that encodes proteins. More commonly, targeted DNA and RNA next-generation sequencing (NGS) panels are utilized to target specific alterations and, depending on the genes included in the panel, can be applied to distinguish a spectrum of glioma subtypes on relatively limited samples. The advantages of targeted NGS panels include high sensitivity, broad genomic coverage, and the ability to assess multiple molecular alterations in a single platform. However, this comes at a higher cost, turnaround times in the order of weeks, and the need for high-quality DNA and/or RNA. When ordering a targeted NGS panel, it is essential to understand which genes and molecular alterations are included therein, as key genes may not be covered, resulting in an inaccurate "false negative" interpretation of the results. Also, keep in mind that a true negative (wildtype) result should be interpreted in the context of the clinical and histological diagnostic considerations.

The choice between WGS, WES, and targeted NGS platforms comes down to what is most practical, readily available, applicable to the sampled tissue, and cost.

1.4.2.3. DNA methylation analysis. DNA methylation profiling provides accurate and objective tumor classification and can also infer chromosomal copy number alterations and *MGMT* promoter methylation status. Methylation profiling is particularly useful for tumor types with characteristic methylation signatures, as well as for diagnostically challenging or unresolved cases, particularly when the output "calibrated score" is above the clinical threshold. According to the 2021 CNS WHO and cIMPACT-NOW update 11[3], an appropriate DNA methylation profile is an essential criterion for certain diagnoses, such as high-grade astrocytoma with piloid features (HGAP) and diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype. Additionally, methylation profiling may indicate an emerging tumor type not included in the 2021 CNS WHO, and for which diagnostic criteria are yet to be established. However, this technique often has a longer turnaround time than NGS/CMA, requires high tissue quantity and tumor cellularity, may yield low-confidence or ambiguous results, and may still require confirmation of indicated mutations/fusions by NGS.

1.5. Conclusions

The diagnostic evaluation of gliomas includes traditional morphologic assessment and clinical-radiologic correlation, supported by appropriately selected immunohistochemical and molecular evaluation. We hope this section has provided a systematic, step-wise approach to clinically informative diagnoses, which can be applied in a variety of practice settings.

2. IDH-wildtype diffuse high-grade gliomas in adults

2.1. Introduction

The diagnosis of diffuse high-grade gliomas (dHGG) in adult patients represents a significant proportion of surgical neuropathology. The 2021 CNS WHO provides a framework to classify dHGG tumor types [1], which continues to be refined through cIMPACT-NOW updates [4]. This section seeks to provide a practical approach to evaluating dHGG, focusing on three major tumor types lacking IDH mutation and corresponding to CNS WHO grade 4: Glioblastoma, IDH-wildtype (GBM-IDHWT); Diffuse midline glioma, H3 K27-altered (DMG-H3K27); and Diffuse hemispheric glioma, H3 G34-mutant (DHG-H3G34).

2.2. Clinical history and radiologic findings

Glioblastoma, IDH-wildtype represents the most common malignant brain tumor in adults, and can occur in any age group, with a peak incidence in patients aged 55–85 years [5]. While GBM-IDHWT most frequently arises in the cerebral hemispheres, it may also involve infratentorial brain and spinal cord. Predominant involvement of midline structures (basal ganglia, thalamus, brainstem, cerebellum, spinal cord) should prompt consideration of *Diffuse midline glioma, H3 K27-altered*, regardless of the age of the patient. A dHGG arising in the cerebral hemispheres of a younger patient (i.e., less than 40 years) warrants consideration for *diffuse hemispheric glioma, H3 G34-mutant* in addition to the significantly more common IDH-mutant diffuse gliomas.

The classic imaging appearance of dHGG is of abnormal T2/FLAIR hyperintense signal extending across grey and white matter structures, harboring heterogeneous regions of often peripheral contrast enhancement with a darker necrotic core. However, some dHGG may demonstrate only limited (if any) contrast enhancement and/or absence of necrosis.

2.3. Morphologic evaluation

A key histologic feature of dHGG is its *infiltrative growth pattern*, best identified distal to the tumor's epicenter. A note of caution is due, as other neoplasia, such as lymphoma, melanoma, poorly differentiated/small cell carcinoma, and certain circumscribed gliomas, may also show infiltrative growth in limited samples, and may require IHCs to establish lineage.

Classic histologic hallmarks indicating a high-grade designation for a diffuse glioma, regardless of subtype, include *high cellularity, frequent mitotic activity, microvascular proliferation* (rapidly-formed blood vessels arising in the context of the hypoxic tumoral environment) and/or *tumor necrosis* (frequently exhibiting surrounding palisading of neoplastic cells). These findings typically correlate with the presence of contrast enhancement and/or presumptive necrosis on pre-operative imaging studies.

