2022

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• ***Context.***—**Integration of molecular data into glioma classification supports diagnostic, prognostic, and therapeutic decision-making; however, testing practices for these informative biomarkers in clinical laboratories remain unclear.**

Objective.—**To examine the prevalence of molecular testing for clinically relevant biomarkers in adult and pediatric gliomas through review of a College of American Pathologists proficiency testing survey prior to the release of the 2021 World Health Organization Classification of Central Nervous System Tumors.**

Design.—**College of American Pathologists proficiency testing 2020 survey results from 96 laboratories performing molecular testing for diffuse gliomas were used to determine the use of testing for molecular biomarkers in gliomas.**

Results.—**The data provide perspective into the testing practices for diffuse gliomas from a broad group of clinical laboratories in 2020. More than 98% of participating laboratories perform testing for glioma biomarkers recognized as diagnostic for specific subtypes, including *IDH*. More than 60% of laboratories also use molecular markers to differentiate between astrocytic and oligodendroglial lineage tumors, with some laboratories providing more comprehensive analyses, including prognostic biomarkers, such as *CDKN2A/B* homozygous deletions. Almost all laboratories test for *MGMT* promoter methylation to identify patients with an increased likelihood of responding to temozolomide.**

Conclusions.—**These findings highlight the state of molecular testing in 2020 for the diagnosis and classification of diffuse gliomas at large academic medical centers. The findings show that comprehensive molecular testing is not universal across clinical laboratories and highlight the gaps between laboratory practices in 2020 and the recommendations in the 2021 World Health Organization Classification of Central Nervous System Tumors.**

(*Arch Pathol Lab Med.* 2023;147:518–524; doi: 10.5858/arpa.2021-0431-CP)

Historically, the classification of diffuse gliomas was based in large part on histologic features, including tumor cell morphology, mitotic index, and the presence of necrosis and/or microvascular proliferation. To delineate between astrocytic and oligodendroglial lineages, histologic assessments in conjunction with immunohistochemical markers were used; however, given the heterogeneous nature of these tumors, significant overlap in these features commonly results in challenges to classify tumor lineages accurately and reproducibly. Because clinical outcomes and treatment strategies vary tremendously for patients with

Accepted for publication March 14, 2022.
Published online July 25, 2022.
Supplemental digital content is available for this article at https://meridian.allenpress.com/aplm in the May 2023 table of contents.

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Table 1. Summary of Laboratories Participating in Glioma 2020 Supplemental Questionnaire	
Practice	**No. (%)**
Laboratory provides diagnostic and/or ancillary testing services for diffuse gliomas	96
Yes	67 (69.8)
No	29 (30.2)
Laboratory has a board-certified neuropathologist	66
Yes	48 (72.7)
No	18 (27.3)

diffuse gliomas, understanding the molecular basis of these tumors has the potential to lead to a molecular classification that will enhance diagnostic accuracy and ensure the optimal care for glioma patients.

In 2016, the fourth edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) recognized the importance of genomic and molecular testing to classify adult and pediatric gliomas.[1] For many entities, this included combining the molecular and histologic features into an integrated diagnosis because it was recognized that specific molecular alterations were strongly correlated with clinical behavior. The status of molecular biomarkers, such as *IDH* mutations, 1p/19q codeletion, histone *H3.3* p.K27M or *H3.3* p.G34R/V mutations, *TERT* promoter mutations, *EGFR* amplification, and *CDKN2A/B* homozygous deletions, among others, is critical for the accurate classification of brain tumors. In 2021, the updated fifth edition (WHO CNS5) advanced the role of molecular biomarkers in the classification of CNS tumors by emphasizing integrated diagnoses and grouping tumors based on genetic alterations.[2] Importantly, molecular biomarkers that provide important prognostic information are now a parameter for establishing tumor grades. Given the increasing utility of genomic testing in CNS tumors, we sought to determine the biomarkers used by clinical laboratories for the characterization of diffuse gliomas prior to WHO CNS5. We surveyed clinical laboratories enrolled in proficiency testing (PT) to investigate the prevalence of testing for biomarkers associated with diagnosis, prognosis, and therapy of diffuse gliomas.

