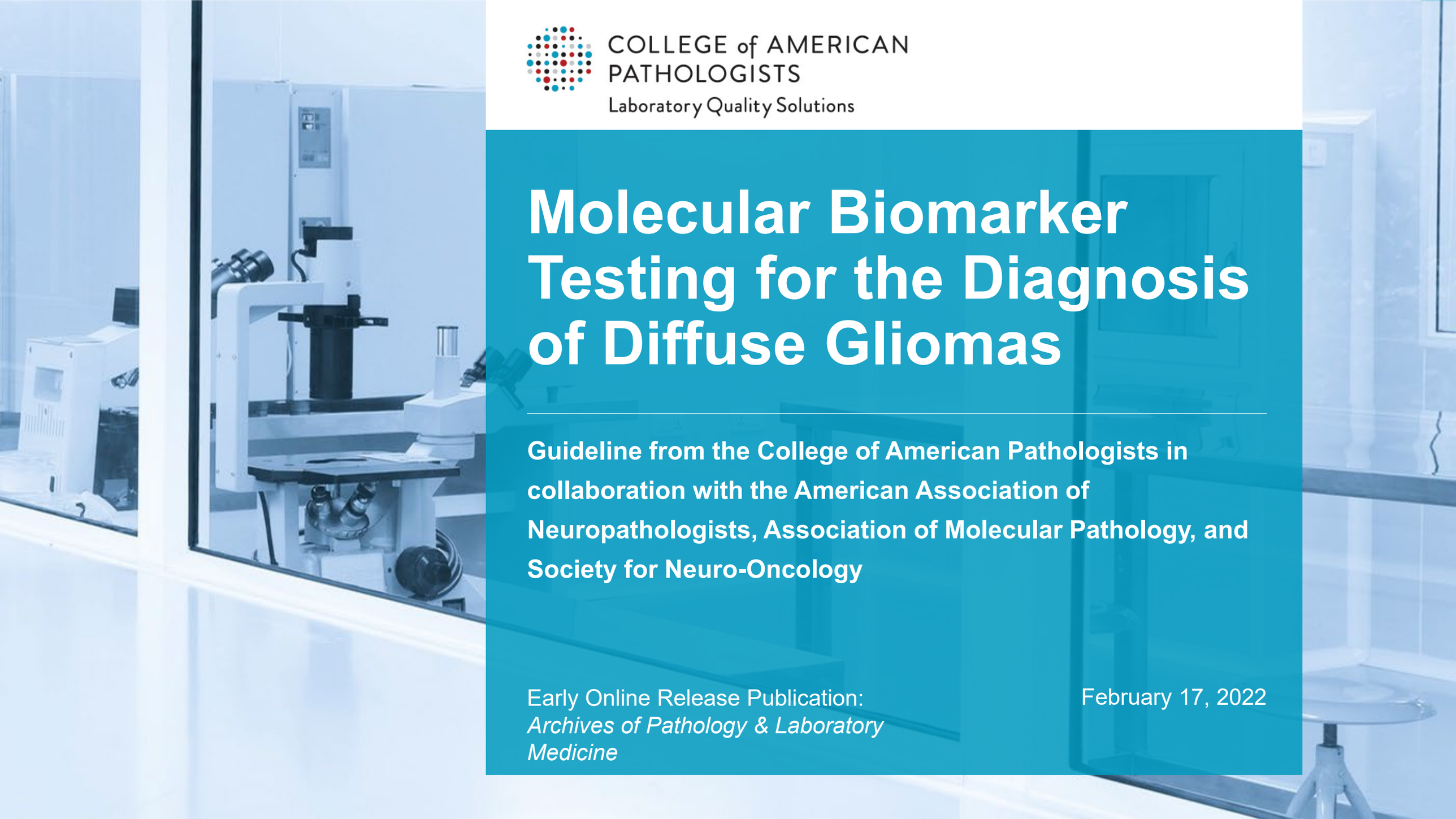

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Molecular Biomarker Testing for the Diagnosis of Diffuse Gliomas

Guideline from the College of American Pathologists in collaboration with the American Association of Neuropathologists, Association of Molecular Pathology, and Society for Neuro-Oncology

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Outline

- Introduction
- Key questions and results
- Guideline recommendations and good practice statements
- Guideline development process
- Conclusion

Introduction

- Diffuse gliomas (DG) are primary central nervous system (CNS) neoplasms characterized by the widespread infiltration of individual tumor cells displaying cytologic and histologic features of glial differentiation.
- These tumors affect patients of all ages and arise throughout the neuro-axis but are most common in older adults and occur most frequently in the cerebral hemispheres.
- Clinical course and treatment varies based on tumor type and grade.