Correlation with pre-operative radiologic studies also helps discern whether a limited biopsy sampling is entirely representative of the targeted lesion. Alternatively, a biopsy showing extensive necrotic material should be interpreted with caution, as this may represent the necrotic core of dHGG, of a metastatic malignancy, or even of a non-neoplastic necro-inflammatory process such as an abscess – all of which may show similar radiologic features.

Glioblastoma, IDH-wildtype is notorious for morphological heterogeneity, earning its historic "multiforme" monicker, and has several well-recognized patterns, including *gemistocytic, giant cell, gliosarcoma, epithelioid,* and *small cell, primitive/PNET-like* components/subtypes (when predominant).

Diffuse midline glioma, H3 K27-altered often lacks high-grade histological features, which is not unexpected as the involvement of critical midline structures necessitates conservative sampling. However, a

diffuse growth pattern is necessary for this diagnosis, and can either be identified histologically or demonstrated by neurofilament IHC.

Diffuse hemispheric glioma, H3 G34-mutant often shows regions with high nuclear-to-cytoplasmic ratios and occasional Homer Wright rosettes (morphologically reminiscent of an embryonal tumor), frequent multinucleated and pleomorphic cells and, rarely, ganglion cell differentiation [6].

2.4. Immunohistochemical studies

2.4.1. Lineage of differentiation

It may sometimes be necessary to confirm the dominant glial lineage of differentiation with GFAP (a cytoplasmic intermediate filament [7]) and/or OLIG2 (a nuclear transcription factor [8]). The attenuated to entirely absent expression of OLIG2 may also serve as a helpful diagnostic adjunct in DHG-H3G34 [9,10]. Neurofilament may similarly be helpful in establishing infiltrative growth, especially in highly cellular tumors.

Additional markers may demonstrate divergent differentiation within GFAP-negative subpopulations, such as synaptophysin and chromogranin highlighting neuronal differentiation, and extensive pericellular reticulin deposition in a malignant spindle cell component indicating mesenchymal differentiation (such as in the gliosarcoma subtype).

2.4.2. Surrogate immunohistochemistry for molecular alterations

IHCs serve as effective surrogate markers of specific molecular alterations (IHC patterns for common IDH-wildtype dHGG subtypes are illustrated in Fig. 1). The initial IHC panel evaluating dHGG includes IDH1-R132H, ATRX, and p53. If pre-operative radiology imaging studies indicate involvement of midline structures, H3K27 M, H3K27me3, and EZHIP should also be included.

Glioblastoma, IDH-wildtype is defined by the absence of *IDH1, IDH2,* and H3 mutations. In patients 54 years of age or older, the most common ("canonical") IDH mutation is *IDH1* p.R132H, with less than 1 % of cases harboring a non-canonical mutation. As such, in dHGG arising in patients aged 55 years or older, the IDH1-R132H IHC serves as an effective surrogate marker for the presence or absence of this mutation, and IDH status overall, for establishing this diagnosis [11].

The negative predictive value of the IDH1-R132H IHC is enhanced by the ATRX IHC. In dHGG with negative IDH1-R132H IHC, loss of nuclear ATRX expression should prompt sequencing studies to evaluate for non-canonical *IDH1* and *IDH2* mutation, as well as for *H3-3A* (p.G34) mutation, regardless of the patient's age. Another entity to consider when loss of ATRX is observed in an astrocytic glioma is high-grade astrocytoma with piloid features (HGAP) - a newly codified entity in the 2021 CNS WHO, which may show infiltrative growth and lack high-grade histological features, and frequently harbor MAPK pathway alterations. While detailed discussion of HGAP is beyond the scope of this review, suffice it to say that loss of ATRX IHC expression in a glioma should broaden the diagnostic considerations and prompt further genomic characterization (see Table 1).

It is essential to assess the ATRX IHC only in regions where background non-neoplastic cells show retained expression (e.g., endothelial nuclei), as suboptimally preserved or artifactually distorted tissue may show widespread absence of ATRX staining in all cell types. Another caveat regarding the interpretation of the IDH1-R132H/ATRX IHC panel is the morphological suspicion for oligodendroglioma, characterized by