MATERIALS AND METHODS

Survey Supplemental Questions

The College of American Pathologists (CAP) Glioma (GLI) A 2020 PT survey included a 20-question supplemental questionnaire (SQ) focused on elucidating current clinical testing practices for diffuse gliomas (Supplemental Table 1, see supplemental digital content at https://meridian.allenpress.com/aplm in the May 2023 table of contents). The SQ was sent to 113 laboratories (including US and international sites) in March 2020 and was returned by April 2020. Laboratories were asked whether they performed clinical testing for specific biomarkers associated with either diagnostic, prognostic, or therapeutic implications for diffuse gliomas. The questions also sought to determine whether this testing was performed internally ("in-house") at the clinical laboratories or sent to a reference laboratory.

A total of 98 laboratories returned the SQ, and 2 SQs were excluded because of missing data. To analyze the responses from the participating laboratories, multivariate stepwise logistic regression models were used to test for laboratory characteristics associated with diffuse glioma testing practices. A stepwise approach was used to address convergence issues due to the small sample size and low frequencies for some testing practices. The models were fit with 3 factors—institution location, institution type, and board-certified neuropathologist on staff. Institution location was defined as a 2-level factor that classified laboratories as domestic (United States) or international. Institution type included 3 categories: independent/commercial reference laboratory, academic hospital/medical center laboratory, and nonacademic hospital/medical center laboratory. Unsure responses were excluded from the analysis. Analyses were performed with SAS 9.4 (SAS Institute, Cary, North Carolina). A significance level of .05 was used for the statistical testing.

RESULTS

Overall Profile of Clinical Laboratories Performing Testing for Diffuse Gliomas

Of the 113 laboratories enrolled in the GLI-A-2020 survey, 104 submitted results for PT, whereas 96 (85%) also submitted all pages of the SQ. The laboratories responding to the SQ were a representative sample in terms of institution type compared with the laboratories participating in the PT survey. The supplemental questions addressed current testing practices in clinical laboratories for diffuse gliomas, including mutational, methylation, and copy number status for multiple biomarkers. Questions about methylation testing were limited to *MGMT* promoter methylation analysis and did not address methylome profiling for glioma classification. Of the 96 laboratories, only 69.8% (67 of 96) affirmed that they provide diagnostic and/or ancillary testing services for diffuse glioma (Table 1). Most of these laboratories were from academic hospital/medical center laboratories (71.6%; 48 of 67; Figure 1). Independent/commercial reference laboratories represented 13.4% (9 of 67) and the remaining 10 laboratories (14.9%) were from nonacademic hospital/medical center laboratories. Additionally, most (71.6%) of the responding laboratories providing services were located domestically (within the United States), whereas the other 19 (28.4%) represented international laboratories (Figure 1).

Given the intrinsic heterogeneity, overlapping morphologic features among different lineages of diffuse gliomas, and significant prognostic differences among subtypes, we first sought to determine whether laboratories had access to a neuropathologist. Most laboratories (72.7%; 48 of 66) that provide diagnostic testing for diffuse gliomas have a board-certified neuropathologist, which provides a greater likelihood that appropriate testing and classification will be made given challenges in classifying these tumors by morphology alone (Table 1). Next, we sought to determine whether responding laboratories perform testing for diffuse gliomas in-house or send out this testing to other laboratories. As highlighted in Table 2, the location of testing depended on which biomarker was being evaluated. For example, *IDH* mutational status was determined by testing performed in-house for 93.8% (61 of 65) of laboratories, whereas only 36% (9 of 25) laboratories performed *MYB/MYBL1* analysis in-house. The type of methodology used by laboratories performing testing in-house was not determined in this survey.

