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 for Molecular Pathology, and Society for Neuro-Oncology

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● ***Context.***—**The diagnosis and clinical management of patients with diffuse gliomas (DGs) have evolved rapidly over the past decade with the emergence of molecular biomarkers that are used to classify, stratify risk, and predict treatment response for optimal clinical care.**

Objective.—**To develop evidence-based recommendations for informing molecular biomarker testing for pediatric and adult patients with DGs and provide guidance for appropriate laboratory test and biomarker selection for optimal diagnosis, risk stratification, and prediction.**

Design.—**The College of American Pathologists convened an expert panel to perform a systematic review of the literature and develop recommendations. A systematic review of literature was conducted to address the overarching question, "What ancillary tests are needed to classify DGs and sufficiently inform the clinical management of patients?" Recommendations were derived from quality of evidence, open comment feedback, and expert panel consensus.**

Results.—**Thirteen recommendations and 3 good practice statements were established to guide pathologists and treating physicians on the most appropriate methods and molecular biomarkers to include in laboratory testing to inform clinical management of patients with DGs.**

Conclusions.—**Evidence-based incorporation of laboratory results from molecular biomarker testing into integrated diagnoses of DGs provides reproducible and clinically meaningful information for patient management.**

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Diffuse gliomas (DGs) are primary central nervous system (CNS) neoplasms characterized by the widespread infiltration of individual tumor cells displaying cytologic and histologic features of glial differentiation.[1] 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 vary based on tumor type and grade.

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Authors' disclosures of potential conflicts of interest and author contributions are found in the Appendix at the end of this article.

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Since the early 1900s, and until the 2016 revised 4th edition of the World Health Organization (WHO) *Classification of Tumours of the Central Nervous System,*[1] DGs were classified based upon the morphologic features of neoplastic cells, with molecular testing playing an ancillary role.

During the past decade, numerous investigations have uncovered molecular genetic alterations that can be used to reliably and reproducibly classify DGs into clinically meaningful subsets, leading the 2016 WHO revised 4th edition update to incorporate diagnostic entities based on the integration of morphologic features with molecular biomarkers.[1–6] More recent advances in our understanding of the pathogenesis and clinical behavior of specific DG subtypes has led to the inclusion of additional molecular biomarkers into clinical practice and the 2021 WHO 5th edition relies even more on molecular test results for diagnosis and grading.[7–11] DNA methylome profiling continues to identify numerous tumor types with specific methylation patterns that have characteristic genetic alterations and clinical behavior.[12] 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 DGs.

DESIGN

This evidence-based guideline was developed following the standards endorsed by the National Academy of Medicine.[13] A detailed description of the methods and the systematic review (including the quality assessment and complete analysis of the evidence) used to create this guideline can be found in the supplemental digital content (SDC) at https://meridian.allenpress.com/aplm in the May 2022 table of contents.

PANEL COMPOSITION

The College of American Pathologists (CAP) in collaboration with the American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology convened a multidisciplinary expert panel consisting of 13 practicing pathologists from a variety of specialties, institution types, and other professional groups, 2 oncologists who were representatives of the American Society of Clinical Oncology, 1 patient advocate, and a research methodologist consultant to develop this guideline. An advisory panel assisted the expert panel at specific key stages in the development of the guideline. All panel members, except for the methodologist consultant, volunteered their time and were not compensated for their involvement. Detailed information about the panel composition can be found in the SDC.

CONFLICT OF INTEREST POLICY

The collaborators agreed upon a conflict of interest policy (effective June 2017), and members of the expert panel disclosed all financial interests from 3 years before appointment through the development of the guideline. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Complete disclosures of the expert panel members are listed in the Appendix. Disclosures of interest judged by the oversight group to be manageable conflicts are as follows: HC – Consultancies with F. Hoffman-La Roche, Ltd (Basel, Switzerland), Genentech USA, Inc. (South San Francisco, California), Upsher-Smith Laboratories, LLC (Grove, Minnesota), Novocure (St. Helier, Jersey), Insys Therapeutics (Phoenix, Arizona), Mateon Therapeutics (formerly OxiGENE) (Agoura Hills, California), CytRx Corp. (Los Angeles, California), Omniox Inc. (San Carlos, California), AbbVie Inc. (North Chicago, Illinois); EH – Consultancies with E.R. Squibb&Sons, LLC (New Brunswick, New Jersey), Honorarium from Arbor Pharmaceuticals, LLC (Atlanta, Georgia); MvdB – Consultancies with Bristol Myers Squibb (New York, New York), Celldex Therapeutics, Inc. (Hampton, New Jersey), Vaximm AG (Basel, Switzerland), Carthera, Nerviano, Agios, Honorarium from Merk Sharp & Dohme Corp (Kenilworth, New Jersey), Research Grants from AbbVie Inc (North Chicago, Illinois).

Most of the expert panel (14 of 17 members) were assessed as having no relevant conflicts of interest. The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. See the SDC for complete information about the conflict of interest policy.

GUIDELINE OBJECTIVES

The expert panel addressed the overarching question, "What ancillary tests are needed to classify DGs to sufficiently inform the clinical management of patients?" To answer this, several more pointed key questions (KQs) were developed as follows:

KQ 1a: What genetic and molecular alterations should be included for optimal classification of DGs?
KQ1b: What are the acceptable techniques/methods for molecular genetic testing of DGs? What are the expected turnaround times for individual assays?
KQ1c: What are the acceptable techniques/methods for assessing whole genome copy number alterations?
KQ 2: What are the core molecular tests/findings that provide sufficient classifying information in the setting of discrete clinicopathologic entities?
KQ 3: What are the acceptable techniques/methods/criteria for determining *MGMT* promoter methylation status?

OUTCOMES OF INTEREST

The panel established outcomes of interest for both clinically based and pathology-based studies/articles. The clinical outcomes of interest included survival rates (overall, 1-year and 3-year survival, progression free), recurrence rates, response to treatment, and accuracy of diagnosis. The pathologic outcomes of interest included sensitivity, specificity, positive predictive value, negative predictive value, concordance, turnaround time, reproducibility of the various tests, as well as mutation/alteration/deletion status (percent, presence, frequency, and association with other alterations) for the molecular targets of interest.

The target audience of this review are those within the neuro-oncology community who care for patients with DGs, including pathologists, neuroradiologists, neurosurgeons, radiation oncologists, neuro-oncologists, medical oncologists, and other members of the patient care team. Recommendations will also be highly relevant to brain tumor investigators, epidemiologists, and cancer registrars.

LITERATURE SEARCH AND COLLECTION

Literature search strategies were developed in collaboration with a medical librarian for the concepts of DGs, molecular markers or gene alterations, and laboratory test methods. In consultation with the expert panel, the search strategies were created using standardized database terms and keywords. Databases searched included Ovid MEDLINE and Embase.com. Additional searches for unindexed literature were conducted in ClinicalTrials.gov, Cochrane Library, Guidelines International Network, National Guideline Clearinghouse, Trip search engine, University of York Centre for Reviews and Dissemination-PROSPERO, and applicable US and international organizational Web sites. In addition, expert panel members were surveyed for any relevant unpublished data at the onset of the project. Initial database searches were completed on November 13, 2017, and refreshed on September 3, 2019, and September 23, 2020. All searches were limited to English language and publication dates of January 1, 2008, to the date of search. Case reports, commentaries, editorials, conference abstracts, and letters were excluded. The Cochrane search filter for humans was applied in Ovid MEDLINE and Embase.com.[14] MEDLINE and conference abstract records were excluded in the Embase searches. A targeted search was performed in Ovid MEDLINE on July 24, 2020, to ensure that emerging evidence about bithalamic glioma, infantile-type hemisphere glioma, and diffuse pediatric-type high-grade glioma and relevant mutations or amplifications was included in anticipation of the WHO 2021 update.[7] The search was limited to publication dates January 1, 2016 to July 24, 2020, English language, and human studies. Case reports, commentaries, editorials, and letters were excluded. The Ovid MEDLINE and Embase search strings and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagrams outlining details of the systematic and targeted review are provided in the SDC as Supplemental Figures 1 through 3.

INCLUSION AND EXCLUSION CRITERIA

Studies were selected for inclusion in the systematic review of evidence if they met the following criteria: the study included human patients; the study was a systematic review with or without meta-analysis, a randomized clinical trial, or a comparative or noncomparative observational study (prospective or retrospective design); the study included a minimum sample size of at least 30, except for studies evaluating B-Raf proto-oncogene (*BRAF*) alterations, fibroblast growth factor receptor (*FGFR*) alterations, and MYB proto-oncogene (*MYB*) and MYB proto-oncogene like 1 (*MYBL1*) alterations, for which the expert panel determined all study sizes should be included based on a lower frequency of these alterations that occur in pediatric brain tumors; the study was published in English; the study addressed 1 of the key questions; the study included measurable data such as accuracy, sensitivity, specificity, negative predictive value, positive predictive value, time to appropriate testing, repeat procedures, overall survival, recurrence rates, turnaround time, concordance; and the study addressed DGs and included at least one of the following genetic and molecular alterations:

1. Isocitrate dehydrogenase (NADP(+))1 (*IDH1*) and isocitrate dehydrogenase (NADP(+))2 (*IDH2)* mutations
2. Histone H3 gene mutations
3. *BRAF* alterations
4. ATRX chromatin remodeler (*ATRX*) alterations
5. Tumor protein p53 (*TP53*) alterations
6. 1p/19q codeletion
7. Chromosome 7 gain
8. Chromosome 10 loss
9. *MYB* and *MYBL1* alterations
10. Telomerase reverse transcriptase (*TERT*) promoter mutations
11. *FGFR* alterations
12. Epidermal growth factor (*EGFR*) alterations
13. Platelet-derived growth factor receptor alpha (*PDGFRA*) alterations
14. MET proto-oncogene, receptor tyrosine kinase (*MET*) alterations
15. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) alterations
16. O-6-methylguanine-DNA methlytransferase (*MGMT*) promoter alterations
17. Phosphatase and tensin homolog (*PTEN*) alterations
18. Neurofibromin 1 (*NF1*) alterations
19. Microsatellite instability status
20. MDM2 proto-oncogene (*MDM2*) alterations
21. Cyclin-dependent kinase 4 (*CDK4*) alteration

Articles were excluded from the systematic review if they were meeting abstracts; case reports, consensus documents, editorials, commentaries, or letters; cell line or animal model studies; full-text articles not available in English; or studies that did not address at least 1 of the key questions with outcomes of interest as agreed upon.

QUALITY ASSESSMENT

A risk of bias assessment was performed for all fully published studies meeting inclusion criteria by the research methodologist. The methodologist assessed key indicators based on study design and methodologic rigor and a rating for the quality of evidence was designated. See Supplemental Tables 1 through 4 for the quality assessment for included studies by study design. See Supplemental Table 5 for the Quality of Evidence definitions. Using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach,[15] an overall GRADE rating was given for each recommendation by outcome (Supplemental Table 6). Refer to the SDC for further details.

ASSESSING THE STRENGTH OF RECOMMENDATIONS

Following the quality of evidence assessment, completion of the GRADE Evidence to Decision framework,[16] and discussion of the definitions and implications of strength of recommendation (Table 1), the expert panel designated the recommendations as either strong or conditional. Refer to the SDC for further details.

GUIDELINE REVISION

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. If necessary, the entire panel will reconvene to discuss potential changes. When appropriate, the panel will recommend revision of the guideline to the CAP and its collaborators for review and approval.

Table 1. Definitions for Strength of Recommendation[a]		
Category	**Definition**	**Implication**
Strong recommendation	One for which the guideline panel is confident that the desirable effects of an intervention outweigh its undesirable effects (strong recommendation for an intervention) or that the undesirable effects of an intervention outweigh its desirable effects (strong recommendation against an intervention)	Implies that most or all individuals will be best served by the recommended course of action
Conditional (weak) recommendation	One for which the desirable effects probably outweigh the undesirable effects (weak recommendation for an intervention), or undesirable effects probably outweigh the desirable effects (weak recommendation against an intervention), but appreciable uncertainty exists	Implies that not all individuals will be best served by the recommended course of action. There is a need to consider more carefully than usual the individual patient's circumstances, preferences, and values

[a] Data derived from Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group materials.[196]

RESULTS

A total of 4821 studies met the search term requirements. Based on review of these abstracts, 703 articles met the inclusion criteria and continued to full-text review. A total of 188 articles were included for data extraction, and 86 articles informed the recommendations. Excluded articles were available as discussion or background references. Additional information about the systematic review is available in the SDC. Note that only the studies listed in the quality summary were used to inform the recommendations. These are studies from our systematic review of the literature. All other references mentioned in the write-up of each recommendation were brought in to provide context or further support. For most recommendations, the evidence base consisted of studies reporting on the molecular alteration frequency of the recommended gene in defined WHO DG subtypes. To highlight this evidence as it relates to establishing an accurate diagnosis while maintaining brevity for publication, each recommendation is accompanied by a table of representative studies. The representative studies chosen for inclusion in the tables are those that the expert panel believed most important in summarizing the evidence for each recommendation. Refer to the diffuse gliomas guideline Web page (www.cap.org) for the complete set of data tables.

The expert panel convened 17 times (15 times by teleconference and 2 in-person meetings) to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the quality of evidence that supports the final recommendations. A nominal group technique was used for consensus decision making to encourage unique input with balanced participation among group members. An initial open comment period was posted on the CAP Web site (www.cap.org) from September 9 to September 30, 2019, during which the draft recommendations were posted for public feedback. To allow for more responses, the CAP reopened the comment period from October 11 to October 31, 2019. Refer to the SDC for further details, including a list of organizations encouraged to participate. The expert panel approved the final recommendations with a supermajority vote.

An independent review panel, masked to the expert panel and vetted through the conflict of interest process, recommended approval by the CAP Council on Scientific Affairs. The manuscript was also approved by American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology. The final recommendations are summarized in Table 2. In addition, a visual representation of the guideline recommendations is provided to help laboratories and clinicians understand the testing involved that ultimately drives WHO-defined categorization. (Figure 1 shows the testing algorithm where *IDH1/2* mutations are present. Figure 2 shows the testing algorithm where *IDH1/2* mutations are not present.)

RECOMMENDATIONS

Strong Recommendation: 1

IDH mutational testing must be performed on all DGs.

The quality of evidence to support this recommendation was assessed as *moderate*.

Quality Summary.—The evidence base informing this recommendation comprises 38 studies.[3–6,17–50] Four studies were genome sequencing studies,[5,6,21,22] while the remaining 34 studies were retrospective cohort studies.[3,4,17–20,23–50] All genome sequencing studies[5,6,21,22] obtained samples retrospectively and were assessed as intermediate-low quality based on risk of selection bias, while no other forms of bias were identified. The retrospective cohort studies all suffered from risk of selection bias and were assessed as low[*] and very low quality[4,18,20,27,29,30,34] based on risk of selection bias in addition to risk in performance, detection, and reporting domains. The aggregate risk of bias across the entire evidence base was serious but quality of evidence was upgraded based on a strong association between IDH mutational testing and DG diagnostic classification. Refer to the SDC for the quality assessment of included genome sequencing studies, retrospective cohort studies, and the GRADE Quality of Evidence Assessment. See Table 3 for a summary of the mutational status of IDH across all DG subtypes.