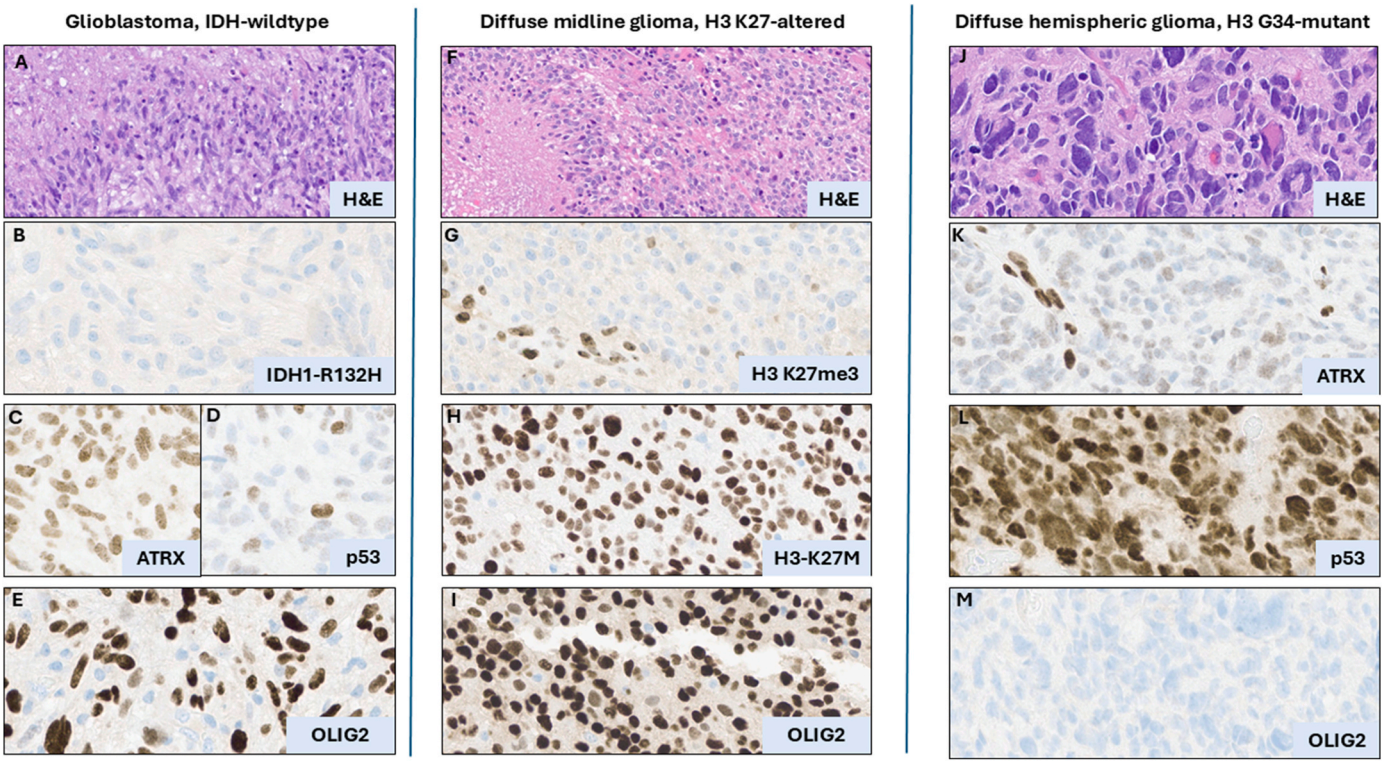

Fig. 1. Common immunohistochemical patterns of selected dHGG types at a glance. *Glioblastoma, IDH-wildtype* is morphologically characterized as a high-grade glioma (A). In patients 55 years and older, absent expression of IDH1-R132H (B) with preserved normal nuclear expression of ATRX (C) and overexpression of p53, even if in a subset (D), is highly supportive of an IDH-wildtype status; expression of OLIG2 is typically present (E). *Diffuse midline glioma, H3 K27-altered* may occasionally show high-grade morphologic features (F), and is defined by aberrant loss of H3 K27me3 across neoplastic nuclei (G), with concomitant expression of H3–K27 M when this mutation is present (H); this tumor type also typically expresses OLIG2 (I). *Diffuse midline glioma, H3 G34-mutant* may also present with high-grade morphology (J), and is notable for a pattern of loss of nuclear expression of ATRX (K, with preserved expression in endothelial cells serving as normal internal controls), widespread overexpression of p53 (L), and absence of OLIG2 (M); this immunophenotype may be a powerful predictor of this tumor type in the absence of dedicated antibodies for *p.G34R* or *p.G34V* mutations.

uniform round-ovoid nuclei, often with perinuclear clearing ("halos"). The p53 IHC may assist in this distinction, as oligodendroglioma typically lacks *TP53* mutation and does not show widespread nuclear p53 overexpression. Given the significantly less aggressive clinical behavior of oligodendroglioma than GBM-IDHWT, a relatively low threshold for requesting *IDH1/IDH2* sequencing in cases showing oligodendroglioma morphology is recommended. Molecular evaluation of the 1p/19q codeletion status may be performed concurrently, or following detection of an IDH mutation.

Diffuse midline glioma, H3 K27-altered is defined by molecular events resulting in loss of the trimethyl (me3) status on the K27 residue of the H3 histone. Aberrant nuclear H3–K27 M expression indicates this specific mutation is associated with loss of nuclear H3 K27me3 expression. EZHIP overexpression is an (uncommon) alternate mechanism by which H3 K27me3 expression is lost in DMG-H3K27, and can also be detected by IHC.