Introduction, continued

- Over the past decade, numerous investigations have uncovered molecular genetic alterations that can be used to reliably and reproducibly classify DG into clinically meaningful subsets.
- The increasing complexity and rapid pace of change in diagnostic criteria, relevant molecular biomarkers, laboratory testing platforms, and clinical practice warrant the development of evidence-based recommendations on biomarker testing for DG.

Introduction, continued

- The CAP Quality and Pathology Laboratory Center for Evidence-based Guidelines developed an expert panel to address the overarching question **"What ancillary tests are needed to classify DG in order to sufficiently inform the clinical management of patients?"**

Key Questions

More specifically, the expert panel sought to answer:

1. **What genetic and molecular alterations should be included for optimal classification of Diffuse Gliomas?**
2. **What are the core molecular tests/findings that provide sufficient classifying information in the setting of discrete clinicopathologic entities?**
3. **What are the acceptable techniques/methods/criteria for determining MGMT promoter methylation status?**

- **Sub questions are listed in the methods supplement**

Results

- A systematic review of the literature was performed
  - 4,821 titles/abstracts screened
  - 703 full text review
  - 188 studies had data extracted
  - 86 studies informed the recommendations
- 13 recommendations and 3 good practice statements were developed
- Refer to the guideline manuscript for a description of the strength of recommendations

Guideline Recommendations

Recommendation 1

IDH mutational testing must be performed on all diffuse gliomas (DG).

Strong Recommendation

Rationale

- The World Health Organization (WHO) requires identification of IDH mutation within a DG for specific neoplastic types
- IDH-mutant DGs are distinct diseases that have specific genetic profiles, clinical courses and therapeutic options that differ from other forms of DG, such as IDH-wild type (WT) and histone H3-mutant DG

Recommendations 2-4

- These recommendations should be considered together after the initial testing of a DG reveals a tumor is IDH-mutant
- ATRX, TP53 and 1p/19q testing support the diagnosis of IDH-mutant astrocytoma or oligodendroglioma, IDH-mutant, 1p/19q codeleted

Recommendation 2-4

2. ATRX status should be assessed in all IDH-mutant DG unless they show 1p/19q codeletion.

Strong Recommendation

3. TP53 status should be assessed in all IDH-mutant DG unless they show 1p/9q codeletion.

Conditional Recommendation

4. 1p/19q codeletion must be assessed in IDH-mutant DG unless they show ATRX loss or TP53 mutation.

Strong Recommendation

Rationale, Recommendation 2

- Loss of nuclear ATRX protein expression in tumor cells of an IDH-mutant DG as determined by IHC serves as a relatively sensitive and specific surrogate marker for astrocytic lineage
- There is a strong inverse relationship among IDH-mutant DG between ATRX loss and the presence of 1p/19q codeletion (ie, molecularly defined oligodendrogliomas)
  - Rare exceptions exist, but for this reason, it has been recommended that 1p/19q testing need not be pursued in IDH-mutant gliomas with immunohistochemically identified ATRX loss or p53 overexpression

Rationale, Recommendation 3

- Among IDH-mutant DG, TP53 mutation and p53 overexpression are associated with astrocytic lineage
- IDH-mutant gliomas that have whole arm 1p/19q codeletion (ie, oligodendroglioma, IDH-mutant, 1p/19q codeleted) only rarely exhibit TP53 mutation or strong p53 overexpression

Rationale, Recommendation 4

- Confirmation of whole arm 1p/19q codeletion in an IDH-mutant DG is essential to render the diagnoses of oligodendroglioma, IDH-mutant and 1p/19q codeleted
- Landmark genomic analyses have established that whole arm 1p/19q codeletion, is entirely restricted to gliomas that also harbor mutations in either IDH1 or IDH2
- 1p/19q codeletion arises with near mutual exclusivity with respect to inactivating alterations of TP53 and ATRX

Recommendation 5

***CDKN2A/B* homozygous deletion testing should be performed on IDH-mutant astrocytomas.**

Conditional Recommendation

Rationale

- CDKN2A/B deletion has been shown by multiple investigations to be an adverse prognostic factor in IDH-mutant astrocytomas
- Since homozygous deletion of CDKN2A/B can be observed in other types of primary brain tumors that have highly variable clinical outcomes, this recommendation pertains specifically to IDH-mutant astrocytomas

Recommendation 6

MGMT **promoter methylation testing should be performed on all glioblastoma (GBM), IDH-wild type (WT).**

Strong Recommendation

Rationale

- The MGMT (O-6-Methylguanine-DNA Methyltransferase) protein, encoded by the MGMT gene, binds to DNA and repairs mutations that occur during DNA replication
- Through these actions, it is a key mediator of resistance to alkylating chemotherapy in the treatment of DG
- Randomized studies have shown that the clinical benefit of adding temozolomide to radiotherapy is predominantly among patients with MGMT promoter methylated GBM

Recommendation 7

For IDH-mutant DG, *MGMT* promoter methylation testing may not be necessary.