Molecular Testing for Markers of Astrocytic Lineage Gliomas

In the 2016 WHO guidelines, *IDH*-mutant gliomas were recognized as a distinct entity and should be distinguished from *IDH*–wild-type (*IDH*-WT) tumors because the prog-

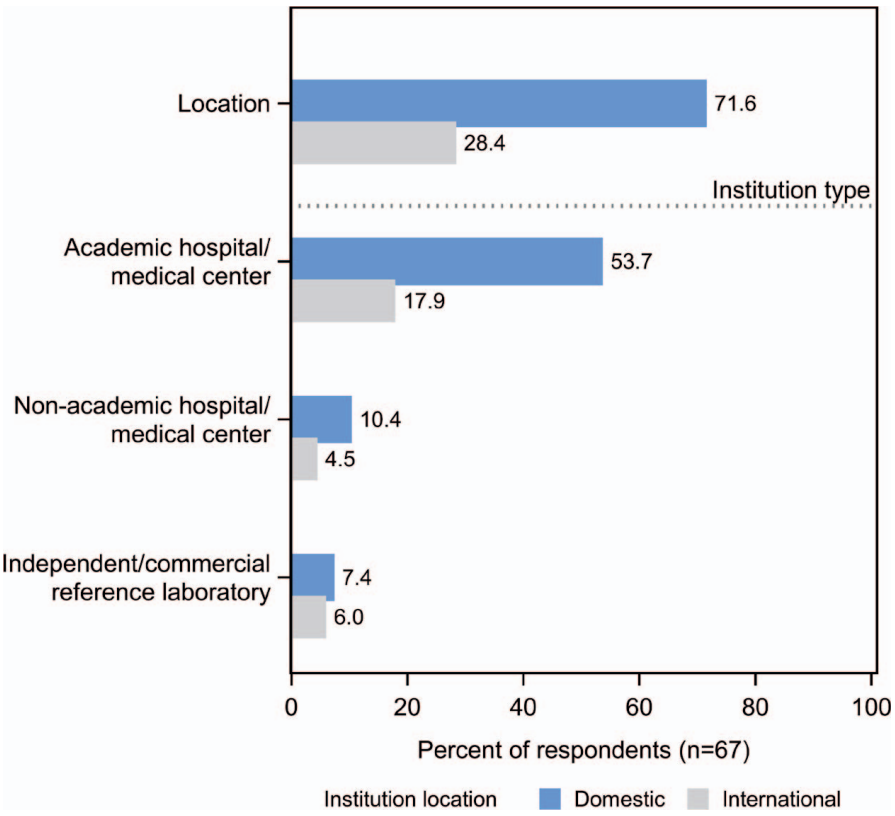

Figure 1. *Respondent demographics. Representation of the types and locations (domestic or international) of the laboratories responding to the supplemental questionnaire.*

nosis differs significantly between the 2 entities.[1] In our survey 98.5% of responding laboratories (65 of 66) indicated that they assess *IDH* mutation status for diffuse gliomas (Figure 2; Table 2) and that this testing is performed in-house for 93.8% (61 of 65) of laboratories. *IDH*-mutant gliomas can represent astrocytic or oligodendroglial lineage tumors; however, co-occurrence of 1p/19q codeletion provides the established diagnostic biomarker for oligodendrogliomas.[1,3–8] Although other mutations and alterations occur in these tumors, such as *TERT* promoter mutations, additional biomarkers are not necessarily required to make a diagnosis of oligodendroglioma. *IDH*-mutant diffuse gliomas that retain 1p/19q, however, can be further characterized by specific biomarkers. For *IDH*-mutant diffuse gliomas without 1p/19q codeletion, we asked whether laboratories routinely test for loss of ATRX expression and only 50% (33 of 66) of responding laboratories confirmed that this testing was performed. We also asked whether laboratories routinely determine *TP53* mutation status in *IDH*-mutant gliomas without 1p/19q codeletion, because the presence of a *TP53* mutation is closely associated with astrocytic tumors.[1,4,9] Similarly to ATRX testing, only 50.7% (34 of 67) perform *TP53* mutational testing. However, 77.6% (52 of 67) of laboratories perform or send out testing for 1p/19q codeletion in *IDH*-mutant diffuse gliomas that retain ATRX expression and are negative for *TP53* mutations. Despite a