Diffuse hemispheric glioma, H3 G34-mutant is defined by missense mutation of *H3-3A*, typically *p. G34R* and occasionally *p. G34V*. Surrogate H3G34R and H3G34V antibodies for these point mutations are available for diagnostic use [12], albeit not widespread. Notably, DHG-H3G34 commonly shows concurrent *ATRX* and *TP53* alterations, translating to aberrant expression of their respective proteins. In contrast to IDH-mutant astrocytoma, however, DHG-G34 also shows reduced-to-absent expression of OLIG2 [13]. In the context of a histologically high-grade infiltrating glioma in the cerebral hemisphere of a younger patient, an ATRX-lost/p53-overexpressed/OLIG2-negative immunophenotype serves as an effective predictor of the presence of H3 G34 alteration, warranting dedicated molecular testing [14].

2.5. Molecular testing

2.5.1. Glioblastoma, IDH-wildtype

Verification of IDH-wildtype and H3-wildtype status by sequencing is the critical first step in the molecular evaluation of GBM-IDHWT. Once an IDH-wildtype infiltrating glioma is established, three molecular hallmarks that may be used as diagnostic criteria for this specific dHGG tumor type: *TERT promoter mutation*, *concurrent chromosomal gain of 7 and loss of 10 (+7/-10),* and *EGFR amplification. TERT* promoter mutation can be assessed by sequencing, while +7/-10 and *EGFR* amplification can be detected by copy number analysis.

Caution is necessary when considering each of these signatures, especially in patients under the age of 40 years, as none of these in isolation is entirely specific for GBM-IDHWT [3]. Rather, these should be detected in combination, or with other typical (non-diagnostic) alterations to definitively support a diagnosis of GBM-IDHWT. *TERT* promoter mutation may be seen in other CNS tumor types and is not recommended as a standalone criterion in diffuse gliomas exhibiting low-grade radiologic (absent enhancement) and histologic (low proliferative activity, absence of microvascular proliferation and tumor necrosis) features. Similarly, +7/-10 and *EGFR* amplification may occasionally be seen in other CNS tumor types (e.g., pleomorphic xanthoastrocytoma, DHG-H3G34).

2.5.2. Diffuse midline glioma, H3 K27-altered

While the H3K27M IHC is an effective surrogate for this mutation, sequencing studies may identify alternative mutations of H3.3 (or H3.1) genes, such as p.K28I (p.K27I). It is important to keep in mind that histone mutation is not the only molecular event driving this tumor type. If clinically and radiologically indicated, such as a diffuse glioma radiologically showing a bithalamic epicenter, sequencing studies for *EGFR* alterations may be considered, while RNA expression microarray may identify tumors driven by EZHIP protein overexpression if the IHC is not available.

2.5.3. Diffuse hemispheric glioma, H3 G34-mutant

Mutation of *H3-3A*, most commonly p.G35R (G34R) or p.G35V (G34V), and the less common *H3–3B* [15] can be assessed for by sequencing, especially if the surrogate IHCs are not available.

DNA methylation profiling may serve as an important orthogonal modality in cases where diagnostic ambiguity persists despite clinical, radiologic, histopathologic, and molecular evaluation.

2.6. Conclusion

This review is intended to provide the conceptual framework for the practical evaluation of dHGG, rather than an exhaustive discussion including less common subtypes. The increasing incorporation of molecular diagnostics in the taxonomy of dHGG will further refine our understanding – and ultimately, treatment - of these malignant neoplasms.

3. Meningioma grading and risk stratification

3.1. Introduction

Meningiomas are the most common primary CNS neoplasm [16]. Outcomes vary significantly based on tumor location, extent of resection, CNS WHO histopathologic grade (hereafter "grade"), and molecular features [17,18]. Grading requires a thorough histologic assessment of an adequately sampled resection specimen, and molecular studies are often indicated to refine grading and risk stratification. Here, we provide a practical overview of meningioma grading in the molecular era.

3.2. Clinical management

The extent of meningioma resection is critically important for local control. Gross total resection improves progression-free survival and, in higher-grade tumors, improves overall survival [18,19]. After resection, grade informs prognosis and the decision to pursue adjuvant radiation therapy; recurrence rates for grade 1, 2, and 3 meningiomas are 7–25 %, 29–52 %, and 50–94 %, and five-year survival rates are 90 %, 80 %, and 30 %, respectively [20,21]. Generally, grade 1 tumors are observed, grade 2 tumors are treated with radiation when resection is incomplete, and grade 3 tumors are almost always treated with radiation, regardless.

3.3. Grossing

Meningiomas should be submitted entirely or sampled with at least one block per linear centimeter for large tumors. This improves detection of brain invasion and enables selection of the most aggressive area for molecular testing, as intratumor heterogeneity can affect results [22, 23].