The identification of an IDH mutation within a DG is required for the diagnosis of specific neoplastic types recognized by the WHO, including astrocytoma, IDH-mutant and oligodendroglioma, IDH-mutant, 1p/19q codeleted.[1,7] More than 70% of histologic grades 2 and 3 infiltrating astrocytomas in adults are IDH-mutated. By definition, all oligodendrogliomas IDH-mutant, 1p/19q codeleted harbor IDH mutations.[4–6,34] 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 DGs.[†] Therefore, testing for IDH mutations

[*] *References 3, 17, 19, 23–26, 28, 31–33, 35–50.*
[†] *References 3–6, 18, 20, 23, 25, 35, 36, 47.*

Table 2. Summary of the Recommendations	
Recommendation	**Strength of Recommendation**
1. IDH mutational testing must be performed on all DGs	Strong
2. ATRX status should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion	Strong
3. TP53 status should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion	Conditional
4. 1p/19q codeletion must be assessed in IDH-mutant DGs unless they show ATRX loss or *TP53* mutations	Strong
5. *CDKN2A/B* homozygous deletion testing should be performed on IDH-mutant astrocytomas	Conditional
6. *MGMT* promoter methylation testing should be performed on all GBM, IDH-WT	Strong
7. For IDH-mutant DGs, *MGMT* promoter methylation testing may not be necessary	Conditional
8. *TERT* promoter mutation testing may be used to provide further support for the diagnosis of oligodendroglioma and IDH-WT GBM	Conditional
9. For histologic grade 2-3 DGs 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
10. H3 K27M testing must be performed in DGs that involve the midline in the appropriate clinical and pathologic setting	Strong
11. H3 G34 testing may be performed in pediatric and young adult patients with IDH-WT DGs	Conditional
12. *BRAF* mutation testing (V600) may be performed in DGs that are IDH-WT and H3-WT	Conditional
13. *MYB/MYBL1* and *FGFR1* testing may be performed in children and young adults with DGs that are histologic grade 2-3 and are IDH-WT and H3-WT	Conditional

Abbreviations: *ATRX*, ATRX chromatin remodeler; *BRAF*, B-Raf proto-oncogene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDKN2B*, cyclin-dependent kinase inhibitor 2B; DGs, diffuse gliomas; *EGFR*, epidermal growth factor; *FGFR1*, fibroblast growth factor receptor 1; GBM, glioblastoma; H3, histone 3; IDH, isocitrate dehydrogenase; *MGMT*, O-6-methylguanine-DNA methlytransferase; *MYB*, MYB proto-oncogene; *MYBL1*, MYB-like; *TERT*, telomerase reverse transcriptase; *TP53*, tumor protein p53; WT, wild-type.

must be performed on DGs to diagnose these tumor types for proper clinical care. Testing may not be necessary if a definitive diagnosis of an IDH-mutant glioma or another diagnosis that is mutually exclusive with IDH-mutant glioma has been previously established. IDH-mutant gliomas are uncommon in pediatric patients and testing may not be necessary in younger children.[51] Testing for IDH mutations also may not be necessary if genetic alterations that are mutually exclusive with *IDH* mutations have been identified in a DG, such as histone H3 mutations. It should be noted that *EGFR* amplifications, gain of chromosome 7/loss of chromosome 10, and *TERT* promoter mutations have been documented in IDH-mutant astrocytomas, albeit at much lower frequency than GBM, IDH-WT, and their identification in a DG should not automatically rule out IDH testing.[3,26,39,47,52] Other clinical or pathologic settings may be encountered in which the diagnosis of an IDH-mutant DG can be formally excluded without testing for the mutation.

IDH1 and *IDH2* mutations result in a substitution for a key arginine at codons R132 and R172, respectively.[53] The most frequent *IDH1* mutation is R132H, which accounts for 89% to 93% of all *IDH1* and *IDH2* mutations.[5,6,27,35,47,53] A highly sensitive and specific monoclonal antibody that recognizes the IDH1-R132H mutant protein is widely used in diagnostic practice. Immunohistochemistry (IHC) for IDH1-R132H is a cost-effective and reliable first-line test for *IDH1* mutation in supratentorial DGs.[39,50,54–57] *IDH1* R132H mutations are followed in frequency by R132C, R132S, R132G, and R132L. *IDH2* mutations represent approximately 3% of all IDH mutations and are more frequent in oligodendrogliomas than IDH-mutant astrocytomas. R172K is the most frequent, followed by R172M and R172W. Testing for these non–IDH1-R132H mutations is accomplished by DNA sequence analysis and is necessary when IHC for IDH1-R132H is negative in appropriate settings, with specific exceptions (see above). Recent studies of primary infratentorial IDH-mutant astrocytomas have shown that they have a different spectrum of IDH mutations, with about 80% harboring non–IDH-R132H mutations,[58] suggesting that IDH sequencing may be required more frequently in this clinical setting.

Sequence analysis for IDH mutations can be performed by pyrosequencing, Sanger sequencing, polymerase chain reaction (PCR), or next-generation sequencing (NGS).[27,34,59–61] Because patients older than 55 years only rarely develop de novo glioblastomas (GBMs) that are IDH-mutant (4%–7%) and the IDH1-R132H antibody recognizes the large majority of IDH mutations, the WHO has recommended that testing for non–IDH1-R132H mutations by sequence analysis may not be necessary in patients older than 55 years whose tumors are negative for IDH1-R132H by IHC.[1,26,27,32,43,55] However, non-R132H mutations are sufficiently common in DGs of all grades in patients less than age 55, such that testing for these mutations is indicated when IHC does not detect the R132H mutation. As above, IDH testing may also be unnecessary if other mutually exclusive genetic alterations are identified.

The finding of an IDH mutation in the setting of glial proliferation strongly supports the diagnosis of a DG because this event does not occur in nonneoplastic diseases and is only rarely, if ever, found in CNS neoplasms other than DGs.[27,46,62–64] IDH mutations are almost always stable and persist through the course of disease, which can be exploited to evaluate residual/progressive tumor[33,65,66]; however, loss of the IDH-mutated allele has been documented in some cases at the time of recurrence and may be associated with greater cell proliferation or high-grade behavior.[67,68]

Public Comment Response to Recommendation 1.—There were 92 respondents, of whom 84 (91.31%) agreed or agreed with modifications, 6 (6.52%) disagreed, and 2 (2.17%) were neutral. There were 31 written comments, including many suggesting that the phrase "appropriate clinical and pathologic setting" needed to be further explained. There were also suggestions that the statement should recommend that IDH testing "should" be performed

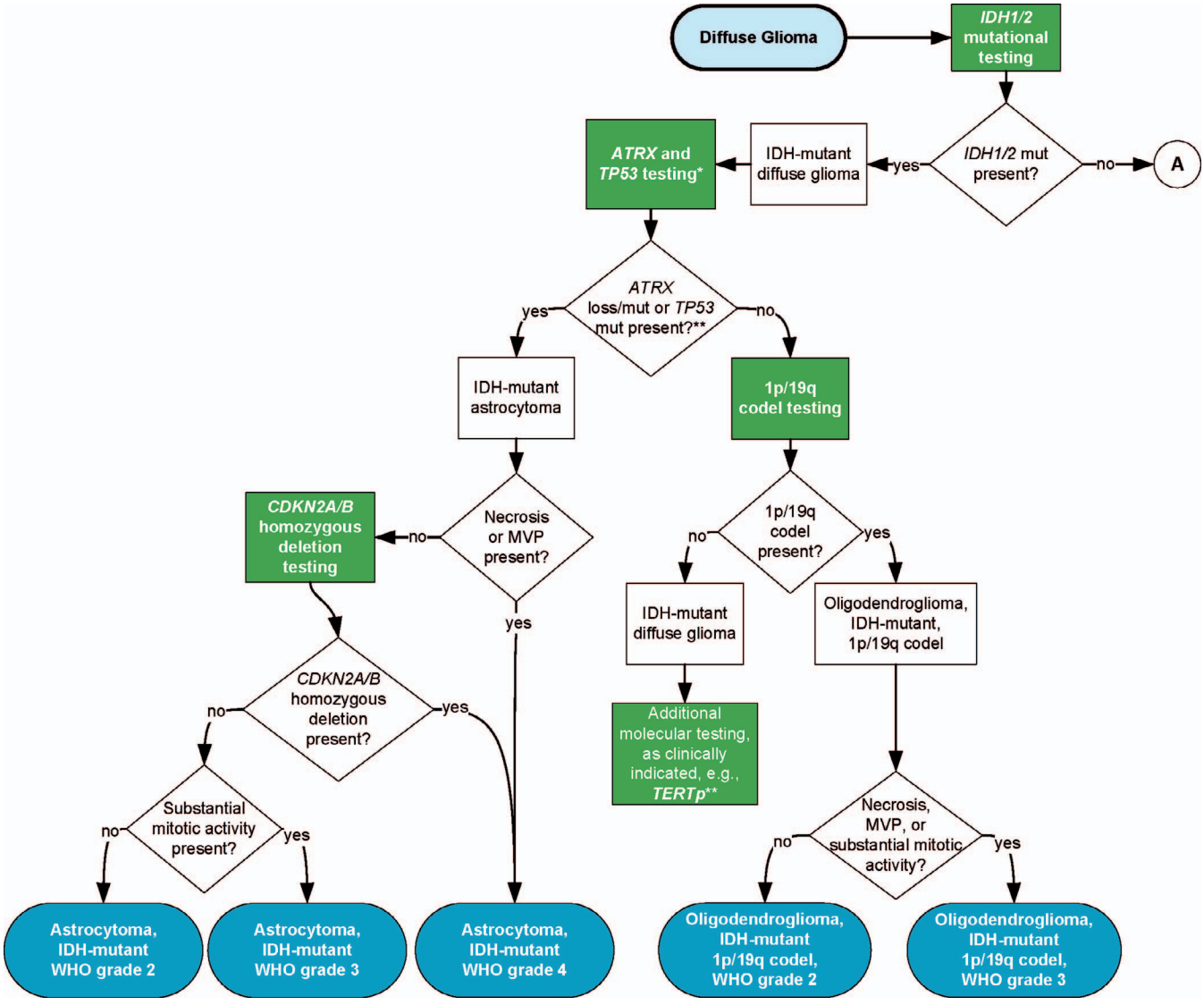

Figure 1. *Testing algorithm for diffuse gliomas with IDH1/2 mutations. A, refer to Figure 2. Blue indicates WHO-defined entities; green indicates recommended tests. *Some institutions/laboratories may prefer to perform 1p/19q codeletion as the initial step for IDH-mutant gliomas. See recommendations 2 through 4. **Additional molecular biomarker testing and DNA methylation profiling may be helpful in establishing a diagnosis for challenging cases. Abbreviations: ATRX,* ATRX *chromatin remodeler;* CDKN2A, *cyclin-dependent kinase inhibitor 2A;* CDKN2B, *cyclin-dependent kinase inhibitor 2B; codel, codeletion; IDH, isocitrate dehydrogenase; MVP, microvascular proliferation; Mut, mutation; TERTp, telomerase reverse transcriptase promoter; TP53, tumor protein p53; WHO, World Health Organization.*

rather than "must" be performed. Others indicated that the methods of testing (eg, IHC versus sequencing) should be described. These comments were taken into consideration. While the recommendation remained the same, the comments were addressed in the text above.

Strong Recommendation: 2

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

Conditional Recommendation: 3

TP53 status should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion.

Strong Recommendation: 4

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

Recommendations 2 through 4 are tightly linked to one another and should be considered together. Once the initial testing of a DG has revealed that the tumor is IDH-mutant, further testing for *ATRX*, *TP53*, and 1p/19q support the diagnosis of IDH-mutant astrocytoma or oligodendroglioma, IDH-mutant, and 1p/19q codeleted. Testing algorithms and workflows differ across practices, institutions, and countries. Some perform IHC for ATRX and p53 first, with follow-up studies for 1p/19q only if these tests are negative. Others perform 1p/19q testing as the initial step, followed by ATRX and/or p53 testing if the test is negative. Multigene panels represent another approach that provides test results simultaneously; proponents assert that it provides information on other biomarkers relevant to classification and grade.[69] Because positive test results from ATRX/p53 and 1p/19q testing are nearly mutually exclusive, it is not necessary to perform additional testing if the initial test results are

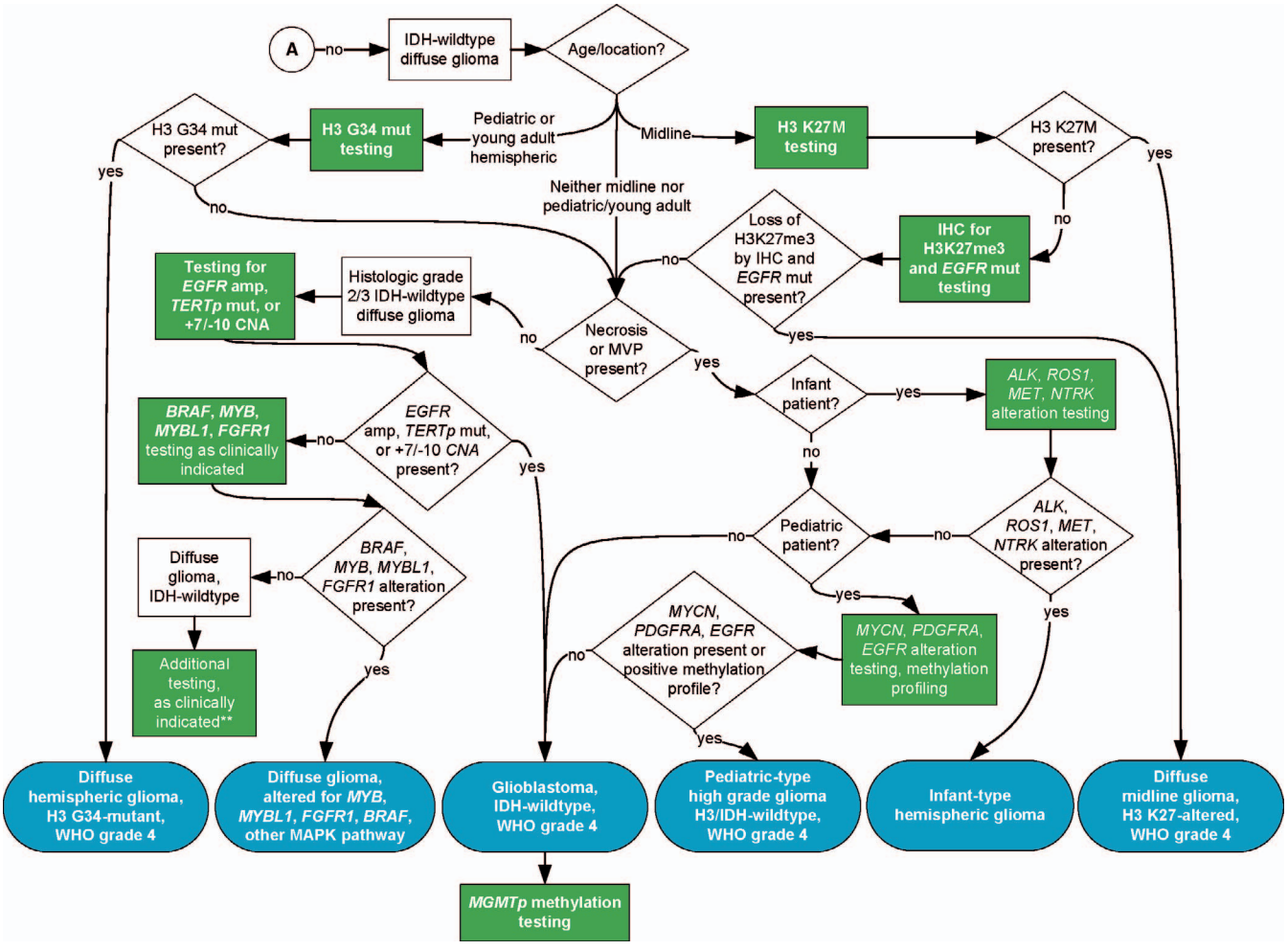

Figure 2. *Testing algorithm for diffuse gliomas without IDH1/2 mutations. A, refer to Figure 1. Blue indicates WHO-defined entities; green indicates recommended tests; italic indicates good practice statements. **Additional molecular biomarker testing and DNA methylation profiling may be helpful in establishing a diagnosis for challenging cases. Abbreviations:* ALK, *ALK receptor tyrosine kinase;* BRAF, *B-Raf proto-oncogene; CNA, copy number alteration;* EGFR, *epidermal growth factor receptor;* FGFR1, *fibroblast growth factor receptor;* H3, *histone 3 gene mutation; H3 K27me3, H3 K27M trimethylation; IDH, isocitrate dehydrogenase (NADP(+)); IHC, immunohistochemistry; MAPK, mitogen-activated protein kinase;* MET, *MET proto-oncogene, receptor tyrosine kinase; MGMTp, O-6-methylguanine-DNA methlytransferase; Mut, mutation;* MYB, *MYB proto-oncogene; MYBL1, MYB-like; MYCN, N-myc proto-oncogene protein; NTRK, neurotrophic tyrosine receptor kinase;* PDGFRA, *platelet-derived growth factor receptor alpha;* ROS1, *ROS proto-oncogene 1; TERTp, telomerase reverse transcriptase promoter; WHO, World Health Organization.*

sufficient to establish a diagnosis. Recommendations 2 through 4 are written in a manner to reflect these considerations.