Table 2. Summary of Testing Location Used to Evaluate Molecular Markers in Diffuse Gliomas

Tests Used for Diffuse Glioma Testing	No. of Laboratories	Test Performed, No. (%)	Testing Location, No. (%)	
			In-house	Send-out
IDH mutational analysis	66	65 (98.5)	61 (93.8)	4 (6.2)
MGMT promoter methylation analysis	67	66 (98.5)	52 (78.8)	14 (21.2)
BRAF p.V600 mutational analysis	67	63 (94.0)	61 (96.8)	2 (3.2)
1p/19q codeletion analysis	66	62 (93.9)	50 (80.6)	12 (19.4)
EGFR amplification analysis	65	50 (76.9)	35 (70.0)	15 (30.0)
TP53 mutational analysis	66	48 (72.7)	34 (70.8)	14 (29.2)
H3 mutational analysis	63	44 (69.8)	20 (45.5)	24 (54.5)
ATRX analysis	64	42 (65.6)	34 (81.0)	8 (19.0)
TERT promoter mutational analysis	65	38 (58.5)	24 (63.2)	14 (36.8)
FGFR1 mutational analysis	63	36 (57.1)	21 (58.3)	15 (41.7)
CDKN2A homozygous deletion analysis	63	33 (52.4)	19 (57.6)	14 (42.4)
Chromosome 7 copy number analysis	63	31 (49.2)	18 (58.1)	13 (41.9)
Chromosome 10 copy number analysis	63	30 (47.6)	18 (60.0)	12 (40.0)
MYB/MYBL1 analysis	62	25 (40.3)	9 (36.0)	16 (64.0)