Conditional Recommendation

Rationale

- The high correlation between IDH-mutation and MGMT promoter methylation suggests that testing for MGMT promoter methylation in IDH-mutant DG may not be necessary
- The clinical relevance of testing for MGMT promoter methylation in IDH-mutant DG is not as firmly established

Recommendation 8

***TERT* promoter mutation testing may be used to provide further support for the diagnosis of oligodendroglioma and IDH-WT GBM.**

Conditional Recommendation

Rationale

- In the setting of IDH-mutation, TERT promoter mutations can provide additional support for the diagnosis of oligodendroglioma, IDH-mutant, 1p/19q codeleted, WHO grade 2 or 3
- It can also provide diagnostic support in cases in which immunostaining for ATRX is equivocal or was not performed
- There are scenarios where it may not be necessary
  - e.g., IDH-mutant gliomas with sufficient evidence of ATRX loss

Recommendation 9

For histologic grade 2-3 DG that are IDH-WT, testing should be performed for whole chromosome 7 gain/whole chromosome 10 loss, *EGFR* amplification, and *TERT* promoter mutation to establish the molecular diagnosis of GBM, IDH-WT, grade 4.

Strong Recommendation

Rationale

- Numerous studies have attempted to identify molecular genetic biomarkers that reliably identify histologic grade 2 and 3 tumors that behave most aggressively
- The strongest evidence indicates that the following markers identify IDH-WT diffuse astrocytic gliomas with grade 4 clinical behavior:
  - 1) whole chromosome 7 gain together with whole chromosome 10 loss (+7/-10);
  - 2) EGFR amplification; or
  - 3) TERT promoter mutation

Recommendation 10

H3 K27M testing must be performed in DG that involve the midline in the appropriate clinical and pathologic setting.

Strong Recommendation

Rationale

- The presence of an H3 K27M mutation in a diffusely infiltrative glioma involving the midline most often predicts clinically aggressive behavior and poor prognosis, leading to its designation of WHO grade 4
- While the number of studies investigating this particular mutation is small, the implications for diagnosis, prognosis and treatment are critically important
- Even though most of these tumors are found in pediatric patients, increasing evidence has found that the midline location of a DG is also tightly linked with H3 K27M mutation, warranting testing of all patients with midline gliomas

Recommendation 11

H3 G34 testing may be performed in pediatric and young adult patients with IDH-WT DG.

Conditional Recommendation

Rationale

- **“Diffuse hemispheric glioma H3 G34-mutant” was recently introduced as a distinct entity in the WHO 5th Edition, underscoring the importance of testing for this molecular alteration**
- **DG with G34R/V mutations should not be lumped together with other IDH WT gliomas, as they carry a disease defining genetic alteration that directs aggressive behavior corresponding to a WHO grade 4 neoplasm regardless of histologic appearance**

Recommendation 12

BRAF **mutation testing (V600) may be performed in DG that are IDH-WT and H3-WT.**

Conditional Recommendation

Rationale

- BRAF V600E mutation is present in a minority of DG, but prevalence is enriched in epithelioid GBMs and other IDH- and H3-WT DG
- This recommendation is influenced by the availability of targeted therapy and the occurrence of BRAF V600E within a variety of brain tumors other than DG, where the mutation is more prevalent

Recommendation 13

MYB/MYBL1 **and** ***FGFR1*** **testing may be performed in children and young adults with DG that are histologic grade 2-3 and are IDH-WT and H3-WT.**

Conditional Recommendation

Rationale

- Recent studies have identified subsets of DG in children and young adults that lack alterations in IDH1, IDH2 or histone H3 genes
- In addition to BRAF, genes that are recurrently altered in this group include MYB, MYBL1, and FGFR1
- Given the diagnostic and prognostic implications for these genetic drivers in gliomas of children and young adults, testing may be advisable, but the quality of evidence is lower than that supporting testing for other more commonly altered genes (eg, IDH1/2 and BRAF)

Good Practice Statements

Good Practice Statements (GPSs)

- High level of certainty that the recommended action will do more good than harm, but has little direct evidence
- Studies identified with a targeted literature search
- Studies are not assessed for quality

Good Practice Statements

1. *EGFR* mutation testing may be performed on diffuse gliomas that involve the midline.