The quality of evidence to support Recommendations 2 and 4 is *moderate*, the quality of evidence to support Recommendation 3 was assessed as *low*.

Quality Summary.—Recommendation 2 was informed by 12 retrospective cohort studies.[24–26,49,50,70–76] Eight of these studies were assessed as low quality,[24–26,49,50,70,74,75] and 4 were assessed as very low quality.[71–73,76] All included studies were limited by a critical risk of selection bias, plus individual studies were further limited by risk of bias in performance,[26,72,73] detection,[24,26,71–76] and reporting[50,71–74,76] domains. Although the aggregate risk of bias across the evidence base was very serious, the evidence was upgraded based on a strong association between *ATRX* assessment and DG WHO classification. Recommendation 3 focuses on the need for *TP53* assessment and was informed by 2 genome sequencing studies,[6,22] 2 prospective cohort studies,[77,78] and 15 retrospective cohort studies.[‡] The retrospective cohort studies were assessed as low[§] and very low quality[29,71,82] based on risk of bias in selection,[‖] performance,[19,29,79] detection,[¶] and reporting[29,42,71,80,82–85] domains. The aggregate risk of bias for the evidence base was very serious and evidence was not further downgraded or upgraded based on any domain. The evidence base supporting Recommendation 4 comprises one genome sequencing study[6] and 11 retrospective cohort studies.[#] All retrospective cohort studies carry a critical risk of selection bias, plus individual studies were further limited by risk of bias in performance,[4] detection,[4,20,42,63,74–76,86] and reporting[**]

[‡] *References 19, 24, 25, 29, 42, 49, 71, 75, 79–85.*
[§] *References 19, 24, 25, 42, 49, 75, 79–81, 83–85.*
[‖] *References 19, 24, 25, 29, 42, 49, 71, 75, 79–85.*
[¶] *References 24, 29, 42, 71, 75, 79–82, 84, 85.*
[#] *References 4, 20, 42, 49, 50, 63, 74–76, 82, 86.*
[**] *References 4, 20, 42, 50, 63, 74, 76, 82, 86.*

Table 3. Representative Studies Reporting on IDH Mutation Testing and Status Across Diffuse Glioma (DG) Subtypes

Study, Study Design	Number DG Cases	Testing Method	Mutation Frequency[a]	Study Conclusion
Brat et al,[6] 2015, GSS	n = 293	Genome sequencing	A: 64/95 (67.4%) OA: 63/74 (85.1%) OD: 96/109 (88.1%) GBM: NR	Genome-wide data delineated 3 molecular classes of histologic grade 2/3 DG that were more concordant with IDH, 1p/19q, *ATRX*, and *TP53* status than with histologic class and correlated with clinical outcome
Ceccarelli et al,[5] 2016, GSS	n = 1132	Genome sequencing	A: 116/168 (69.1%) OA: 99/114 (86.8%) OD: 154/173 (89.0%) GBM: 34/453 (7.5%)	DG classification based on IDH and 1p/19q status was further refined using DNA methylation profiles to identify clinically relevant genetic subsets
Chan et al,[23] 2015, RCS	n = 237	Sequencing	A: 98/168 (58.3%) OA: 36/48 (75.0%) OD: 20/21 (95.2%) GBM: NR	When combined with IDH and 1p/19q status, *TERTp* mutation contributed to prognostic subgroups of lower-grade DG
Killela et al,[34] 2014, RCS	n = 473	Sequencing	A: 69/88 (78.4%) OA: 50/58 (86.2%) OD: 84/87 (96.6%) GBM: 24/240 (10.0%)	Genetic signatures of DG based on *TERTp* and IDH status stratifies patients into prognostically distinct cohorts
Mellai et al,[39] 2011, RCS	n = 287	Sequencing, IHC	A: 7/18 (38.9%) OA: 4/4 (100.0%) OD: 33/62 (53.2%) GBM: 19/186 (10.2%)	IDH mutation was specific for DG, was correlated with *MGMTp* methylation and was anticorrelated with *EGFR* amp
Sanson et al,[47] 2009, RCS	n = 404	Sequencing	A: 19/30 (63.3%) OA: 60/88 (68.2%) OD: 65/103 (63.1%) GBM: NR	*IDH1* mutation was closely linked to prognosis, *MGMTp* status and molecular profile of DG, grades 2-4
Labussiere et al,[35] 2010, RCS	n = 764	Sequencing	A: NR OA: NR OD: NR GBM: NR	*IDH1*/*IDH2* mutation was tightly linked to the finding of t(1;19) translocation in DG, but not with other focal 1p and 19q losses
Lee et al,[36] 2017, RCS	n = 168	Sequencing, MSP	A: 23/38 (60.5%) OA: NR OD: 65/65 (100.0%) GBM: 13/65 (20.0%)	*TERTp* mutation strongly correlated with poor survival outcome in patients with IDH-WT GBM
Eckel-Passow et al,[4] 2015, RCS	n = 615	Sequencing	A: NR OA: NR OD: NR GBM: NR	DG can be classified into 5 clinically relevant groups based on IDH, *TERT*, and 1p/19q
Korshunov et al,[3] 2015, RCS	n = 202	450K BeadChip array	A: NR OA: NR OD: NR GBM: 10/202 (5.0%)	Pediatric GBM showed low frequency of *IDH1* mutation and high frequency of histone H3 mutations, leading to refined prognostic model
Ebrahimi et al,[26] 2016, RCS	n = 1064	IHC	A: 156/284 (54.9%) OA: 66/71 (93.0%) OD: 81/83 (97.6%) GBM: 27/364 (7.4%)	ATRX is a potential marker for predicting IDH/*H3F3A* mutations and substratification of DG into prognostic groups
Boots-Sprenger et al,[20] 2013, RCS	n = 561	MLPA	A: 55/76 (72.4%) OA: NR OD: NR GBM: 36/226 (15.9%)	IDH, 1p/19q, and *MGMTp* status were correlated with patient outcome in DG, yet some exceptions were noted
Dubbink et al,[25] 2016, RCS	n = 133	NGS	A: 20/20 (100.0%) OA: 0/21 (0.0%) OD: 48/49 (98.0%) GBM: 0/55 (0.0%)	DG can be subclassified into prognostic groups based on the molecular status of IDH, 1p/19q, *TERTp*, 7+/10q−, and *H3F3A*

Abbreviations: 7+/10q−, chromosome 7 gain/chromosome 10q loss; amp, amplification; A, astrocytoma histology; ATRX, ATRX chromatin remodeler; *BRAF*, B-Raf proto-oncogene; *EGFR*, epidermal growth factor; GBM, glioblastoma; GSS, genome sequencing study; mut, mutation; H3, histone 3; IDH, isocitrate dehydrogenase; *IDH1*, isocitrate dehydrogenase (NADP(+))1; *IDH2*, isocitrate dehydrogenase (NADP(+))2; IHC, immunohistochemistry; *MGMT*, O-6-methylguanine-DNA methlytransferase; *MGMTp*, *MGMT* promoter; MLPA, multiplex ligation-dependent probe amplification; MSP, methylation-specific polymerase chain reaction; NGS, next-generation sequencing; NR, not reported; OA, oligoastrocytoma histology; OD, oligodendroglioma histology; RCS, retrospective cohort study; t(1;19), translocation (1;19); *TERT*, telomerase reverse transcriptase; *TERTp*, *TERT* promoter; *TP53*, tumor protein p53; WT, wild-type.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

domains. Although the aggregate risk of bias across the evidence base was very serious, the evidence was upgraded based on a strong association between 1p/19q codeletion status and diffuse glioma WHO classification. Refer to the SDC for the quality assessment of included genome sequencing studies, retrospective cohort studies, and the GRADE Quality of Evidence Assessment. See Table 4 for a summary of the mutational status of *ATRX*, *TP53*, and 1p/19q across all DG subtypes.

Recommendation 2

As with all cancers, DGs must employ a mechanism to prevent telomeres from shortening to escape cellular senescence. In the case of IDH-mutant astrocytomas (WHO grades 2–4), this is primarily accomplished by alternative lengthening of telomeres via inactivation of the *ATRX* gene.[87] Most oligodendrogliomas, IDH-mutant, 1p/19q-codeleted, and IDH-WT GBMs accomplish this via overexpression of telomerase resulting from activating *TERT* promoter mutations. Owing to these associations, loss of nuclear ATRX protein expression in tumor cells of an IDH-mutant DG as determined by IHC serves as a relatively (albeit not completely) sensitive and specific surrogate marker for astrocytic lineage.[††] Because strong and diffuse nuclear p53 immunoreactivity is also commonly encountered in IDH-mutant astrocytomas, the ATRX immunostain is often run simultaneously with the IDH1-R132H (Recommendation 1) and p53 stains (discussed in Recommendation 3). Less common astrocytoma subtypes, which are more common in children, may also show loss of ATRX expression, including diffuse midline gliomas with H3 K27M mutation (~15% of cases), diffuse hemispheric gliomas with H3 G34 mutation (nearly all cases), and anaplastic astrocytoma with piloid features.[26,88–93] Consistent with their associations with glial lineage, there is a strong inverse relationship among IDH-mutant DGs between ATRX loss and the presence of 1p/19q codeletion (ie, molecularly defined oligodendrogliomas).[49,50,72,76,94] Only rare examples of "dual genotype" IDH-mutant DGs have been described in which ATRX loss, *TP53* mutation, and 1p/19q codeletion were identified in the same neoplasm.[95–97] 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.[98] By the same token, ATRX testing is not considered necessary for IDH-mutant DGs with established codeletion of chromosome 1p/19q.

Beyond these basic testing recommendations, a few practical issues are worth highlighting. First, the ATRX immunostain can be technically challenging and appropriate positive and negative controls are therefore critical for accurate assessment. Internal positive controls, such as entrapped neurons, nonneoplastic glia, and endothelial cells, should show retained expression within the same regions where tumor nuclei are immunonegative. If all nuclei (including nonneoplastic cells) are immunonegative, this should be interpreted as a technical failure of the immunostain rather than true loss of expression in the tumor. A mosaic reactivity pattern, wherein some tumor nuclei appear positive and others negative, often represents a technical failure as well and as such, should not be interpreted as loss of expression within just a subset of tumor cells. Regardless, in the case of equivocal immunostaining or in diagnostic practices that do not initially screen for ATRX inactivation using IHC, *ATRX* gene alterations can be identified instead using other molecular techniques (eg, NGS). Second, because only 70% to 80% of IDH-mutant astrocytomas (WHO grades 2–4) show *ATRX* inactivation, retained ATRX immunoexpression does not exclude the possibility of this diagnosis. Infratentorial IDH-mutant astrocytomas show loss of ATRX in only approximately 50% of cases.[58] For cases suspected to be IDH-mutant astrocytoma based on morphology, but showing retention of ATRX on IHC, evidence of astrocytic lineage should depend instead on other findings, such as p53 protein overexpression, *TP53* gene mutation, or a lack of 1p/19q codeletion. Last, ATRX loss may also be encountered in rare IDH-WT GBMs of older adults and in several pediatric/young adult IDH-WT astrocytoma subtypes, as already discussed. As such, ATRX loss is not entirely specific for IDH-mutant astrocytomas and this finding should not be interpreted in isolation, but rather in the context of other alterations, including IDH mutation. DNA methylation profiling has rapidly emerged as a platform that is capable of reproducibly identifying IDH-mutant astrocytomas, as well as IDH-mutant subtypes that are infratentorial, supratentorial low grade and supratentorial high grade. Although the technology is not widely available at this time, methylation profiling may be useful in diagnostically challenging cases and will likely emerge as an important diagnostic platform.[12,58]

Public Comment Response to Recommendation 2.—There were 92 respondents, of whom 78 (84.8%) agreed or agreed with modification, 12 (13.1%) disagreed, and 2 (2.2%) were neutral. There were 37 written comments, including many requesting clarification of recommended testing methodologies and a specific algorithm outlining the recommended order of testing, including the relation of ATRX to 1p/19q, p53, histone H3, or *TERT* promoter analyses. One respondent commented that ATRX status is not needed if 1p/19q is done routinely, whereas another stated that 1p/19q results can be misleading, and as such, ATRX data provide useful quality assurance. Although some of these issues are beyond the scope of this paper, a few practical guidelines for testing were nonetheless discussed in the prior paragraph. A few respondents also commented that they did not believe ATRX testing was sufficiently cost effective or diagnostically informative to warrant its routine use, whereas a few felt that Recommendation 2 did not go far enough, because ATRX testing could be potentially useful for all gliomas, including nondiffuse and pediatric astrocytoma variants. These useful comments were taken into consideration and while the final recommendation remained the same, some of these specific concerns were addressed in the text above.