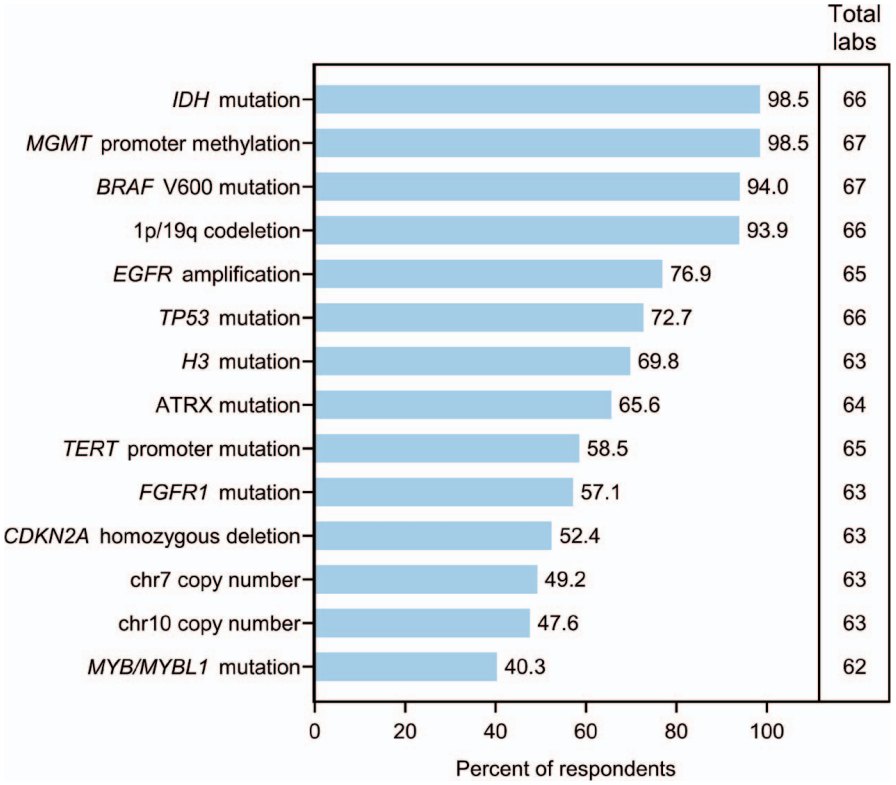

Figure 2. *Testing performed for diffuse gliomas. Graphical representation of the percentage of responding laboratories that performed testing for specific biomarkers associated with either diagnostic, prognostic, or therapeutic implications for diffuse gliomas.*

significant number of laboratories testing for 1p/19q codeletion in this glioma subtype, it should be noted that only 58.2% (39 of 67) always perform this testing, and 19.4% (13 of 67) indicated this testing occurs sometimes (Table 3).

Molecular Testing for Prognostic Biomarkers in Diffuse Gliomas

In addition to the utility of integrating molecular biomarkers into the diagnosis of diffuse gliomas, they can also provide significant prognostic information. Homozygous deletion of *CDKN2A/B*, 2 genes that encode cyclin-dependent kinase inhibitors, is a common feature of high-grade gliomas.[1,5] Loss of these 2 genes in the absence of high-grade histologic features is indicative of a more aggressive tumor.[2,10] In the SQ, we asked if laboratories performed testing to determine *CDKN2A* deletion status in *IDH*-mutant diffuse astrocytic gliomas, and less than half (41.8%; 28 of 67) indicated that this biomarker is included in their workflow, with 16 laboratories indicating they always perform testing and 12 laboratories only performing the test sometimes (Table 4). Similarly, *TERT* promoter mutations serve as an important prognostic indicator in grade 2 and 3 diffuse gliomas. These mutations are most common in oligodendrogliomas (>95% of cases) and primary *IDH*-WT glioblastomas (70%–80%) but can also be detected in lower-grade infiltrating (diffuse and anaplastic) astrocytomas.[4–7] In the SQ, laboratories were asked whether they perform or send out testing to detect *TERT* promoter mutations to support the diagnosis of oligodendroglioma, and 28.8% of laboratories (19 of 66) confirmed that this testing is performed. Notably, *TERT* promoter mutation testing was performed by 40.3% (27 of 67) of laboratories to support the diagnosis of *IDH*-WT glioblastomas; however, only 12 of these laboratories always perform this testing (Table 4).

Although much less common than *IDH* mutations, *BRAF* p.V600 mutations are also present in a subset of adult diffuse gliomas and are important to discern given the potential therapeutic intervention with BRAF-targeted therapies.[11] Moreover, *BRAF* p.V600E mutations are enriched in epithelioid glioblastomas, a rare morphologic variant, and initial case reports demonstrate potential for using targeted therapies for these tumors.[1,3] Given the prognostic and therapeutic importance of this alteration, we surveyed whether clinical laboratories perform or send out testing for *BRAF* p.V600 status. For diffuse grade 2 or 3 *IDH*-WT and *H3*-WT tumors, 50.7% (34 of 67) of laboratories indicated this was being done, whereas 66.7% (44 of 66)

Table 3. Molecular Testing Practices for Markers of Astrocytic Lineage Gliomas

Laboratory Practice (In-house or Send-out Testing)	No. (%)
Routine testing for ATRX loss in *IDH*-mutant diffuse gliomas without 1p/19q codeletion	66
Yes	33 (50.0)
No	19 (28.8)
Unsure	14 (21.2)
Routine *TP53* mutational testing in *IDH*-mutant diffuse gliomas without 1p/19q codeletion	67
Yes	34 (50.7)
No	23 (34.3)
Unsure	10 (14.9)
Testing for 1p/19q codeletion status in *IDH*-mutant diffuse gliomas without ATRX loss or *TP53* mutations	67
Yes, always	39 (58.2)
Yes, sometimes	13 (19.4)
No	5 (7.5)
Unsure	10 (14.9)