3.4. Histologic aspects of meningioma grading

Refinements to meningioma grading appeared in the 2021 CNS WHO and the 2025 cIMPACT-NOW update 8[21,24]. The grade incorporates histologic and molecular features (see Table 2). The majority of meningiomas (81.3 %) are grade 1 or so-called "benign", 17.2 % are grade 2 "atypical", and 1.4 % are grade 3 "anaplastic/malignant" [16].

Mitotic count is the most reproducible and common histologic criterion to upgrade a tumor [25,26]. Accurate counting requires close scrutiny to identify the best "hotspot", defined as a continuous 1.6 mm^2 region (corresponding to seven 40x fields of 0.23–0.24 mm^2). Digitized slides can be used, however, studies show decreased accuracy in identifying mitotic figures on whole slide images compared to light microscopy [27,28]. Immunohistochemistry for Ki-67 and PHH3 can identify proliferative hotspots but does not substitute for mitotic count.

Presence of three out of the five atypical features warrants upgrading of a meningioma to grade 2 (Fig. 2). The five features are hypercellularity (practically a subjective impression, but technically >53 nuclei per 0.23 mm), small cell change (i.e., clusters of cells with high nuclear:

Table 2
Meningioma grading by cIMPACT-NOW Update 8.

	Grade 1	Grade 2	Grade 3
Mitoses/1.6 mm^2	0–3 **AND**	4–19 **OR**	≥20 **OR**
Atypical histologic features[a]	0–2	≥3 **OR**	**OR**
Special histologic subtype		Clear cell **OR** Chordoid **OR**	Overtly malignant cytomorphology[b] **OR**
Molecular		1p loss (with 22q loss/*NF2* oncogenic variant)	*CDKN2A/B* homozygous deletion **OR** *TERT*p mutation
Brain invasion	Report If otherwise benign, leave ungraded until molecular data provide support for grade	Report Does not affect grade	Report Does not affect grade

[a] Hypercellularity, sheeting, small cell change, spontaneous necrosis, macronucleoli.
[b] Carcinoma-, melanoma-, sarcoma-like.

cytoplasmic ratio resembling lymphocytes at low power), sheeting architecture (i.e., loss of whorls or fascicular growth), macronucleoli (visible at 100x magnification), and spontaneous necrosis (i.e., not induced by embolization or radiation) [21,29].

Histologic subtypes that automatically warrant at least grade 2 classification are chordoid and clear cell meningioma (Fig. 2). Chordoid meningiomas have meningothelial cells growing in chords or trabeculae of variably vacuolated cytoplasm, embedded in a mucin-rich matrix, often admixed with areas of more classic meningioma. To assign a chordoid subtype, a significant proportion (suggested >50 %) of the resected tumor should display chordoid morphology. Clear cell meningioma is essentially a distinct entity, with *SMARCE1* gene alterations specific for this subtype and found in nearly all cases [30]. They have a separate methylation class, lack the typical cytogenetic alterations seen in other high-risk meningiomas, and have unique clinical features, occurring in young patients often in the spine or cerebellopontine angle [21]. Clear cell meningiomas often have sheeting architecture with round nuclei and clear cytoplasm containing abundant PAS-positive, diastase-digestible glycogen; they lack psammoma bodies or whorls, tend to grow along or in between dense hyalinized collagen, and typically harbor inconspicuous mitotic activity.

Rhabdoid and papillary subtypes no longer affect grade but can be an indication for molecular testing [24]. *BAP1* mutations or deletions are often seen in these two morphologic variants, which can be somatic or germline, and translate into loss of BAP1 immunostaining.