Recommendation 3

Among IDH-mutant DGs, *TP53* mutation and p53 overexpression are associated with astrocytic lineage and test results for these biomarkers are used to support the diagnosis of IDH-mutant astrocytomas, especially in the setting of ATRX loss or mutation.[22,49,75,78,82,85,99] IDH-mutant gliomas that have whole-arm 1p/19q codeletion (ie, oligodendroglioma, IDH-mutant, 1p/19q codeleted) only rarely (2%–3%) exhibit *TP53* mutation or strong p53 overexpression.[19,42,71,83]

[††] *References 24–26, 49, 50, 70, 71, 73–75.*

Table 4. Representative Studies Reporting on ATRX, *TP53*, and 1p/19q Status Across Diffuse Glioma (DG) Subtypes

Study, Study Design	Number of DG Cases	Marker	Testing Method	Mutation Frequency[a]	Study Conclusion
Brat et al,[6] 2015, GSS	n = 293	1p/19q, IDH, *TP53*, *ATRX*	Genome sequencing	A: IDH + 1p/19q, 2/95 (2.1%) OA: IDH + 1p/19q, 13/74 (17.6%) OD: IDH + 1p/19q, 69/109 (63.3%) GBM: NR	Genome-wide data delineated 3 molecular classes of histologic grade 2/3 DG that were more concordant with IDH, 1p/19q, *ATRX*, and *TP53* status than with histologic class and correlated with clinical outcome
McClendon et al,[22] 2008, GSS	n = 91	*TP53*	Genome sequencing	A: NR OA: NR OD: NR GBM: NR	Highlighted core molecular pathways consistently altered in GBM, including p53, RTK, and RB networks
Cai et al,[70] 2014, RCS	n = 169	ATRX	Sequencing	A: ATRX, 42/68 (61.8%) OA: NR OD: NR GBM: ATRX, 42/101 (41.6%)	ATRX expression is tightly correlated with IDH1/2 mutation and can be used to define prognostic DG subgroups
Rajmohan et al,[74] 2016, RCS	n = 91	ATRX, IDH, 1p/19q	Sequencing, IHC, FISH	A: ATRX, 15/18 (83.3%); 1p/19q, 1/18 (5.6%) OA: ATRX, 8/33 (24.2%); 1p/19q, 19/33 (57.6%) OD: ATRX, 4/40 (10.0%); 1p/19q, 28/40 (70.0%) GBM: NR	Histologic grade 3 DG can be stratified into prognostic groups based on IDH, 1p/19q, and ATRX status
Mukasa et al,[42] 2012, RCS	n = 250	*TP53*, 1p/19q, IDH	Sequencing	A: 1p/19q, 4/58 (6.9%); TP53, 23/58 (39.7%) OA: 1p/19q, 1/12 (8.3%); *TP53*, 5/12 (41.7%) OD: 1p/19q, 29/40 (72.5%); TP53, 3/40 (7.5%) GBM: 1p/19q, 1/122 (0.8%); TP53, 25/122 (20.5%)	IDH mutation in DG was tightly correlated with 1p/19q codeletion and the combination was associated with prolonged survival
Sahm et al,[82] 2014, RCS	n = 43	IDH, ATRX, *TP53*, 1p/19q	Sequencing, IHC	A: NR OA: NR OD: NR GBM: NR	Histologically defined oligoastrocytomas can be reclassified as astrocytoma or oligodendroglioma based on IDH, *TP53*, ATRX, and 1p/19q
Eckel-Passow et al,[4] 2015, RCS	n = 615	IDH, *TERT*, 1p/19q	Sequencing	A: NR OA: NR OD: NR GBM: NR	DG can be classified into 5 clinically relevant groups based on IDH, *TERTp*, and 1p/19q
Shao et al,[75] 2016, RCS	n = 135	ATRX, *TP53*, 1p/19q, IDH	IHC	A: ATRX, 20/61 (32.8%); *TP53*, 29/57 (50.9%); 1p/19q, 4/25 (16.0%) OA: NR OD: NR GBM: ATRX, 2/10 (20.0%); *TP53*, 6/11 (54.5%); 1p/19q, 0/4 (0.0%)	DG with IDH mutations and ATRX loss also had p53 overexpression and MGMTp methylation and were mutually exclusive with 1p/19 codeletion
Cryan et al,[71] 2014, RCS	n = 108	ATRX, *TP53*	IHC, NGS	A: ATRX, 15/28 (53.6%); *TP53*, 20/28 (71.4%) OA: ATRX, 9/15 (60.0%); *TP53*, 12/15 (80.0%) OD: ATRX, 2/65 (3.1%); *TP53*, 4/65 (6.2%) GBM: NR	Oligodendroglioma and astrocytoma can be distinguished by their IDH, 1p/19q, *ATRX*, and *TP53* profile. Histologic oligoastrocytomas harbored *TP53* and *ATRX* mutations at frequencies like astrocytomas
Ebrahimi et al,[26] 2016, RCS	n = 1064	ATRX, IDH	IHC	A: ATRX, 136/284 (47.9%) OA: ATRX, 23/71 (32.4%) OD: ATRX, 1/83 (1.2%) GBM: ATRX, 27/364 (7.4%)	ATRX is a potential marker for predicting IDH/*H3F3A* mutations and substratification of DG into prognostic groups

Table 4. Continued

Study, Study Design	Number of DG Cases	Marker	Testing Method	Mutation Frequency[a]	Study Conclusion
Ikemura et al,[73] 2016, RCS	n = 193	ATRX, IDH, *TP53*, 1p/19q	IHC	A: ATRX, 24/44 (54.5%); *TP53*, 13/32 (40.6%); 1p/19q, 3/30 (10.0%) OA: ATRX, 4/13 (30.8%); *TP53*, 2/9 (22.2%); 1p/19q, 7/11 (63.6%) OD: ATRX, 0/18 (0.0%); TP53, 0/15 (0.0%); 1p/19q, 17/18 (94.4%) GBM: ATRX, 15/118 (12.7%); *TP53*, 18/82 (22.0%); 1p/19q, 3/85 (3.5%)	In grade 2/3 DG, ATRX loss was strongly correlated with IDH mutation and p53 overexpression, while mutually exclusive of 1p/19q codeletion
Wiestler et al,[76] 2013, RCS	n = 133	ATRX, 1p/19q	IHC	A: ATRX, 44.62% (29/65) OA: ATRX, 27.08% (13/48) OD: ATRX, 20.00% (2/10) GBM: NR	Among patients with anaplastic DG, ATRX loss strongly correlated with IDH mutations, anticorrelated with 1p/19q codeletion, and provided prognostic value
Hewer et al,[72] 2016, RCS	n = 54	ATRX, IDH, 1p/19q	IHC	A: NR OA: NR OD: NR GBM: NR	Histologic oligoastrocytomas that had IDH mutations showed either ATRX loss or 1p/19q LOH, but not both alterations
Bienkowski et al,[49] 2018, RCS	n = 165	ATRX, IDH, *TP53*, 1p/19q	IHC, PCR, MLPA	A: ATRX, 24/78 (30.8%); *TP53*, 38/80 (47.5%); 1p/19q, 1/68 (1.5%) OA: ATRX, 25/53 (47.2%); *TP53*, 30/53 (56.6%); 1p/19q, 17/53 (32.1%) OD: ATRX, 3/31 (9.7%); *TP53*, 9/31 (29.0%); 1p/19q, 18/29 (62.1%) GBM: NR	For assessing IDH, 1p/19q, and *TERTp* status in DG, a combination of IHC, direct sequencing, and MLPA is a practical approach
Alentorn et al,[19] 2014, RCS	n = 126	*TP53*	IHC	A: *TP53*, 7/20 (35.0%) OA: *TP53*, 19/49 (38.8%) OD: *TP53*, 8/51 (15.7%) GBM: NR	Among IDH-mutant DG, p53 overexpression is mutually exclusive with 1p/19q codeletion
Wang et al,[83] 2016, RCS	n = 670	*TP53*, IDH	IHC	A: *TP53*, 42/65 (64.6%) OA: *TP53*, 85/137 (62.0%) OD: *TP53*, 11/80 (13.8%) GBM: *TP53*, 57/300 (1.9%)	IDH mutation was strongly correlated with high p53 expression among histologically defined astrocytic neoplasms
Rajeswarie et al,[50] 2018, RCS	n = 449	IDH, ATRX, 1p/19q	IHC, FISH	A: NR OA: NR OD: NR GBM: NR	In a resource-limited set up, histology with IHC for IDH1-R132H and ATRX form the baseline testing to derive DG subgroups
Dubbink et al,[25] 2016, RCS	n = 133	ATRX, 1p/19q, IDH	NGS	A, 1p/19q intact: ATRX, 65.00% (13/20); *TP53*, 95.00% (19/20) OA: NR OD, 1p/19q codeletion: ATRX, 2.04% (1/49); *TP53*, 8.16% (4/49) GBM: ATRX, 0.00% (0/55); *TP53*, 20.00% (11/55)	DG can be subclassified into prognostic groups based on the molecular status of IDH, 1p/19q, *TERTp*, 7+/10q−, and *H3F3A*
Chaurasia et al,[24] 2016	n = 163	*TP53*	NR	A: NR OA: NR OD: NR GBM: ATRX, 15.34% (25/163); *TP53*, 49.08% (80/163)	IHC for ATRX, IDH1, and p53 can be used to stratify GBM patients as individual markers and in combination

Abbreviations: 7+/10q−, chromosome 7 gain/chromosome 10q loss; A, astrocytoma histology; ATRX, ATRX chromatin remodeler; FISH, fluorescence in situ hybridization; GBM, glioblastoma; GSS, genome sequencing study; H3, histone 3; *IDH1*, isocitrate dehydrogenase (NADP(+))1; *IDH2*, isocitrate dehydrogenase (NADP(+))2; IHC, immunohistochemistry; LOH, loss of heterozygosity; *MGMTp*, O-6-methylguanine-DNA methlytransferase; MLPA, multiplex ligation-dependent probe amplification; NGS, next-generation sequencing; NR, not reported; OA, oligoastrocytoma histology; OD, oligodendroglioma histology; PCR, polymerase chain reaction; RB, radial basis; RCS, retrospective cohort study; RTK, receptor tyrosine kinase; *TERTp*, telomerase reverse transcriptase promoter; *TP53*, tumor protein p53.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

In one large series, *TP53* mutations were noted in 94% of IDH-mutant DGs that did not demonstrate 1p/19q codeletion.[6] Thus, 1p/19q codeletion and *TP53* mutations are nearly, but not entirely, mutually exclusive in IDH-mutant DGs. In IDH-mutant DGs with *TP53* mutations, the 17p-arm typically exhibits copy neutral loss of heterozygosity (cnLOH); thus, there are generally 2 identical copies of the mutant *TP53* gene.[4–6,65,100] In a small percentage of IDH-mutant astrocytomas there are 2 distinct *TP53* mutations; 1 involves each of the *TP53* alleles on the two 17p arms.[4–6,65,100] *TP53* mutations that occur in IDH-mutant astrocytomas tend to activate the expression and/or enhance the stability of the p53 protein. Thus, p53 IHC is an excellent surrogate of *TP53* mutation in IDH-mutant gliomas, especially if the expression of ATRX is not retained.[101,102] Unlike IHC for ATRX, however, p53 expression is more of a continuum, and the interpretation may not be straightforward. For higher sensitivity and specificity, more than 10% of tumor cells must show strong nuclear positivity, although most *TP53*-mutant gliomas show even more widespread (>50%) p53 expression.[102] Using this threshold, only a small subset of DGs with strong p53 immunoreactivity in more than 10% of nuclei were found to be *TP53* WT by sequencing.[102] If tumor DNA sequencing is performed, the combination of IDH, *TP53* and *ATRX* mutations and the associated copy number patterns are distinctive for IDH-mutant astrocytomas.[4–6,65,100] It should be noted that *TP53* mutation is also encountered in other DGs, especially IDH-WT GBMs.[24,29,41,42,86,103] In IDH-WT gliomas, one *TP53* gene typically undergoes mutation, and the WT *TP53* gene is lost through deletion. In IDH-WT gliomas, *TP53* mutation less predictively generates p53 overexpression.[75,83,104] Nevertheless, p53 overexpression is encountered in up to 30% of IDH-WT GBM, so this finding should not be used as support for IDH-mutant status in isolation.

Public Comment Response to Recommendation 3.—There were 87 respondents, of whom 64 (73.6%) agreed or agreed with modifications, 16 (18.4%) disagreed, and 7 (8.0%) were neutral. There were 35 written comments received during the public comment period. These comments could be classified as follows: (1) the word "should" could be replaced with "must" or "may." The "should" language of the recommendation was retained based on the strength of the evidence in the literature. (2) The method of *TP53* mutation assessment should be addressed. As described in the text above, IHC can be used to assess the expression of p53 protein in the context of IDH-mutant gliomas based on its correlation with *TP53* mutation; sequencing methods can also be employed. (3) Some commented that *TP53* status should be evaluated in all IDH-mutant DGs. Based on the evidence, it was concluded that this is not necessary if whole-arm 1p/19q codeletion is known to be present. All comments were taken into consideration and the recommendation remained as initially stated.

Recommendation 4

Confirmation of whole-arm 1p/19q codeletion in an IDH-mutant DG is essential to render the diagnosis of oligodendroglioma, IDH-mutant and 1p/19q codeleted. Landmark genomic analyses have firmly established that whole-arm 1p/19q codeletion, the product of an unbalanced translocation involving chromosomes 1 and 19, is entirely restricted to gliomas that also harbor mutations in either *IDH1* or *IDH2*.[4,6] Therefore, assessment of 1p/19q codeletion status is only relevant in IDH-mutant DGs. 1p/19q testing is not recommended in IDH-WT gliomas, as false-positive test results (see below) may complicate effective diagnostic categorization and the results have no bearing on patient outcome or treatment.[105,106]

As described above, 1p/19q codeletion arises with near mutual exclusivity with respect to inactivating alterations of *TP53* and *ATRX*.[4,6] Multiple studies have concluded that loss of nuclear ATRX expression by IHC is strongly predictive of a 1p/19q noncodeleted status.[74,76,82] Confirmed *TP53* mutation or p53 overexpression is similarly inversely associated with 1p/19q codeletion.[74,75] Loss of ATRX expression or *TP53* mutation has been noted in a small minority of IDH-mutant gliomas that also exhibit 1p/19q codeletion (2%–8%).[4,6,74,76,82] The extent to which these cases reflect false-positive testing for 1p/19q codeletion is unclear. Of note, The Cancer Genome Atlas analysis, which employed cytogenomic arrays to confirm whole-arm 1p/19q codeletion, revealed low rates of coinciding *ATRX* or *TP53* mutations (2%–3%),[6] suggesting that under strict testing criteria, the mutual exclusivity between *ATRX/TP53* mutations and 1p/19q codeletion is near absolute. Nevertheless, the small degree of overlap between these biomarkers may justify 1p/19q codeletion assessment in some IDH-mutant gliomas with established ATRX loss or *TP53* mutation/p53 overexpression if oligodendroglioma is suspected based on histopathologic features. As mentioned above, rare cases of carefully documented "dual genotype" IDH-mutant DGs have demonstrated the co-occurrence of ATRX loss, *TP53* mutation, and whole-arm 1p/19q codeletion.[95–97] Nonetheless, for most cases, 1p/19q testing is no longer needed if an IDH-mutant DG demonstrates ATRX loss and *TP53* mutation, as highlighted in cIMPACT-NOW update 2.[98]

1p/19q codeletion is optimally assessed by cytogenomic array or NGS methodologies capable of definitively demonstrating deletion of whole chromosomal arms of 1p and 19q.[35,107] Other approaches, such as fluorescence in situ hybridization (FISH) and PCR-based testing, have also been effectively applied to 1p/19q analysis[4,74,76,82] and have lower cost and quicker turnaround time than their genomic counterparts. DNA methylation profiling can identify a methylome highly characteristic of oligodendroglioma and has the added benefit of providing copy number alterations that can directly inform the status of 1p/19q codeletion.[12]

FISH is the most widely used test and has the advantage of assessing a limited sample of tissue (2 slides; a minimum of 50 tumor nuclei required per slide), which is often the case in small biopsy samples. One must consider the possibility of false-positive results, attributable to partial arm deletions that are detected by FISH, yet do not represent whole-arm deletions associated with the unbalanced translocation. False-positive test results on 1p/19q testing by FISH are also noted in IDH-WT GBMs, because they are genomically unstable,[105,106] underlying our recommendation that 1p/19q assessment be exclusively restricted to IDH-mutant gliomas.

Public Comment Response to Recommendation 4.—There were 89 respondents, of whom 76 (85.4%) agreed or agreed with modifications, 10 (11.2%) disagreed, and 3 (3.7%) were neutral. Thirty comments were received. One comment suggested that the term "codeletional status" used in the prior version of the recommendation be removed and replaced with "codeletion" for simplicity. The text was modified in response to this recommendation. Multiple comments suggested that 1p/19q testing be performed more

broadly, on all DGs or on all IDH-mutant DGs. The panel did not reach these conclusions based on review of the evidence, and the detailed reasoning is explained in the preceding paragraph. A related series of comments was made regarding the importance of p53 IHC or *TP53* mutational analysis in the classification of DGs. One suggested using the phrase "ATRX loss with or without *TP53* mutations" instead of "ATRX loss or *TP53* mutations." While it was acknowledged that IHC for p53 has limitations, and that *TP53* mutations may rarely co-occur with 1p/19q codeletion in IDH-mutant gliomas (see above), the evidence suggests that *TP53* mutations are one of the defining genetic characteristics of IDH-mutant astrocytoma (see preceding sections) and its inclusion is justified in the diagnostic workup of IDH-mutant DGs. Multiple comments argued for the formal incorporation of histopathological criteria in addition to or in lieu of biomarkers for the classification of oligodendroglioma. Based on our review of the evidence, the designation of glioma subclasses based on IDH mutation and 1p/19q codeletion is more robust and clinically meaningful than histopathology alone.[4,6,74,76,82] There is also considerable evidence showing that the interpretation of histopathologic features varies considerably, even among experienced pathologists. Multiple comments suggested that pediatric gliomas be explicitly excluded from this recommendation. The sentiment underlying this suggestion is correct, given that oligodendroglioma, IDH-mutant and 1p/19q codeleted is rare in children. However, because IDH mutations occur predominantly in adults and our recommendation for 1p/19q codeletion assessment is only for IDH-mutant DGs, testing in pediatric gliomas is essentially ruled out in most cases. Finally, multiple comments urged specific recommendations regarding the most appropriate testing modalities for 1p/19q assessment. This point was addressed in the preceding paragraphs.