Table 4. Molecular Testing Practices for Prognostic Biomarkers in Diffuse Gliomas

Laboratory Practice (In-house or Send-out Testing)	No. (%)
Testing for *CDKN2A* homozygous deletions in *IDH*-mutant diffuse astrocytic gliomas	67
Yes, always	16 (23.9)
Yes, sometimes	12 (17.9)
No	29 (43.3)
Unsure	10 (14.9)
Testing for *TERT* promoter mutations to further support the diagnosis of oligodendrogliomas	66
Yes, always	9 (13.6)
Yes, sometimes	10 (15.2)
No	39 (59.1)
Unsure	8 (12.1)
Testing for *TERT* promoter mutations to further support the diagnosis of *IDH*-WT glioblastomas	67
Yes, always	12 (17.9)
Yes, sometimes	15 (22.4)
No	32 (47.8)
Unsure	8 (11.9)
Routine testing for *BRAF* p.V600 mutational analysis in diffuse gliomas of histologic grade 2 or 3 that are *IDH*-WT and *H3*-WT	67
Yes	34 (50.7)
No	18 (26.9)
Unsure	15 (22.4)
Routine testing for *BRAF* p.V600 mutational analysis in epithelioid grade IV astrocytic glioma (glioblastoma)	66
Yes	44 (66.7)
No	12 (18.2)
Unsure	10 (15.2)

Abbreviation: WT, wild type.

of laboratories indicated this testing was performed for epithelioid glioblastomas (Table 4).

Molecular Testing for Malignant Midline Gliomas

In addition to *IDH*-mutant gliomas, the 2016 WHO guidelines recognized the diagnostic importance of molecular biomarkers for midline gliomas.[1] These tumors develop in midline structures, such as the thalamus, brainstem, and spinal cord, and are particularly challenging to sample adequately for optimal histologic analysis. However, genomic characterization of these tumors demonstrates that they frequently harbor a specific mutation at amino acid 27 of the histone *H3.3* gene (*H3F3A*). In the 2016 WHO Classification, the presence of an *H3* p.K27M mutation was diagnostic for a subset of diffuse midline gliomas (WHO grade 4) and was critical for distinguishing between low- and high-grade tumors irrespective of histologic features.[1] Additionally, the presence of an *H3F3A* p.G34R/V mutation was also recognized as a molecular subtype of diffuse hemispheric gliomas.[1,3,6,12] In the WHO CNS5, the *H3* p.K27-mutant glioma subtype has been refined to *H3* p.K27-altered in order to reflect more diverse mechanisms other than single-base substitutions that can produce a similar disease profile.[2] In children and young adults that present with diffuse gliomas that involve the midline, 51.5% (34 of 66) of responding laboratories always perform testing to identify *H3* p.K27 mutations and another 12.1% (8 of 66) of laboratories perform this testing sometimes (Table 5).

Table 5. Molecular Testing Practices for Malignant Midline Gliomas

Laboratory Practice (In-house or Send-out Testing)	No. (%)
Testing to identify *H3* p.K27 mutations in diffuse gliomas that involve the midline in children and young adults	66
Yes, always	34 (51.5)
Yes, sometimes	8 (12.1)
No	12 (18.2)
Unsure	12 (18.2)
Testing for *H3* p.G34 mutations in children and young adults with *IDH*-WT diffuse gliomas	67
Yes, always	18 (26.9)
Yes, sometimes	12 (17.9)
No	23 (34.3)
Unsure	14 (20.9)
Routine testing for histologic grade 2 or 3 diffuse gliomas that are *IDH*-WT and *H3*-WT to evaluate the molecular signature of a grade IV astrocytic glioma (glioblastoma), to include whole chromosome 7 and 10 copy number alterations, *EGFR* amplification, or *TERT* promoter mutations	67
Yes	27 (40.3)
No	25 (37.3)
Unsure	15 (22.4)

Abbreviation: WT, wild type.