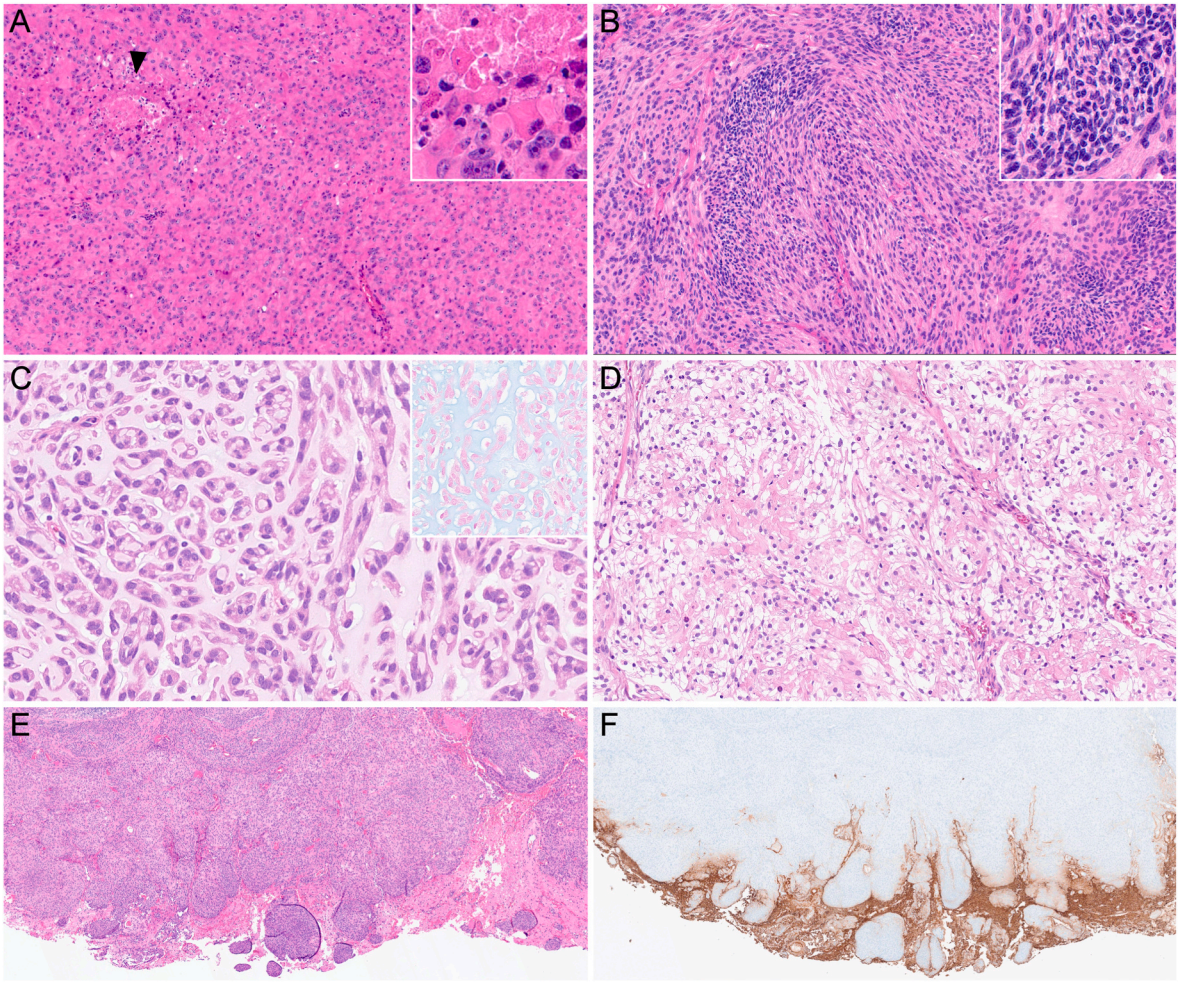

Fig. 2. Histologic features of meningioma. (**A**) Atypical meningioma with sheeting architecture, necrosis (arrowhead and inset), and macronucleoli (inset). (**B**) Meningioma with small cell change, with a low-power appearance reminiscent of a lymphoid aggregate. (**C**) Chordoid meningioma (inset showing Alcian blue stain highlighting myxoid material). (**D**) Clear cell meningioma. (**E**) Brain-invasive meningioma with irregular tongue-like extension into parenchyma, (**F**) accentuated by GFAP IHC. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Brain invasion was removed as an automatic criterion for WHO grade 2 designation in the cIMPACT-NOW 8 update but is considered an indication for molecular testing [24]. GFAP staining can help identify areas of irregular tongue-like invasion (Fig. 2). Growth along Virchow-Robin spaces without pial breach does not qualify.

3.5. Differential diagnosis

During the evaluation of a clinically suspected meningioma, a paucity or absence of the characteristic lobular and/or whorled architecture and meningothelial cytomorphology should prompt consideration of other (much less common) dura-based tumor entities. While typically not necessary, demonstrating expression of epithelial membrane antigen (EMA), progesterone receptor, and SSTR2A by IHC may help support the meningioma diagnosis in such instances. A comprehensive discussion of alternative diagnostic considerations is beyond the scope of this review, but some key considerations are summarized below.

Schwannoma can mimic fibrous meningioma but is distinguished by diffuse and strong S100 immunoreactivity and is typically negative for EMA and SSTR2A. Another mimic is solitary fibrous tumor, which is generally monomorphic and demonstrates nuclear STAT6 immunostaining. Chordoma can resemble chordoid meningioma and is EMA-positive, but will also express brachyury, cytokeratins, and S100.

Other sarcomas that enter the differential include malignant peripheral nerve sheath tumor, which shows H3K27me3 loss in most cases (which can also be seen in high-grade meningiomas), and rhabdomyosarcomas, which express myogenin and MyoD1. Primary intracranial sarcoma, DICER1-mutant exhibits PAS-positive globules and shows positivity for desmin, smooth muscle actin, and p53, with loss of H3K27me3 and ATRX.