2. Testing for alterations in *ALK, ROS1, MET* and *NTRK* genes may be performed on cerebral hemispheric diffuse gliomas of infancy that are wildtype for IDH and histone H3.

3. Testing for DNA methylation class or for alterations in *PDGFRA*, *EGFR*, *MYCN* may be performed in pediatric high grade diffuse gliomas that are wildtype for IDH and histone H3.

Guideline Development Process

Collaboration

- The CAP collaborated with the American Association of Neuropathologists (AANP), Association for Molecular Pathology (AMP), and Society for Neuro-Oncology (SNO). They provided members to participate on the guideline panels and approved the guideline prior to submission to publication.
- Two oncologists representing the American Society of Clinical Oncology (ASCO) also served on the expert panel.

Expert Panel Members

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Development Process

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Evidence-based Guideline (EBG) Development and Review Process

The Pathology and Laboratory Quality Center for Evidence-based Guidelines (the Center) develops recommendations related to the practice of pathology and laboratory medicine. Through them, we continually improve the quality of diagnostic medicine and patient outcomes.

1	Submit & Select Ideas	The Center Guideline Committee vets all topics submitted via the CAP Center website and recommends approval for those meeting the appropriate criteria to the Council on Scientific Affairs (CSA).
2	Determine Scope & Form Panel	A rigorous and transparent screening is conducted, including conflicts of interest, for the volunteer expert panel who defines the scope and key questions and for the advisory panel.
3	Research & Review Evidence	A systematic review of the literature of the literature using the GRADE approach offers a transparent and sensible method to grading the quality (certainty) of the evidence and strength of recommendations.
4	Draft Recommendations	The expert panel develops draft recommendations based upon the extracted data, the strength of evidence, and the considered judgment process including assessment of benefits to harms.
5	Open Comment Period	The draft recommendations undergo a public peer review during which stakeholder feedback is collected.
6	Complete Recommendations & Draft Manuscript	The expert panel finalizes the recommendations and the guideline manuscript based on updated literature and stakeholder feedback.
7	Review & Approve	The independent review panel, comprised of unconflicted individuals with topic expertise, acts on behalf of the CSA as the CAP approval body.
8	Publish & Implement	The guideline manuscript is submitted for publication to the *Archives of Pathology & Laboratory Medicine* (and partner journals if applicable). The Center develops tools and educational activities to support the adoption and implementation of guideline.
9	Maintain & Monitor	Center EBGs are reviewed every four years (or earlier if evidence becomes available that could potentially alter the original guideline recommendations). Upon review, the guideline will either be reaffirmed, updated, or retired.

Reaffirm	Update	Retire
Confirmation complete guideline is accurate and up to date and then place into step 9	Refresh guideline and start at step 2 of process	Guideline inactive (i.e., no updated systematic review)

Email center@cap.org for questions, comments, or to report concerns including conflict of interest issues.

Literature Search and Comment Period

- **Ovid MEDLINE and Embase were searched November 13, 2017**
  - **Search dates 1/1/2008 through 11/13/2017**
  - **2 literature refreshes 9/3/2019 and 09/23/2020**
- **The draft recommendations were released to the public for comments September 9 through 30 and again October 11 through 31, 2019 to increase the number of participants**
  - **A total of 315 comments were submitted from 155 participants.**
  - **Final recommendations were approved with a majority vote by the expert panel members**
  - **Further details can be found in the methods supplement**

Conclusions

Conclusions

- Thirteen evidence-based recommendations and three good practice statements are offered to help pathologists and their clinical colleagues select biomarker testing for the diagnosis of diffuse gliomas.
- As testing evolves, the guideline will need to be updated.

Disclosure

Practice guidelines and consensus statements are intended to assist physicians and patients in clinical decision-making. New evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for a patient. Refer to the guideline manuscript for complete details about the recommendations. The CAP and its collaborators make no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The CAP and its collaborators assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

References

- Brat DJ, Aldape K, Bridge JA, et al. Molecular biomarker testing for the diagnosis of diffuse gliomas: Guideline from the College of American Pathologists in collaboration with the American Association of Neuropathologists, Association of Molecular Pathology, and Society for Neuro-Oncology. *Arch Pathol Lab Med*. 2022; 146(5): 547–574. doi:10.5858/arpa.2021-0295-CP

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