Similarly for this patient population that has *IDH*-WT diffuse gliomas, 26.9% (18 of 67) of laboratories always perform testing for *H3* p.G34 mutations, whereas 17.9% (12 of 67) sometimes perform this testing. For grade 2 or 3 tumors that do not harbor *IDH* or *H3* mutations, laboratories were queried if they routinely evaluated for the molecular glioblastoma signature, including chromosomes 7 and 10 copy number alterations, *EGFR* amplification, and *TERT* promoter mutations.[3,5] Of the 67 responding laboratories, 27 (40.3%) indicated that this level of testing was performed.

Molecular Testing for Therapeutic Indicators

For diffuse gliomas, molecular biomarkers have distinct roles in classifying tumors, some serving as both diagnostic and prognostic (eg, 1p/19q codeletion) biomarkers, whereas others serve as adjunct evidence for a particular lineage. Another critical role for molecular biomarkers is predictive, determining which patients will respond more favorably to chemotherapy regimens, thus guiding treatment decisions. For glioblastoma patients, *MGMT* methylation status is the single most informative molecular biomarker that will predict which patients will benefit from the alkylating chemotherapy agent temozolomide.[13] *O*6-methylguanine methyltransferase (MGMT) is a DNA repair enzyme that is associated with resistance to alkylating chemotherapy. In cells with reduced MGMT levels, *O*6-methylguanine accumulates in DNA, inducing DNA damage and subsequent cell death.[14,15] A common mechanism leading to a reduction of MGMT expression is through promoter methylation. As such, glioblastoma patients with methylated *MGMT* promoters have increased sensitivity to alkylating agents. In our survey 90.9% (60 of 66) of laboratories perform or send out testing for *MGMT* promoter methylation status in glioblastoma. For *IDH*-mutant grade 2 or 3 diffuse gliomas, 40.9%

Table 6. Molecular Testing Practices for Therapeutic Indicators	
Laboratory Practice (In-house or Send-out Testing)	**No. (%)**
Testing for *MGMT* promoter methylation status in grade IV astrocytic gliomas (glioblastomas)	66
Yes	60 (90.9)
No	1 (1.5)
Unsure	5 (7.6)
Testing for *MGMT* promoter methylation status on *IDH*-mutant diffuse gliomas of histologic grade 2 or 3	66
Yes, always	27 (40.9)
Yes, sometimes	29 (43.9)
No	5 (7.6)
Unsure	5 (7.6)

(27 of 66) always perform this testing, whereas 43.9% (29 of 66) indicated it is performed sometimes (Table 6).

Molecular Testing for Pediatric Diffuse Gliomas

Although diffuse gliomas mainly arise in adult populations, children also present with histologically similar lesions, albeit to a much lesser extent than the more common circumscribed pilocytic astrocytomas. Despite having similar morphologic features, the genetic composition of pediatric diffuse gliomas rarely overlaps with that of adult diffuse gliomas. Indeed, *IDH*-mutant gliomas are rarely identified in pediatric patients, whereas *BRAF* p.V600E, *FGFR1* alterations, and *MYB* or *MYBL1* rearrangements represent molecular subtypes of gliomas in children.[16] These alterations can direct treatment decisions and/or provide prognostic information and importantly are now recognized as new tumor types in the WHO CNS5 as diffuse astrocytoma, *MYB*- or *MYBL1*-altered and diffuse low-grade glioma, MAPK pathway-altered[2]; however, in our survey cohort, only 25.4% (17 of 67) and 19.4% (13 of 67) perform or send out testing for *FGFR1* and *MYB/MYBL1* alterations, respectively, in grade 2 or 3 *IDH*- and *H3*-WT gliomas (Table 7).