Conditional Recommendation: 5

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

The quality of evidence to support this recommendation was assessed as *moderate*.

Quality Summary.—The evidence base informing this recommendation comprises 2 prospective cohort studies[77,78] and 9 retrospective cohort studies.[41,84,85,99,108–112] The included studies were assessed as intermediate,[77] intermediate-low,[78] low,[41,84,85,99,110–112] and very low quality[108,109] based on risk of bias in selection,[‡‡] performance,[78,99,108,112] detection,[§§] and reporting[‖‖] domains. In addition, 2 of the studies reported statistical analyses that were underpowered,[109,112] and 1 did not report on sources of funding.[109] Although the aggregate risk of bias across the evidence base was very serious, the evidence was upgraded based on a strong association between *CDKN2A/B* deletion and diffuse glioma WHO classification. Refer to the SDC for the quality assessment of included retrospective cohort studies and the GRADE Quality of Evidence Assessment. See Table 5 for a summary of the mutational status of *CDKN2A/B* across all DG subtypes.

CDKN2A lies adjacent to *CDKN2B* in a region of chromosome 9 that is frequently mutated and deleted in a wide variety of human cancers. *CDKN2A/B* deletion has been shown by multiple investigations to be an adverse prognostic factor in IDH-mutant astrocytomas.[¶¶] Subsequent analyses of multiple cohorts of IDH-mutant astrocytomas showed that homozygous deletion of *CDKN2A/B* was the most relevant adverse prognostic indicator.[110] Patients with histologic grade 3 IDH-mutant astrocytomas that harbored homozygous deletion of *CDKN2A/B* had shorter survivals than patients with histologic grade 4 tumors that did not have *CDKN2A/B* homozygous deletion. Because homozygous deletion of *CDKN2A/B* can be observed in other types of primary brain tumors that have highly variable clinical outcomes, including IDH-WT GBM, pleomorphic xanthoastrocytoma (PXA), and anaplastic astrocytoma with piloid features, this recommendation pertains specifically to IDH-mutant astrocytomas.

In addition to studies captured in our systematic review, other studies[1,113] have shown similar associations between *CDKN2A/B* homozygous deletion and shorter survival among patients with IDH-mutant astrocytomas. Such findings culminated in the cIMPACT-NOW recommendation that *CDKN2A/B* homozygous deletion could be used as a grade 4 criterion in a histologic grade 2 or 3 IDH-mutant astrocytoma[11] and led to its inclusion as a grade 4 criterion within the WHO, 5th edition.[7] While not a part of the current recommendations, *CDKN2A/B* homozygous deletion has also been shown to be a marker of poor prognosis in oligodendroglioma, IDH-mutant and 1p/19q codeleted, which may be relevant to future recommendations or grading schemes for these tumors.[111]

Public Comment Response to Recommendation 5.—Of 86 total respondents to this recommendation, 66 (76.7%) agreed or agreed with modification, 8 (9.3%) disagreed, and 12 (14.0%) were neutral. Twenty-three written comments were received. Some respondents expressed a desire to strengthen the language of the recommendation from "may be performed" to "should be performed." In response to this suggestion, the recommendation was strengthened to "should be performed" based on review of the evidence. Others requested advice regarding methods, desired reconciliation with subsequent cIMPACT-NOW 5 statements, or underscored the importance of the distinction between heterozygous and homozygous loss of *CDKN2A/B*. The evidence suggests that only homozygous deletion of *CDKN2A/B* is strongly and reproducibly associated with decreased survival that is independent of tumor grade. These comments were taken into consideration and specific concerns were addressed and clarified in the preceding paragraphs.

Strong Recommendation: 6

MGMT promoter methylation testing should be performed on all GBM IDH-WT. The quality of evidence to support this recommendation was assessed as *moderate*.

Quality Summary.—This recommendation was informed by 2 meta-analyses,[114,115] 3 genome sequencing studies,[5,21,22] and 5 retrospective cohort studies.[86,108,116–118] The included meta-analyses were assessed as high[115] and high-intermediate[114] quality. Neither of these 2 studies reported on using publication status as a study selection inclusion criteria,[114,115]

[‡‡] *References 41, 77, 78, 84, 85, 99, 108–112.*
[§§] *References 41, 77, 84, 85, 108–110, 112.*
[‖‖] *References 41, 77, 78, 84, 85, 99, 108–112.*

[¶¶] *References 41, 85, 99, 108, 109, 111, 112.*

Table 5. Representative Studies Reporting on *CDKN2A* Status Across Diffuse Glioma (DG) Subtypes

Study, Study Design	Number of DG Cases	Testing Method	*CDKN2A* Mutation Frequency[a]	Study Conclusion
Reis et al,[99] 2015, RCS	n = 270	FISH	A: loss, 48.15% (52/108) OA: loss, 38.78% (19/49) OD: loss, 23.96% (23/96) GBM: NR	*CDKN2A* loss was associated with shorter survival among grade 2/3 IDH-mutant DGs that had *TP53* mutations and ATRX loss
Purkait et al,[109] 2013, RCS	n = 67	FISH	A: NR OA: NR OD: NR GBM: loss, 43.1%[b]	*CDKN2A* deletion was observed GBMs and showed strong correlation with loss of p16 expression by IHC
Collins et al,[108] 2014, RCS	n = 267	CGH array	A: hemizygous loss, 30.56% (11/36); homozygous loss, 2.78% (1/36) OA: hemizygous loss, 33.33% (2/6); homozygous loss, 0.00% (0/6) OD: NR GBM: hemizygous loss, 29.78% (67/225); homozygous loss, 31.56% (71/225)	Homozygous deletion of *CDKN2A* was seen at greater frequency in DGs of higher histologic grade, especially GBM
Molenaar et al,[41] 2014, RCS	n = 98	MLPA	A: NR OA: NR OD: NR GBM: hemizygous loss, 27.6%; homozygous loss, 43.9%	*CDKN2A* alteration was not associated with survival in patients with histologically diagnosed GBM
Shirahata et al,[110] 2018, RCS	n = 211	450k array	A: NR OA: NR OD: NR GBM: NR	*CDKN2A/B* homozygous deletion significantly associated with overall survival in *IDH* mutant astrocytomas
Yang et al,[85] 2020, RCS	n = 160	FISH	A: 15.00% (24/160) OA: NR OD: NR GBM: NR	*CDKN2A* deletion can be used to risk stratify grade 2/3 astrocytomas

Abbreviations: A, astrocytoma histology; ATRX, ATRX chromatin remodeler; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDKN2B*, cyclin-dependent kinase inhibitor 2B; CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization; GBM, glioblastoma; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; MLPA, multiplex ligation-dependent probe amplification; NR, not reported; OA, oligoastrocytoma histology; OD, oligodendroglioma histology; RCS, retrospective cohort study; *TP53*, tumor protein p53.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

[b] Study did not report raw values for *CDKN2A* loss in primary GBMs.

and 1 did not report on conflict of interest or sources of funding.[114] The genome sequencing studies were all assessed as intermediate-low quality based on retrospective acquisition of samples in all of them,[5,21,22] plus individual moderate risk of reporting[21] and detection bias.[5,21] Finally, the retrospective cohort studies were assessed as low[86,116,117] and very low quality[108,118] based on risk of bias. The aggregate risk of bias of the evidence base was serious and the evidence was not further upgraded or downgraded for any domain. Refer to the SDC for the quality assessment of genome sequencing studies, retrospective cohort studies, meta-analyses, and the GRADE Quality of Evidence Assessment. See Table 6 for a summary of the promoter methylation status of *MGMT* in GBMs.

The MGMT 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 DGs. Randomized studies have shown that the clinical benefit of adding temozolomide to radiotherapy is predominantly among patients with *MGMT* promoter methylated GBM ("predictive marker").[119,120] Temozolomide monotherapy in newly diagnosed GBM is only effective in patients with tumors that have *MGMT* promoter methylation.[121,122] In GBM patients treated with combined temozolomide chemoirradiation, *MGMT* promoter methylation is an established prognostic parameter. In a large randomized trial on temozolomide dose intensification, *MGMT* promoter methylation was associated with improved overall survival (21.2 versus 14 months; hazard ratio, 1.74; $P < .001$).[123] Clinical benefit of treatment with temozolomide or lomustine at the time of tumor progression after initial therapy is particularly observed in patients with methylated *MGMT* promoter at the time of first diagnosis.[124–126] Assessment of *MGMT* promoter methylation can be useful for making treatment decisions for patients when either radiotherapy or chemotherapy is considered contraindicated, and in patients with recurrent tumors for whom second line alkylating chemotherapy is unlikely to be successful.

The expression of the MGMT protein is inhibited by methylation of specific CpG sites in the *MGMT* promoter and laboratory testing is therefore focused on these regions.[116,127] Despite the overall correlation between *MGMT* promoter methylation and clinical outcome, test characteristics remain suboptimal. *MGMT* promoter methylation results are affected by the choice of CpG islands and

Table 6. Representative Studies Reporting on *MGMT* Promoter Methylation in Glioblastomas (GBMs)				
Study, Study Design	**Number of GBM Cases**	**Testing Method**	**Promoter Methylation Frequency[a]**	**Study Conclusion**
Houdova Megova et al,[31] 2017, RCS	n = 145	MSP	IDH-WT GBM: 47.78% (43/90) IDHmut GBM: 87.50% (7/8)	*IDH1* mutations are closely associated with *MGMTp* methylation in DGs. *IDH1* mutations in GBMs were a stronger marker of overall patient survival than MGMTp methylation
Lee et al,[36] 2017, RCS	n = 65	Sequencing, MSP	IDH-WT GBM: 50.00% (26/52) IDHmut GBM: 76.92% (10/13)	*MGMTp* methylation was much more common in IDH-mutant DGs than in IDH-WT DGs
Mulholland et al,[143] 2012, RCS	n = 172	Pyrosequencing, MSP, RT-PCR	GBM: 52.33% (90/172)	Nearly all IDH-mutant DGs (127/129) showed *MGMTp* methylation by quantitative analysis of CpG sites following pyrosequencing
Bady et al,[116] 2012, RCS	n = 174	BeadChip array	GBM: NR	Two sites within *MGMTp* were critical for gene silencing. A strong link was noted between *MGMTp* methylation and G-CIMP status

Abbreviations: DGs, diffuse gliomas; G-CIMP, Glioma-CpG island methylator phenotype; *IDH1*, isocitrate dehydrogenase (NADP(+))1; *MGMT*, O-6-methylguanine-DNA methyltransferase; *MGMTp*, O-6-methylguanine-DNA methyltransferase; MSP, methylation-specific polymerase chain reaction; mut, mutation; NR, not reported; RCS, retrospective cohort study; RT-PCR, real-time polymerase chain reaction; WT, wild-type.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

promoter regions that are tested, as well as the platform employed. Techniques used to assess *MGMT* promoter methylation status include qualitative or quantitative methylation-specific PCR,[128] pyrosequencing,[129] multiplex ligation-dependent probe amplification,[130] genome-wide methylation analysis,[116] and IHC.[131] With any of these available assays, the determination of the optimal technical cut off between methylated and unmethylated remains a challenge.[132] In 1 study that compared 5 techniques for measuring *MGMT* promoter methylation on a series of 100 GBMs, the percentage of tumors with promoter methylation varied between 30% and 60%, a difference that was attributed to both variations between techniques and between laboratories.[123,133,134]

A major obstacle for current studies on the interpretation and optimization of these techniques is the lack of randomized trials with an untreated control arm that would allow assessment of the predictive value of *MGMT* promoter methylation. Current studies therefore assess the prognostic significance of various *MGMT* promoter methylation tests in GBM patients being treated with chemoirradiation with temozolomide[134–136] or the concordance of results between various techniques.[129] Individual detection techniques show only modest correlation when compared with one another.[137,138] MGMT protein expression by IHC was found unreliable in 1 series due to large interobserver variation, yet another study concluded that IHC results correlated with methylation-specific PCR.[131,139,140] Also, in some studies *MGMT* promoter status was found to vary between paired samples obtained at the time of first diagnosis and progression.[141] With a quantitative methylation-specific PCR technique, analysis within cohorts has revealed a clinically relevant gray zone between *MGMT* promoter clearly methylated and unmethylated tumors. Establishing an appropriate threshold within this test gray zone has substantial implications for patients in clinical trials in which temozolomide is excluded from therapy based on *MGMT* methylation status.[142]

Public Comment Response to Recommendation 6.—Of 86 respondents, 66 (76.7%) agreed or agreed with modifications, 8 (9.3%) disagreed, and 12 (14.0%) were neutral. Twenty-five comments were received. Comments emphasized that patients with GBM that had an unmethylated *MGMT* promoter may also benefit from the addition of temozolomide to radiotherapy, assumed the impact was mainly prognostic and stated that the need for testing depended on clinical decision-making and treatment planning. While most of the statement remained unchanged, the panel clarified that the recommendation applies to all GBM IDH-WT.

Conditional Recommendation: 7

For IDH-mutant DGs, *MGMT* promoter methylation testing may not be necessary. The quality of evidence to support this recommendation was assessed as *low*.

Quality Summary.—The evidence base informing this recommendation comprises 1 genome sequencing study[5] and 4 low-quality retrospective cohort studies.[31,36,40,143] The genome sequencing study[5] was assessed as intermediate-low quality based on a serious risk of selection bias and a moderate risk of detection bias. All retrospective cohort studies were limited by critical risk of selection bias.[31,36,40,143] Individual studies were further limited by risk of bias in performance,[31,40] reporting,[31,36,40,143] and detection domains,[36,143] as well as underpowered statistical analyses in 2,[31,143] and a lack of reporting funding sources in 1.[40] The aggregate risk of bias across these studies was very serious but evidence was not further downgraded for any domain. Refer to the SDC for the quality assessment of included genome sequencing studies, retrospective cohort studies, and the GRADE Quality of Evidence Assessment.