Histiocytic lesions such as Langerhans cell histiocytosis and Rosai-Dorfman disease often harbor BRAF-V600E mutation and can be characterized with CD1a, S100, and CD68 as appropriate. Metastases (epithelial, melanocytic, hematologic) may also present as meningial-based lesions, as can inflammatory conditions such as IgG4-related disease, rheumatoid nodule, granulomatosis with polyangiitis. Immunohistochemical stains remain valuable to resolve these differential diagnoses, and molecular studies such as genome-wide DNA methylation profiling may be considered to assist in challenging cases.

3.6. Molecular testing

The recent updates recommend molecular testing of a subset of meningiomas to refine grade and further inform the risk of recurrence.

Copy-number variations (CNV) are common and prognostically important in meningioma. Losses of 22q (which includes the *NF2* locus) and 1p are the most prevalent. The cIMPACT-NOW update 8 recommends meningiomas with both 1p loss and 22q deletion/*NF2* oncogenic variant should be assigned to "CNS WHO grade 2", unless grade 3 features are present [24]. Approximately 14 % of histologic grade 1 tumors have both alterations, and these behave similarly to grade 2 tumors [31]. Less than 1 % of meningiomas show loss of 1p without loss of 22q, and the behavior of these tumors is not well understood [31]. The presence or absence of other high-risk CNVs (e.g., losses of 6, 10, 14, 18) does not affect grade but should be reported, as they can further inform the molecular risk by various novel scoring systems [32,33]. Chromosomal SNP arrays are the gold standard for CNV assessment, but DNA sequencing-based approaches, methylation arrays, and FISH may also be applied. It should be noted, however, that even segmental deletions (suggested cutoff $\geq$5 %) of the 1p chromosomal arm confer a higher risk [34], meaning FISH may be less sensitive for this purpose.

Homozygous deletion of *CDKN2A* and/or *CDKN2B* automatically upgrades a meningioma to grade 3 by 2021 CNS WHO criteria [21]. This can be tested via FISH, chromosomal SNP array, or NGS testing. Loss of p16 or MTAP immunostaining has been explored as a surrogate marker for *CDKN2A/B* homozygous deletion [35,36], but is not formally recommended in current guidelines.

Meningiomas with a *TERT* promoter (*TERT*p) mutation have reduced time to recurrence and are automatically assigned grade 3 per 2021 WHO criteria [21,37]. However, a large 2025 study found that *TERT*p mutation alone did not independently affect overall or recurrence-free survival after controlling for factors such as adjusted WHO grade, extent of resection, and *CDKN2A/B* status [38]. Thus, meningiomas with *TERT*p mutation that are otherwise histologically and molecularly benign appear to behave indolently, and grade 3 meningiomas had poor outcomes regardless of *TERT*p status [38]. The authors advise *TERT*p mutation testing in grade 2 tumors only, where it retained an independent predictor of recurrence-free survival, but not overall survival [38]. This may be formally incorporated into future consensus guidelines.

Genome-wide DNA methylation profiling has become a tool to help stratify the risk of recurrence in a subset of meningiomas. DNA is extracted, bisulfite-treated, and analyzed using commercial methylation arrays, and the resulting data are analyzed using one or multiple classifiers [2], which output a "calibrated score" to one of several meningioma classes corresponding to benign, intermediate, or malignant behavior.

The utility of methylation profiling varies by tumor grade. Methylation profiling is less impactful in WHO grade 1 tumors, as only about 10 % match to a higher risk methylation class (MC) [39]. Grade 2 tumors benefit the most, with 65 % matching to MC-benign, 30 % to MC-intermediate, and 4 % to MC-malignant [39]. Grade 3 meningiomas match to all classes roughly evenly [39], but current guidelines do not suggest "downgrading" a grade 3 tumor based on such data [24]. However, methylation profiles can help predict the likelihood of radiation resistance and could help inform clinical decision-making in all aggressive tumors [18]. Methylation profiling is also useful to diagnose clear cell meningioma, which has a distinct methylation class [40].

Disadvantages to methylation profiling include cost, accessibility, time to results, and potential challenges in interpretation when data are unclear, such as the ~19 % of cases that do not achieve a "matching" score exceeding 0.9[39]. Interpretation of DNA methylation results is best done by an experienced neuropathologist for integration of morphologic, CNV data, methylation data, and other molecular findings.

Although not routinely assessed, meningiomas driven by alterations in *AKT1*, *KLF4*, *TRAF7*, *SMO*, or *PIK3CA* generally are low-grade and lack chromosomal alterations [41].

3.7. Assessment of germline variants predisposing to meningioma

Around 95 % of meningiomas arise sporadically [42]. A minority arise in the setting of prior irradiation or tumor predisposition syndromes (e.g., NF2, Gorlin, Cowden, Proteus, Werner, BAP1, SMARCE1, SMARCB1, and MEN1 syndromes) [43,44]. Less than 0.4 % of meningiomas occur in the pediatric population (0–19 years), and less than 3 % occur in patients younger than 35 years of age [16]. Genetic counseling and germline testing may be indicated in young patients who develop a meningioma.