In the section above, data supporting *MGMT* promoter methylation testing in GBM, IDH-WT were presented. The clinical relevance of testing for *MGMT* promoter methylation in IDH-mutant DGs is not as firmly established. IDH mutations lead directly to global DNA hypermethylation and the establishment of the CpG island methylator phenotype, as demonstrated experimentally and in numerous investigations of clinical specimens.[5,144,145] Therefore,

Table 7. Representative Studies Reporting on *TERT* Promoter, *EGFR*, Chromosome 7, and Chromosome 10 Status Across Diffuse Glioma (DG) Subtypes

Study, Design	Number of DG Cases	Marker(s)	Testing Method	Mutation Frequency[a]	Study Conclusion
McLendon et al,[22] 2008, GSS	n = 91	*EGFR*	Genome sequencing	A: NR OA: NR OD: NR GBM: NR	Highlighted core molecular pathways consistently altered in GBM, including p53, RTK, and RB networks
Brat et al,[6] 2015, GSS	n = 293	IDH, *TP53*, ATRX, 1p/19q, EGFR	Genome sequencing	A: NR OA: NR OD: NR GBM: NR	IDH-WT lower grade DG had genomic alterations and clinical behavior like IDH-WT GBM
Brennan et al,[21] 2013, GSS	n = 543	Chr 7, Chr 10, *TERTp*, *EGFR*	Genome sequencing	A: NR OA: NR OD: NR GBM: *TP53*, 27.9%[b]; *TERT*, 84.0%[b]	*EGFR* amp, *TERTp* mutation, Chr 7 gains, and Chr 10 losses were frequent events in IDH-WT GBM
Chan et al,[23] 2015, RCS	n = 237	*TERT*	Sequencing	A: *TERT*, 19.05% (32/168) OA: *TERT*, 39.58% (19/48) OD: *TERT*, 76.19% (16/21) GBM: NR	Among histologic grade 2/3 IDH-WT DG, *TERTp* mutation was associated with short survival, like IDH-WT GBM
Gao et al,[150] 2016, RCS	n = 389	*TERT*	Sequencing, pyro-sequencing	A: *TERT*, 26.72% (66/247) OA: *TERT*, 41.30% (19/46) OD: *TERT*, 22.73% (10/44) GBM: *TERT*, 32.69% (17/52)	*TERTp* mutations are prognostic factors among DG and inversely associated with ATRX inactivation
Chan et al,[160] 2015, RCS	n = 214	*TERT*, *EGFR*	Sequencing, FISH	A: NR OA: NR OD: NR GBM: NR	Among patients with grade 2/3 IDH-WT DG, *EGFR* amp and *TERTp* mutation were associated with shorter survival, like IDH-WT GBM
Eckel-Passow et al,[4] 2015, RCS	n = 615	*TERT*	Sequencing	A: NR OA: NR OD: NR GBM: NR	DG classified based on IDH, *TERTp*, and 1p/19q showed that those with *TERTp* mutation only had short overall survival
Labussiere et al,[151] 2014, RCS	n = 807	*TERT*	Array, PCR	A: *TERT*, 30.23% (13/43) OA: *TERT*, 31.95% (54/169) OD: *TERT*, 61.00% (122/200) GBM: *TERT*, 75.70% (299/395)	Among IDH-WT GBM, the presence of *TERTp* mutation or *EGFR* amp was associated with shorter survival
Collins et al,[108] 2014, RCS	n = 267	Chr 7, Chr 10, *EGFR*	CGH array	A: Chr 7 tri/poly, 5.56% (2/36); mono, 0.00% (0/36); Chr 10 mono, 11.11% (4/36); 10q partial del, 38.89% (14/36); *EGFR* amp, 11.11% (4/36) OA: Chr 7 tri/poly, 0.00% (0/6); mono, 0.00% (0/6); Chr 10 mono, 0.00% (0/6); 10q partial del, 33.33% (2/6); *EGFR* amp, 0.00% (0/6) OD: NR GBM: Chr 7 tri/poly, 63.56% (143/225); mono, 0.44% (1/225); Chr 10 mono, 64.89% (146/225); 10q partial del, 20.00% (45/225); *EGFR* amp, 44.44% (100/225)	Among patients with recurrent grade 3/4 astrocytoma, *EGFR* amp Chr 7 gains and Chr 10 losses were associated with shorter survival
Little et al,[162] 2012, RCS	n = 342	*EGFR*	FISH	A: *EGFR* amp, 15.38% (2/13) OA: NR OD: *EGFR* amp, 9.76% (4/41) GBM: *EGFR* amp, 44.17% (106/240)	Detailed FISH analysis of GBMs showed that 44% have *EGFR* amp, 21% have *PDGFRA* amp and 9% contain both *EGFR* amp and *PDGFRA* amp
Guillaudeau et al,[161] 2009, RCS	n = 35	*EGFR*	FISH, IHC	A: *EGFR* amp, 0% (0/8) OA: NR OD: NR GBM: *EGFR* amp, 72.73% (8/11)	*EGFR* amp was associated with increased protein expression of EGFR by IHC in DGs

Table 7. Continued

Study, Design	Number of DG Cases	Marker(s)	Testing Method	Mutation Frequency[a]	Study Conclusion
Houdova Megova et al,[31] 2017, RCS	n = 145	*EGFR*	Not reported	A: *EGFR* amp, 7.69% (2/26) OA: *EGFR* amp, 0% (0/4) OD: *EGFR* amp, 0% (0/7) GBM: *EGFR* amp, 36.56% (30/82)	*EGFR* amp and Chr 10 losses are more frequent in IDH-WT than IDH-mut DGs and are most frequent in IDH-WT GBM
Aibaidula et al,[18] 2017, RCS	n = 166	*TERT*	Patient records	A: NR OA: NR OD: NR GBM: NR	Among adults with IDH-WT grade 2/3 DGs, *EGFR* amp and mutations of *TERTp* or *H3F3A* K27M were associated with shorter survival

Abbreviations: A, astrocytoma histology; amp, amplification; ATRX, ATRX chromatin remodeler; Chr, chromosome; del, deletion; *EGFR*, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; GBM, glioblastoma; GSS, genome sequencing study; H3, histone 3; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; mono, monopoly; NR, not reported; OA, oligoastrocytoma histology; OD, oligodendroglioma histology; PCR, polymerase chain reaction; *PDGFRA*, platelet-derived growth factor receptor alpha; poly, polysomy; RCS, retrospective cohort study; TERT, telomerase reverse transcriptase; *TERTp*, TERT promoter; *TP53*, tumor protein p53; tri, trisomy; WT, wild-type.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

[b] Study did not report raw values for mutation frequency.

the prevalence of *MGMT* promoter methylation in IDH-mutant DGs is very high.[40,144,146,147]

Data supporting this recommendation from our systematic review documents the tight correlation between IDH-mutant DGs and *MGMT* promoter methylation.[31,36,40,143,148,149] Using bisulfite treatment, pyrosequencing, and focusing on 16 selected CpG sites, Mulholland et al found that 127 of 129 gliomas (98.5%) with IDH mutations were *MGMT* methylated, irrespective of pathologic group.[143] A strong association between IDH mutation and *MGMT* promoter methylation has also been demonstrated by others.[31,40] Thus, the high correlation between IDH-mutation and *MGMT* promoter methylation suggests that testing for *MGMT* promoter methylation in IDH-mutant DGs may not be necessary.

Public Comment Response to Recommendation 7.—There were 89 respondents, of whom 65 (73.0%) agreed or agreed with modifications, 15 (16.9%) disagreed, and 9 (10.1%) were neutral. There were 17 written comments, many of which stated that *MGMT* testing decisions should be left to the treating oncologist and questioned whether it is worthwhile to issue a recommendation for every test that should not be performed. We have carefully taken these comments into consideration and have kept the recommendation as is, because *MGMT* promoter methylation testing is one of the most frequently requested tests for DGs, and we did not find sufficient evidence for reflexively ordering it in the group of IDH-mutant DGs. The strong association between IDH mutation and *MGMT* promoter methylation supports a role for IDH mutation (when present) resulting in *MGMT* promoter methylation, with the limitations of predominantly retrospective evidence. The recommendation is listed as conditional, which leaves room for individual and institutional treatment decisions.

Conditional Recommendation: 8

TERT promoter mutation testing may be used to provide further support for the diagnosis of oligodendroglioma and IDH-WT GBM. The quality of evidence to support this recommendation was assessed as *low*.

Quality Summary.—This recommendation was informed by 2 genome sequencing studies[5,6] and 11 retrospective cohort studies.[##] The genome sequencing studies[5,6] were assessed as intermediate-low quality while the retrospective cohort studies were assessed as low[***] and very low quality.[4,18,34,152] Included studies suffered from risk of bias in selection,[†††] performance,[4,18,34,150] reporting,[‡‡‡] and detection[§§§] domains. The aggregate risk of bias across these studies was very serious but evidence was not further downgraded for any domain. Refer to the SDC for the quality assessment of included genome sequencing studies, retrospective cohort studies, and the GRADE Quality of Evidence Assessment. See Table 7 for a summary of the mutational status of the *TERT* promoter across all DG subtypes.

As discussed for ATRX (Recommendation 2), cancerous proliferation requires escape from cellular senescence. In oligodendrogliomas, IDH-mutant and 1p/19q codeleted, and IDH-WT GBM this predominantly occurs via point mutations in the *TERT* promoter, which leads to enhanced TERT expression. Point mutations occur in 2 hotspots located 124 base pairs (bp) and 146 bp upstream of the translation start site and are referred to as C228T and C250T.[34,151] Testing for promoter mutation is performed by DNA sequence analysis, either by Sanger methods, pyrosequencing, PCR, or targeted NGS.[25,34,84,150,154,155]

In contrast to oligodendrogliomas and IDH-WT GBM, IDH-mutant astrocytomas primarily use the alternative lengthening of telomeres pathway to escape senescence, which is driven by inactivation of *ATRX*. Of note, *TERT* promoter mutations and *ATRX* loss/mutations are mutually exclusive,[4,6,150] consistent with our understanding that these 2 genetic mechanisms confer equivalent selective growth advantages. Thus, in the setting of IDH-mutation, *TERT* promoter mutations can provide additional support for the diagnosis of oligodendroglioma, IDH-mutant and 1p/19q codeleted, WHO grade 2 or 3. It can also provide diagnostic support in cases in which immunostaining for ATRX is

[##] *References 4, 18, 23, 34, 49, 84, 150–154.*

[***] *References 23, 49, 84, 150, 151, 153, 154.*

[†††] *References 4–6, 18, 23, 34, 49, 84, 150–154.*

[‡‡‡] *References 4, 18, 23, 34, 84, 150–152, 154.*

[§§§] *References 4, 5, 23, 34, 84, 150–152, 154.*

equivocal or was not performed. By the same logic, *TERT* promoter sequencing may not be necessary in IDH-mutant gliomas with sufficient evidence of ATRX loss. Similarly, if there is laboratory evidence that clearly establishes the diagnosis of oligodendroglioma (IDH-mutant and 1p/19q codeleted), then *TERT* promoter testing may not be necessary.

TERT promoter mutations were initially noted in the majority of histologically defined GBMs.[156,157] Subsequently, IDH mutations and *TERT* promoter mutations were shown to be mutually exclusive in GBMs, with those GBMs harboring *TERT* promoter mutations associated with shorter survivals compared with those with IDH mutations.[34,151,155] Further comprehensive investigations demonstrated that those adult DGs that harbored *TERT* promoter mutations but did not have IDH mutations, were tightly associated with genetic signatures of IDH-WT GBM and short overall patient survivals.[4,5,158] *TERT* promoter mutations are not specific for IDH-WT GBM, because they have been documented in other forms of CNS neoplasia in addition to oligodendroglioma, IDH-mutant and 1p/19q codeleted.[156,158,159] However, in the proper clinical, radiologic and neuropathologic setting, *TERT* promoter mutations may be used to support the diagnosis of IDH-WT GBM.

Public Comment Response to Recommendation 8.—There were 88 respondents, of which 69 (73.9%) agreed or agreed with modifications, 9 (17.1%) disagreed, and 10 (10.2%) were neutral. There were 15 comments, including several that reiterated the importance of interpreting *TERT* promoter mutations in the context of IDH-status, as well as its established relationships with *ATRX* mutations and 1p/19q codeletions, as discussed above. The recommendation was unchanged.

Strong Recommendation: 9

For histologic grade 2 to 3 DGs 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 IDH-WT GBM, grade 4. The quality of evidence to support this recommendation was assessed as *moderate*.

Quality Summary.—The evidence base informing this recommendation includes studies evaluating testing of chromosome 7, chromosome 10, *EGFR*, and *TERT* promoter. One genome sequencing study[21] and 1 retrospective cohort study[108] comprise the evidence base for chromosome 7, 1 retrospective cohort study was included for chromosome 10,[108] 2 genome sequencing studies,[21,22] and 8 retrospective cohort studies[18,31,49,108,160–163] for *EGFR*, and 1 genome sequencing study[5] and 8 retrospective cohort studies for *TERT*.[4,18,23,49,150–153] Included studies were assessed as intermediate-low through very low quality and suffered from risk of bias in selection,[|||||] performance,[4,18,31,108,150,163] reporting,[¶¶¶] and detection[###] domains. Quality of evidence was assessed for each target individually and overall for the recommendation. For both chromosome 7 and *EGFR* testing, the aggregate risk of bias for studies included in the evidence base was serious and evidence was not further downgraded, resulting in moderate quality of evidence. For chromosome 10 the risk of bias of the 1 included study was very serious, thus carrying a low quality of evidence. Finally, the quality of evidence for *TERT* was also low based on a very serious aggregate risk of bias and no further downgrading of quality. The overall recommendation was assessed as *moderate*. Refer to the SDC for the quality assessment of included genome sequencing studies, retrospective cohort studies, and the GRADE Quality of Evidence Assessment. See Table 8 for a summary of the mutational status of *EGFR*, *TERT* promoter, chromosome 7, and chromosome 10 across all DG subtypes.

A substantial subset of IDH-WT diffuse astrocytic gliomas in adults are considered grade 2 or 3 based on histologic criteria (no microvascular proliferation or necrosis), yet have an aggressive clinical course, with overall survival times similar to patients with IDH-WT GBM. 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. Studies that have assessed the frequency of +7/–10, *EGFR* amplification and *TERT* promoter mutations have demonstrated higher percentages of these alterations in histologically defined GBMs as compared to histologic grade 2 or 3 DGs, lending support to the association between these markers and the molecular signature of IDH-WT GBM.[****]

IDH-WT diffuse astrocytic gliomas of histologic grade 2 or 3 that carry *EGFR* amplification are associated with significantly shorter patient survival and have outcomes similar to patients with histologically defined IDH-WT GBM.[5,6,18,158,160] *EGFR* amplification has excellent specificity for gliomas with aggressive behavior and is not present in other glioma subtypes that display a more indolent clinical course. It is typically accompanied by gain of chromosome 7 and loss of chromosome 10 in adult DGs, as well as in a smaller subset of pediatric high-grade gliomas.[158,164]

Similarly, those histologic IDH-WT diffuse astrocytic gliomas of histologic grade 2 or 3 with the +7/–10 signature are associated with short patient survival, similar to histologically defined IDH-WT GBM.[5,158,165] The +7/–10 signature also has excellent specificity for DGs, with the rare exception of PXAs.[158] Most investigations have focused on the prognostic role of whole gains of chromosome 7 and whole losses of chromosome 10 (+7/–10), because these are most frequent; the prognostic association of other, far less common combinations of imbalances, such as +7q/–10q, +7/–10q or +7q/–10, await further evaluation.

TERT promoter mutation in IDH-WT diffuse astrocytic gliomas of histologic grade 2 or 3 is associated with significantly shorter patient survival.[4,18,23,160] However, other types of IDH-WT glial neoplasms without WHO grade 4 histology or aggressive behavior also occasionally harbor *TERT* promoter mutations.[156,158,159] Therefore, *TERT* promoter mutations can be considered a marker for grade 4 behavior if the clinical, radiologic and histopathologic features are those of diffusely infiltrative astrocytic glioma. Further, more recent investigations have concluded that a subset of histologic grade 2 IDH-WT DGs that harbor only *TERT* promoter mutations (no other genetic alterations

[|||||] *References 4, 5, 18, 21–23, 31, 49, 108, 150–153, 160–163.*
[¶¶¶] *References 4, 18, 21, 23, 31, 108, 150–152, 160, 161, 163.*
[###] *References 4, 5, 21, 23, 108, 150–152, 160–162.*

[****] *References 18, 21, 22, 31, 108, 151, 161, 162.*

Table 8. Representative Studies Reporting on Histone H3 Mutation Frequency Across Diffuse Glioma (DG) Subtypes

Study, Study Design	Number DG Cases	Marker(s)	Testing Method	Mutation Frequency[a]	Study Conclusion
Castel et al,[168] 2015, RCS	n = 62	H3 K27M	Sequencing, IHC	Midline glioma: H3 K27M, 95.16% (59/62)	There was a high frequency of H3 K27M mutations in DIPGs and the correlation between Sanger sequencing and IHC for H3 K37M mutations was strong
Khuong-Quang et al,[88] 2012, RCS	n = 42	H3 K27M	Sequencing	Midline glioma: H3 K27M, 71.43% (30/42)	H3 K27M mutations were found in high frequency in DIPG and had a poor prognosis compared to DIPG without this mutation. *TP53* mutations co-occurred with H3 K27M mutations with high frequency
Gessi et al,[171] 2013, RCS	n = 123	H3 G34, H3 K27	Pyrosequencing, MLPA	GBM: H3 G34/H3 K27, 31.71% (39/123) CNS-PNET: H3 G34/H3 K27, 12.12% (4/33)	Histone H3 G34R mutations were identified in a subset of pediatric tumors histologically classified as GBM and as CNS-PNET
Korshunov et al,[3] 2015, RCS	n = 202	H3 G34, H3 K27M	450k BeadChip array	GBM: H3 G34, 11.88% (24/202); H3 G34/H3 K27M, 46.04% (93/202)	Pediatric tumors diagnosed histologically as GBM had specific genetic and clinical subsets, defined by mutations in H3 K27M, H3 G34R and *IDH1*

Abbreviations: CNS-PNET, pediatric primitive neuroectodermal tumors of the central nervous system; DIPG, diffuse intrinsic pontine glioma; GBM, glioblastoma; *IDH1*, isocitrate dehydrogenase (NADP(+))1; IHC, immunohistochemistry; MLPA, multiplex ligation-dependent probe amplification; RCS, retrospective cohort study; *TP53*, tumor protein p53.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies.

involving canonical IDH-WT GBM pathways) have longer clinical outcomes than expected for a grade 4 neoplasm.[166,167] The combination of *TERT* promoter mutation with other markers, such as *EGFR* amplification and +7/–10 adds specificity as a marker of grade 4 behavior.[158]

cIMPACT-NOW update 3 has recommended that IDH-WT DGs of histologic grade 2 or 3 that have +7/–10, *EGFR* amplification or *TERT* promoter mutation should be considered grade 4 and more recently, these molecular alterations have been added as criteria for the diagnosis of GBM, IDH-WT, WHO grade 4 by the WHO 5th edition.[7,10] Clinical implications for the use of these biomarkers to establish the molecular diagnosis of IDH-WT GBM, grade 4 include the use of combined chemotherapy and radiotherapy and expanded clinical trial inclusion.

Public Comment Response to Recommendation 9.—Of 89 respondents, 77 (86.5%) agreed or agreed with modifications, 6 (6.7%) disagreed, and 6 (6.7%) were neutral. Twenty written comments were received, many expressing concern about the use of "grade IV astrocytic glioma." As "astrocytic glioma" is not currently an official WHO class, respondents requested that the statement be changed to "molecular diagnosis of GBM." Additional comments requested that the recommendation include 'and' instead of 'or' for the target testing to reduce turnaround time. These suggestions were incorporated into the final recommendation.

Strong Recommendation: 10

H3 K27M testing must be performed in DGs that involve the midline in the appropriate clinical and pathologic setting. The quality of evidence to support this recommendation was assessed as *moderate*.

Quality Summary.—This recommendation was informed by 2 retrospective cohort studies.[88,168] Both retrospective studies were limited by risk of bias in selection, performance, reporting, and detection domains.[88,168] In addition, 1 study reported underpowered statistical analyses.[168] The aggregate risk of bias for the evidence base was very serious and the quality of evidence was upgraded based on a strong association between H3 K27M testing and DG diagnosis in the WHO classification. Refer to the SDC for the quality assessment of included retrospective cohort studies and the GRADE Quality of Evidence Assessment. See Table 8 for a summary of the mutational status of histone H3 in GBM, midline glioma, and pediatric primitive neuroectodermal tumors of the CNS.

Diffuse midline glioma with histone H3 K27M mutation was a newly recognized diagnostic entity in the 4th edition update of the *WHO Classification of Tumours of the Central Nervous System*.[1] The diagnosis relies on the histologic identification of diffusely infiltrating glioma within midline CNS structures such as spinal cord, brainstem, or thalamus and the identification of K27M mutation in either *H3F3A* or *HIST1H3B/C* using anti-H3 K27M IHC or by sequencing methods.[90,169] By histology, tumors can show a large degree of histopathologic and cytopathologic variability, ranging from a low-density infiltrate to an undifferentiated, high-grade neoplasm. Regardless of histologic features, 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.[88,168] While the number of studies investigating this particular mutation is small, the implications for diagnosis, prognosis, and treatment are critically important. This underlies the strong recommendation that H3 K27M testing must be performed in DGs that involve the midline in the appropriate clinical and pathologic setting. As most of these tumors occur in the pediatric population, this recommendation for additional testing is

essential for children and young adults. For older adults there is increasing evidence that the midline location of a DG is also tightly linked with H3 K27M mutation, warranting testing of all patients with midline gliomas to guide appropriate care.[91]

H3 K27M testing can be performed using anti-H3 K27M IHC, which displays strong nuclear staining with relatively high sensitivity for the mutation, ranging from approximately 71% to 100% when compared with sequencing methods.[88,168,170–172] IHC can be particularly useful in small biopsy samples, when tissue is limited. Several anti-H3 K27M antibodies are commercially available for on-site laboratory development, and reference laboratories also offer testing. Of note, the staining pattern of a positive test is nuclear, and therefore staining of cytoplasmic and/or cellular processes should not be interpreted as positive. Confirmatory molecular sequencing may be of utility in equivocal cases. DGs that have H3 K27M mutations also show loss of H3 K27 trimethylation (H3 K27me3), which can be detected by loss of nuclear immunoreactivity for H3 K27me3 by IHC.[173] A smaller subset of diffuse midline gliomas that show loss of H3 K27me3 do not have H3 K27M mutations, but rather have *EGFR* mutations (see Good Practice Statement 1) or overexpression of EZHIP.[172,174,175]

Public Comment Response to Recommendation 10.—There were 88 respondents, of whom 85 (96.6%) agreed or agreed with modifications and 3 (3.4%) disagreed. There were 24 written comments. Originally, the statement included that the recommendation applied to "the appropriate clinical and pathologic setting." Some commented that this phrase needed to be further explained. There were also suggestions that the statement should recommend that the H3 K27M test phrasing of "should" be performed rather than "must" be performed. Others indicated that the methods of testing (eg, IHC versus sequencing) should be described. These comments were taken into consideration. While the recommendation remained the same, these comments have been addressed in the preceding paragraphs.

Conditional Recommendation: 11

H3 G34 testing may be performed in pediatric and young adults with IDH-WT DGs. The quality of evidence to support this recommendation was assessed as *low*.

Quality Summary.—The evidence base informing this recommendation comprises 2 low-quality retrospective cohort studies.[3,171] In addition to suffering from critical risk of selection bias, both studies were limited by a moderate risk of reporting bias[3,171] and one was also limited by risk of performance and detection bias.[3] The aggregate risk of bias for the studies was very serious and the evidence was not further downgraded for any domain. Refer to the SDC for the quality assessment of included retrospective cohort studies and the GRADE Quality of Evidence Assessment. See Table 8 for a summary of the mutational status of histone H3 in GBM, midline glioma, and pediatric embryonal tumors in the CNS.

A hotspot mutation in the histone gene *H3F3A* found within a subset of pediatric and young adult high-grade gliomas confers either a G34R or G34V substitution in the gene product.[3,171,176,177] G34R/V mutant DGs occur in a somewhat older age group in comparison with K27M cases (median age, 19 years for G34R/V mutation; median age, 6 for K27M mutation) and tend to involve the cerebral hemispheres rather than midline locations.[176] Prognosis for G34R/V-mutant gliomas is poor but somewhat better than the K27M-mutant gliomas (median survival of 12 months for K27M and 24 months for G34R or G34V). "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.[7] Of note, these DGs 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.[3,171] Testing by sequencing or with mutation-specific (H3 G34R) antibodies is recommended for nonmidline DGs in pediatric and young adult populations.

Public Comment Response to Recommendation 11.—There were 89 respondents, of whom 75 (83.2%) agreed or agreed with modification, 4 (4.5%) disagreed, and 11 (12.4%) were neutral. There were several written comments, including 1 that suggested that the age of the patient should be considered but that older adults might also benefit from testing in some cases. Others indicated that the methods of testing (ie, IHC versus sequencing) should be described. These comments were taken into consideration. While the recommendation remained the same, the comments were addressed in the text above.

Conditional Recommendation: 12

BRAF mutation testing (V600) may be performed in DGs that are IDH-WT and H3-WT. The quality of evidence to support this recommendation was assessed as *low*.

Quality Summary.—The evidence base informing this recommendation consists of 1 intermediate quality prospective cohort study[77] and 4 retrospective cohort studies.[18,178–180] Two included retrospective studies were assessed as very low quality based on serious or critical risk of bias across more than 1 domain.[18,178] The other 2 retrospective studies were also assessed as very low quality and this was based on critical risk of selection bias, moderate risk of bias in other domains, underpowered statistical analyses, and a lack of funding source reporting.[179,180] The aggregate risk of bias for the studies was very serious but evidence was not further downgraded for any domain. Refer to the SDC for the quality assessment of retrospective cohort studies and the GRADE Quality of Evidence Assessment. See Table 9 for a summary of the mutational status of *BRAF*, *MYB/MYBL1*, and *FGFR1* across DG subtypes.

BRAF V600E mutation is a driver mutation that is present in a wide variety of neoplasms, including melanoma, hairy cell leukemia, papillary thyroid carcinoma, colorectal carcinoma, lung carcinoma, Langerhans cell histiocytosis, Erdheim Chester disease, papillary craniopharyngioma, and ameloblastoma. It is present in a minority of DGs, but prevalence is enriched in epithelioid GBMs[178–180] and other IDH- and H3-WT DGs.[2,18] Of note, *BRAF* V600E mutation is distinct from the *KIAA1549*::*BRAF* fusion and other types of *BRAF* fusion, which are especially frequent in cerebellar pilocytic astrocytomas.

In addition to these studies, the recommendation was influenced by the availability of targeted therapy[181] and the occurrence of *BRAF* V600E within a variety of brain tumors other than DGs, where the mutation is more prevalent; these include PXAs, ganglioglioma (GG), and a minority of pilocytic astrocytomas.[2,182] Although *BRAF* V600E mutation occurs in a higher proportion of these generally circum-

Table 9. Representative Studies Reporting on *BRAF*, *MYB*/*MYBL1*, and *FGFR1* Status Across Diffuse Glioma (DG) Subtypes

Study, Study Design	Number DG Cases	Marker	Testing Method	Mutation Frequency[a]	Study Conclusion
Korshunov et al,[179] 2018, RCS	n = 64	*BRAF* V600E	450k array	E-GBM: *BRAF*, 56.25% (36/64)	Epithelioid GBMs resolve into 3 genetic entities. Those clustering with PXA and IDH-WT GBM by methylation are enriched for *BRAF* V600E mutations
Kleinschmidt-DeMasters et al,[178] 2013, RCS	n = 24	*BRAF* V600E	IHC, PCR	E-GBM: *BRAF*, 53.85% (7/13)	*BRAF* V600E mutations were noted in a substantial percentage of epithelioid GBMs, but not rhabdoid or giant cell GBMs
Nakajima et al,[180] 2018, RCS	n = 14	*BRAF* V600E	Sequencing, CGH array	E-GBM: *BRAF*, 92.86% (13/14)	Epithelioid GBMs are characterized by *BRAF* V600E mutations, *TERTp* mutations, and *CDKN2A/B* deletions, most often in combination
Aibaidula et al,[18] 2017, RCS	n = 166	*BRAF* V600E, *MYB*	Sequencing, FISH	IDH-WT (all DGs): *BRAF*, 6.02% (10/166); *MYB*, 19.88% (33/166)	Subset of adult IDH-WT histologic grade 2/3 DGs harbor clinically relevant *BRAF* V600E mutations and *MYB* amps
Tatevossian et al,[183] 2010, RCS	n = 77	*MYB*	SNP array	A: *MYB* amp, 14.29% (2/14); poly, 7.14% (1/14)	*MYB* amp was described in a subset of pediatric low-grade DA, leading to increased gene and protein expression. Focal truncating deletion of *MYB* was noted in AG
Ramkissoon et al,[184] 2013, RCS	n = 44	*MYB*	Copy number analysis	A: *MYBL1* rearrangement, 26.32% (5/19)	Recurrent truncating duplications of MYBL1 were present in a subset of low-grade pediatric DA. Focal truncating deletion of *MYB* was noted in AG
Qaddoumi et al,[2] 2016, RCS	n = 91	*FGFR1*	Sequencing	A: *FGFR1/3*, 23.53% (4/17) OD: *FGFR1/3*, 61.90% (26/42)	Alterations in *BRAF*, *FGFR1*, or *MYB*/*MYBL1* were solitary pathogenic alterations in subsets of low-grade pediatric DGs, often corresponding to histologic class

Abbreviations: A, astrocytoma histology; AG, angiocentric glioma; amp, amplification; *BRAF*, B-Raf proto-oncogene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDKN2B*, cyclin-dependent kinase inhibitor 2B; CGH, comparative genomic hybridization; DA, diffuse astrocytoma; E-GBM, epithelioid glioblastoma histology; *FGFR1*, fibroblast growth factor receptor 1; FISH, fluorescence in situ hybridization; GBM, glioblastoma; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; *MYB*, MYB proto-oncogene; *MYB1*, MYB proto-oncogene like 1; NR, not reported; OD, oligodendroglioma histology; PCR, polymerase chain reaction; poly, polysomy; PXA, pleomorphic xanthoastrocytomas; RCS, retrospective cohort study; SNP, single nucleotide polymorphism; *TERTp*, telomerase reverse transcriptase promoter; WT, wild-type.

[a] Mutational frequencies refer to histologic classifications. See full data tables in the supplemental digital content for all outcomes reported by included studies

scribed neuroepithelial tumors, PXA, GG, and pilocytic astrocytoma fell outside of the scope and search criteria used to develop these guidelines. However, histologic overlap between these entities can be considerable; given the presence of targeted therapy, a liberal approach to testing was favored in borderline or ambiguous cases where the distinction between a diffuse and circumscribed neoplasm could not be made with certainty.

This recommendation is most relevant to IDH-WT and H3-WT DGs in which *BRAF* V600E mutations have been shown to be enriched, including epithelioid GBMs and cases overlapping with PXA or anaplastic PXA; pediatric DGs or tumors overlapping with GG or pilocytic astrocytoma; and histologic grade 2 and 3 DGs lacking a "GBM molecular signature."[10] Evidence for *BRAF* V600E testing in the setting of an IDH-WT GBM with typical histologic features or with canonical genetic alterations in older adults is currently lacking.

While many of the markers considered in this guideline pertain principally to DGs in adults, it should be noted that a small number of pediatric DGs with *BRAF* V600E mutation have been identified,[2] and that *BRAF* V600E testing should be considered in the context of IDH-WT and H3-WT DGs in children.[9] This is particularly important given relatively common histologic overlap between circumscribed and DGs in children.