3.8. Practical approach to molecular testing

The approach to molecular testing of meningiomas will vary per local capabilities and resources. Table 3 summarizes a suggested approach to molecular testing after histologic evaluation.

Approximately 10 % of grade 1 tumors have molecular higher-risk features [39]. Rather than test all grade 1 meningiomas, it is suggested to prioritize those with concerning clinical features (e.g., rapid growth or recurrence) or "borderline" histologic features (e.g., 3 mitoses/1.6 mm^2, 2 atypical cytologic features, or chordoid/clear cell/papillary/rhabdoid histology). Identification of 1p loss results in upgrading to grade 2. Although this can be accomplished through various techniques, comprehensive CNV profiling and/or methylation

Table 3
Suggested approach to molecular testing of meningioma following histologic evaluation.

Histologic evaluation	Additional Molecular	Suggested Molecular Testing
Grade 1, not "borderline"	No, unless there are worrisome clinical features (e.g., recurrent tumor, serial scans showing rapid growth, or young patient)	None
Grade 1 with "borderline" atypical features falling short of grade 2[a]	Yes	For grading: 1p loss assessment For molecular risk stratification: Comprehensive CNV profile DNA methylation profiling
Brain invasion but otherwise benign (BIOB) Grade 1	Yes (leave ungraded until molecular data provide support for grade assignment)	
Grade 2	Yes	For grading: *TERT*p *CDKN2A/B* homozygous deletion For molecular risk stratification: Comprehensive CNV profile DNA methylation profiling
Clear cell subtype in differential diagnosis	Yes	*SMARCE1* sequencing/ immunostaining and/or DNA methylation profiling
Papillary or rhabdoid subtypes	Yes (consider evaluation for BAP1 loss by immunohistochemistry)	If otherwise, grade 1: Comprehensive CNV profile **AND** DNA methylation profiling If otherwise, grade 2: *TERT*p *CDKN2A/B* homozygous deletion Comprehensive CNV profile DNA methylation profiling
Grade 3	No	

[a] 3 mitoses/1.6 mm^2, 2 of the 5 atypical histologic features.

array can also inform whether recurrence risk is higher than average for the assigned WHO grade.

For brain-invasive, otherwise benign (BIOB) meningiomas, it is recommended not to assign a preliminary WHO grade and to pursue comprehensive CNV profiling and/or methylation studies before assigning a grade.

Grade 2 tumors benefit the most from ancillary molecular studies, and it is recommended that all tumors in this group be assessed for *CDKN2A/B* homozygous deletion and *TERT*p mutation analysis. At this point, the presence of one or both of those upgrades the tumor to grade 3. DNA methylation profiling does not alter grade but could inform risk of recurrence.

3.9. Conclusions

In meningiomas, appropriate tumor grading is essential to help inform clinicians of the need for adjuvant therapy. Adjuvant radiation is often considered for grade 2 and 3 tumors. Histologic grade relies on mitotic activity and atypical cytologic features. One only needs 4 or more mitoses per 1.6 mm^2 area to upgrade the tumor to 2; therefore, one must properly sample and examine the tumor, including any possible brain parenchyma if grossly identified. Certain types of meningiomas benefit from additional molecular testing to confirm grading and inform prognosis. Key indications include clinical recurrence or rapid growth, grade 1 meningiomas with borderline features (3 mitoses/1.6 mm^2, 2 out of 5 atypical features), grade 1 meningioma with brain invasion, clear cell, chordoid, rhabdoid morphology, papillary morphology, and all grade 2 meningiomas. Loss of 1p and 22q deletion in a histologic grade 1 meningioma warrants upgrading to grade 2. The presence of homozygous deletion of *CDKN2A/B* or *TERT*p mutation in a histologic grade 2 meningioma is now considered sufficient to upgrade to grade 3. Genome-wide DNA methylation profiling does not affect grading but informs of the risk of aggressive behavior and may help clinicians modify the type and dose of radiation therapy.

The benefits of grading and risk stratification are only realized if there is clear communication of the implications among the clinical team and, ultimately, with the patient.

CRediT authorship contribution statement

Yi Zhu: Conceptualization, Writing – original draft, Writing – review & editing. **Mark A. Rudolf:** Conceptualization, Writing – original draft, Writing – review & editing. **Kyle Conway:** Writing – original draft, Writing – review & editing, Conceptualization. **Jorge A. Trejo-Lopez:** Conceptualization, Writing – original draft, Writing – review & editing. **Sandra Camelo-Piragua:** Conceptualization, Writing – original draft, Writing – review & editing. **Aditya Raghunathan:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors of this article have no relevant financial relationships with commercial interests to disclose.

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