Public Comment Response to Recommendation 12.—Of 89 respondents to this recommendation, 75 (84.3%) agreed or agreed with modifications, 10 (11.2%) disagreed, and 4 (4.5%) were neutral. Of 24 written comments, 7 solicited advice as to the best method of testing, and 5 emphasized the importance of clinical and pathologic correlation. The remaining 12 comments expressed diverse thoughts, both supportive and skeptical, including concern about the difference between the strength of the recommendation and the level of evidence (2 comments). These helpful comments were considered and the initial recom-

mendation was streamlined to encompass all IDH-WT and H3-WT DGs (within the appropriate clinicopathologic context) instead of epithelioid GBM and "other" IDH-WT and H3-WT DGs. We also included a more specific description of the appropriate clinicopathologic context for testing in the text above.

Conditional Recommendation: 13

MYB/MYBL1 and *FGFR1* testing may be performed in children and young adults with DGs that are histologic grade 2 to 3 and are IDH-WT and histone H3-WT. The quality of evidence to support this recommendation was assessed as *low*.

Quality Summary.—The evidence base informing this recommendation includes three retrospective cohort studies evaluating *MYB/MYBL1* testing[18,183,184] and 1 retrospective cohort study evaluating *FGFR1* testing.[2] The 4 studies were assessed as low[183,184] and very low quality[2,18] based on risk of bias in selection,[2,18,183,184] performance,[18] reporting,[2,18,183,184] and detection[2,183,184] domains. Quality of evidence was assessed for each target individually and then overall for the entire statement. The aggregate risk of bias for *MYB/MYBL1* studies was very serious but evidence was not further downgraded for any domain. The risk of bias for the one *FGFR1* study was very serious. As only one study was identified, quality of evidence is dependent only on the risk of bias. The quality of evidence for the entire recommendation was assessed as *low*. Refer to the SDC for the quality assessment of included retrospective cohort studies and the GRADE Quality of Evidence Assessment. See Table 9 for a summary of the mutational status of *BRAF*, *MYB/MYBL1*, and *FGFR1* across DG subtypes.

Recent studies have identified subsets of DGs in children and young adults that lack alterations in *IDH1*, *IDH2*, or histone H3 genes. In addition to *BRAF*, mentioned above, genes that are recurrently altered in this group include MYB proto-oncogene (*MYB*), MYB proto-oncogene like 1 (*MYBL1*), and *FGFR1*. These tumors are technically IDH-WT and H3-WT, but in retrospective studies they seem to exhibit an indolent clinical course, especially when compared with most DGs in adults.[18] Genetic mechanisms of activation of these drivers are heterogeneous, which may affect the availability of specific assays for detection. These include duplications, truncations, amplifications, and rearrangements resulting in fusion genes (*MYB* and *MYBL1*) and internal tandem duplications of tyrosine kinase domains and single nucleotide variants (*FGFR1*). The prevalence of these alterations is difficult to assert with certainty, because most of the early data were from small retrospective studies. Integrated molecular and clinical analyses of a large cohort of pediatric low-grade gliomas provide insight into the spectrum and frequency of these alterations.[185] Specific genetic alterations seem to correlate with glioma subtypes, with *MYBL1* rearrangements being reported in up to 50% pediatric diffuse astrocytoma,[184] *MYB* amplifications/copy number gains in 2 of 14 (14.3%) pediatric diffuse astrocytoma[183] and 33 of 166 (19.9%) adult IDH-WT lower grade gliomas.[18] Conversely, in one study *FGFR1* alterations were frequent in rare low-grade neoplasms with 'oligodendroglial' phenotype affecting children and young adults, 39 of 42 (92.9%).[2] In addition, *MYB*::*QKI* fusions are found in nearly all angiocentric glioma (WHO grade 1), a low-grade DG often associated with epilepsy.[186] Given the diagnostic and prognostic implications for these genetic drivers in gliomas of children and young adults, testing for them may be advisable depending on laboratory capabilities, but the quality of evidence is lower than that supporting testing for other more commonly altered genes (eg, *IDH1*/2 and *BRAF*).

Public Comment Response to Recommendation 13.—There were 88 respondents, of whom 66 (75.0%) agreed or agreed with modifications, 8 (9.1%) disagreed, and 14 (15.9%) were neutral. There were 19 written comments, including some that suggested that evidence for the recommendation was not strong and the targets are not actionable; questions about testing methods; requests to clarify what is meant by "in the appropriate clinical setting" and inclusion of other genetic drivers (ie, panels). These comments were taken into consideration and the recommendation was revised or addressed by the other recommendations (eg, testing for *BRAF*).

Table 10. Summary of the Good Practice Statements (GPS)

GPS1. *EGFR* mutation testing may be performed on DGs that involve the midline
GPS2. Testing for alterations in *ALK*, *ROS1*, *MET*, and *NTRK* genes may be performed on cerebral hemispheric DGs of infancy that are wild-type for IDH and histone H3
GPS3. Testing for DNA methylation class or for alterations in *PDGFRA*, *EGFR*, and *MYCN* may be performed in pediatric high-grade DGs that are wild-type for IDH and histone H3

Abbreviations: DGs, diffuse gliomas; IDH, isocitrate dehydrogenase.

GOOD PRACTICE STATEMENTS

During the period of the systematic literature review and drafting of recommendations, several clinically relevant investigations were published on the topic of biomarker testing for DG subtypes not originally covered by the Patient/population, Intervention, Comparator, and Outcome frameworks. To include guidance on these subtypes, we performed a focused literature review and included Good Practice Statements based on this second review. A description of the methods used to formulate these statements is included in the SDC.

The targeted search included alterations of ALK receptor tyrosine kinase (*ALK*), *EGFR*, ROS proto-oncogene 1 (*ROS1*), neurotrophic tyrosine receptor kinase (*NTRK*), *MET*, *PDGFRA*, and MYCN proto-oncogene (*MYCN*) within bithalamic gliomas, infantile-type hemisphere gliomas, or diffuse pediatric-type high-grade gliomas. The panel viewed the statements below as important, but there was not a formal rating for the certainty of evidence. The decision to form these statements was based on (1) emerging evidence with limited number of studies and samples and (2) anticipated updates to the WHO occurring at the time that this manuscript was being written. The panel believed that the tests would provide prognostic or treatment-related information for clinicians to consider. Future updates to the guideline will address these tests using a formal rating of the evidence and will include a grade for the strength of recommendation. Refer to Table 10 for the list of Good Practice Statements.

Statement 1

EGFR mutation testing may be performed on DGs that involve the midline.

A small subset of diffusely infiltrating midline gliomas that harbor *EGFR* mutations have a distinct methylation pro-

file.[174,187] While most of these DGs with *EGFR* mutations are histone H3-WT, some contain H3 K27M mutations. Like H3 K27M mutant gliomas involving the midline, *EGFR*-mutant DGs in this location that are histone H3-WT typically show loss of H3 K27me3, which can be detected by IHC. Small in-frame insertions/duplications of the tyrosine kinase domain and point mutations of the extracellular domain are detected by NGS and are potential therapeutic targets.

Statement 2

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

DGs that occur in infancy are usually molecularly distinct from those of older childhood and adults. These large cerebral hemispheric tumors are typically wild type for IDH and histone H3 and often harbor fusions (rarely other activating alterations) involving *ROS1*, *ALK*, *MET*, or the *NTRK* family.[188,189] The identification of such fusions or other activating alterations could lead to targeted therapy. Fusions can be identified by NGS and by FISH if appropriate probes are available.

Statement 3

Testing for DNA methylation class or for alterations in *PDGFRA*, *EGFR*, *MYCN* may be performed in pediatric high-grade DGs that are WT for IDH and histone H3.

Among pediatric diffuse high-grade gliomas that are IDH-WT and histone H3-WT, 3 molecular subclasses can be identified by DNA methylation profiling that have differing molecular genetic alterations and clinical outcomes. One class is generally characterized by *PDGFRA* amplifications or mutations; a second class enriched for *EGFR* amplifications is associated with the longest survival; and the third often harbors *MYCN* amplifications and is associated with the shortest survival.[164,190]

FUTURE CONSIDERATIONS

The recommendations provided are based on evidence available at the time of literature review. It is expected that additional studies and new technologies will emerge that will need to be incorporated into future guidelines. In the category of IDH-WT diffuse astrocytic gliomas of adults, it has been recommended that histologic grade 2 and 3 DGs should be tested for chromosome 7 gain/whole chromosome 10 loss, *EGFR* amplification, and *TERT* promoter mutation to establish the molecular diagnosis of GBM, grade 4. It might be expected that additional biomarkers or combinations of biomarkers will be identified and validated in histologic grade 2 and 3 DGs that are also capable of predicting grade 4 clinical behavior, including alterations in *PDGFRA*, *FGFR3*, *MET*, *TP53*, *NF1*, *PTEN*, among others.[10] For IDH-mutant astrocytomas of histologic grade 2 and 3, future studies could uncover and validate additional markers that can more optimally stratify risk or predict grade 4 clinical behavior in addition to *CDKN2A/B* homozygous deletion, such as *PDGFRA*, *MYCN*, or *CDK4* amplifications, mutations in *CDKN2A/B* or *RB1*, among others.[11,65,85] *CDKN2A/B* homozygous deletion have recently been shown to be a marker of poor prognosis in oligodendroglioma, IDH-mutant and 1p/19q codeleted, which along with other biomarkers, may be relevant to future recommendations or grading schemes.[111] Similarly, it is likely that genetic or epigenetic markers will be uncovered and validated that are capable of stratifying risk among patients with DGs driven by histone H3, mitogen-activated protein kinase pathway or *MYB/MYBL1* genes.[9,191]

The laboratory findings of tumor mutational burden (TMB), mismatch repair (MMR) loss and microsatellite instability are of interest in multiple forms of cancer, because the findings from these tests predict response to immunotherapies, including immune checkpoint inhibitors in some cancer types.[192–194] Initial randomized clinical trials of checkpoint inhibitors in newly diagnosed and recurrent GBM have failed to demonstrate improved survival.[195] Guidelines for testing of tumor mutational burden, mismatch repair, or microsatellite instability in DGs will be developed if and when clinical care warrants them.

Whole-genome DNA methylation profiling represents a robust and reproducible method for precisely segregating tumor types that have similar genetic alterations, epigenetic signatures, and clinical behaviors.[5,12] IDH-WT GBM, IDH-mutant astrocytomas, oligodendrogliomas, IDH-mutant and 1p/19q codeleted, and histone H3-mutant gliomas cluster tightly within their own class with little overlap based on DNA methylation profiling.[5,6,22] Other DGs, such as those that occur in children and are driven by mitogen-activated protein kinase pathway or *MYB/MYBL1* genomic alteration also cluster together, indicating a high degree of specificity for each signature.[164,190] While the literature indicates this method is superior for classification purposes and could have a role in future classification and grading, it currently lacks widespread clinical implementation due to regulatory challenges, prevailing practice patterns, and uncertainties in reimbursement. Currently, DNA methylation profiling is an attractive alternative for identifying DG classes that will likely be implemented into clinical practice soon.

LIMITATIONS

We were unable to answer KQs 1b—What are the acceptable techniques/methods for mutation testing of DGs and what are the expected turnaround times for individual assays? While we collected the various methods of testing, none performed poorly in our assessment. Each have varying strengths and weaknesses. For the latter question, no studies from the literature search reported turnaround time data and so, no recommendation was made. It is up to each laboratory to determine the best methodology and acceptable turnaround times. Although the individual DG entities have prognostic characteristics that have been well defined in the literature,[2–7] most of the studies that comprised the evidence base for the recommendations correlated the reported outcomes to DG entities and not to survival outcomes. Additional studies that investigate outcomes related to biomarkers and DG subtypes would increase certainty of the evidence base and lead to stronger recommendations.

CONCLUSIONS

The 13 evidence-based recommendations and 3 good practice statements that are provided for biomarker testing of DGs, together with their explanations and justifications, are intended to guide practice and improve the clinical care of patients with these diseases. For more than a century, the diagnosis of DG was based primarily on histologic appearance, yet recent clinically relevant molecular genetic discoveries have forced a re-evaluation of diagnostic definitions and criteria. We are now firmly within the molecular era in which

integrated diagnoses are formulated by directly incorporating molecular biomarker test results. The complexity of establishing an integrated diagnosis has increased and pace of change has been rapid, warranting the recommendations that we have provided. Neuropathologists, molecular pathologists, and treating physicians will continue to work closely together to provide optimal clinical care by using molecular biomarkers for diagnostic and treatment purposes.

DISCLAIMER

The CAP developed the Pathology and Laboratory Quality Center for Evidence-based Guidelines as a forum to create and maintain laboratory practice guidelines (LPGs). Guidelines are intended to assist physicians and patients in clinical decision-making, and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time an LPG is developed and when it is published or read. LPGs are not continually updated and may not reflect the most recent evidence. LPGs address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines 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 the patient. Accordingly, adherence to any LPG is voluntary, with the ultimate determination regarding its application to be made by the physician considering each patient's individual circumstances and preferences. The CAP and its collaborators make no warranty, express or implied, regarding LPGs 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.

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APPENDIX

Disclosed Interests and Activities From July 2017 to April 2021

Daniel J. Brat, MD, PhD

Position of Influence: Society for Neuro-Oncology, American Association of Neuropathologists

Julia A. Bridge, MD

Research Grant: Cepheid

Consulting fees or Advisory Board: Bayer, Bristol Myers Squibb/National Comprehensive Cancer Network, Epizyme, Merck, Moffitt Cancer Center, Translational Genomics Research Institute, VENTANA/Roche

Position of Influence: United States & Canadian Academy of Pathology

Howard Colman, MD, PhD

Consulting fees or Advisory Board: AbbVie, Adastra Pharmaceuticals, Bayer, Best Doctors, CytRx Corporation, F. Hoffman-La Roche, Forma Therapeutics, Foundation Medicine, Genentech, Insys Therapeutics, Karyopharm Therapeutics, NewLink Genetics, Novocure, Omniox, Orbus Therapeutics, OXiGENE, Private Health, Upsher-Smith

Speakers' bureau/lecture fees/honoraria: Merck

Institutional contracts/clinical trials: AbbVie, Array BioPharma, Bayer, BeiGene, Bristol Myers Squibb, DNAtrix, Forma Therapeutics, Global Coalition for Adaptive Research, Kadmon Holdings, Karyopharm Therapeutics, Merck, NewLink Genetics, Nuvation Bio, Orbus, Plexxikon

Meera R. Hameed, MD

Consulting fees or Advisory Board: Novartis

Eyas M. Hattab, MD, MBA

Consulting fees or Advisory Board: Arbor Pharmaceuticals, Cell Marque, E.R. Squibb & Sons, Bristol Myers Squibb, Ventana Medical System/Roche Diagnostics

Speakers' bureau/lecture fees/honoraria: Arbor Pharmaceuticals, Roche Diagnostics

Position of Influence: College of American Pathologists, United States & Canadian Academy of Pathology

Other: International Collaboration on Cancer Reporting member

Jason T. Huse, MD, PhD

Research Grants: Taiho Oncology, Inc.

Martin J. van den Bent, MD, PhD

Consulting fees or Advisory Board: AbbVie, Agios, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, CarThera, Celgene, Celldex Therapeutics, Genenta Science, Karyopharm Therapeutics, Nerviano Medical Sciences, VAXIMM

Speakers' bureau/lecture fees/honoraria: Merck Sharp & Dohme Corp

Research Grants: AbbVie

No Disclosures to Report

Arie Perry, MD; Brent T. Harris, MD, PhD; Michelle Hawks Yount, MS; Fausto J. Rodriguez, MD; Robert B. Jenkins, MD, PhD; William C. McDonald, MD; Kenneth Aldape, MD; Peter Canoll, MD, PhD; Dolores H. Lopez-Terrada, MD, PhD; Lesley H. Souter, PhD; Carol Colasacco, MLIS, SCT(ASCP); Nicole E. Thomas, MPH, CT(ASCP)^{cm}