Table 7. Molecular Testing Practices for Pediatric Diffuse Gliomas	
Laboratory Practice (In-house or Send-out Testing)	**No. (%)**
Routine testing for *FGFR1* alterations in children and young adults with diffuse gliomas that are histologic grades 2–3 and *IDH*-WT and *H3*-WT	67
Yes	17 (25.4)
No	33 (49.3)
Unsure	17 (25.4)
Routine testing for *MYB/MYBL1* alterations in children and young adults with diffuse gliomas that are histologic grades 2–3 and *IDH*-WT and *H3*-WT	67
Yes	13 (19.4)
No	36 (53.7)
Unsure	18 (26.9)

Abbreviation: WT, wild type.

DISCUSSION

In this manuscript we summarize 2020 survey results from 67 clinical laboratories that perform or send out molecular testing as part of their routine clinical workflow for diffuse gliomas. These responding laboratories represent an 85% response rate, which demonstrates the utility of using PT programs as an effective means of assessing the current practices and needs of clinical laboratories. Overall, the responses from clinical laboratories, both domestic and international, demonstrate that most laboratories surveyed provide diagnostic testing for gliomas; however, the respondents overwhelmingly represented large academic hospital or medical center laboratories. Given the specialized care required for brain tumor patients, this finding is not surprising but does highlight resource gaps for smaller laboratories that are not able to offer testing for these types of tumors. Additionally, our results illustrate the widespread adoption of testing for diagnostic biomarkers like *IDH*, which is performed in 98.5% (65 of 66) of responding laboratories. Similarly, in our cohort of 67 responding laboratories, 66 of them also routinely evaluate *MGMT* methylation status, which is a strong predictor of response to therapy. Incorporation of this testing by almost all clinical laboratories likely reflects the changes made by the WHO wherein *IDH*-mutant gliomas are now recognized as molecularly defined entities and include astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant, and 1p/19q-codeleted.[2]

Testing for lineage-defining molecular biomarkers, such as ATRX, *TP53*, and 1p/19q codeletion is performed by most of the responding laboratories but not to the same extent as the 2016 WHO-defined molecular biomarkers. Given the overlapping histologic features typically present in diffuse gliomas, distinguishing between a diffuse astrocytoma and oligodendroglioma by microscopic features alone results in high interobserver discordance with ensuing, significant prognostic and therapeutic differences. As highlighted by WHO CNS5, IDH mutant status alone is not sufficient to characterize tumors, because *IDH* mutations are diagnostic in both lineages. The new guidelines advocate for additional molecular profiling to include genes that are characteristically altered in the various tumor lineages. For example, oligodendroglioma, IDH-mutant, 1p/19q-codeleted may also include *TERT* promoter, *CIC*, *FUBP1*, and *NOTCH1* analysis because mutations in these genes are characteristic of the oligodendroglial lineage.[2] Furthermore, molecular markers can supersede histologic features present in a sample and therefore should be included in diagnostic testing algorithms. Similarly, co-occurring ATRX and *TP53* alterations are characteristic for astrocytoma, IDH-mutant and should be evaluated in adult diffuse gliomas; however, the method by which laboratories choose to interrogate these markers should be selected with caution. Although immunohistochemistry is more affordable for most clinical laboratories, ATRX and p53 immunohistochemistry interpretation requires careful assessment. For ATRX, there must be clear loss of staining only in the tumor cells, but positive staining must be present in background endothelial, glial, or hematopoietic cells. For p53 immunohistochemistry, only strong and extensive nuclear positivity should be interpreted as positive, which can be challenging to demonstrate because there is significant variability in staining intensity, and the absence of staining does not exclude the presence of a mutation.[17,18] Given that 27.3% of participating clinical laboratories do not have access to a board-certified neuropathologist who is trained to accurately identify tumor cells and interpret these stains, molecular testing to identify loss-of-function ATRX or

clinically relevant *TP53* mutations may be a more reliable approach for most clinical laboratories.

As noted above, the incorporation of genetic alterations in CNS tumor diagnoses not only improves accuracy but also can provide clinically relevant tumor grading and prognostic information. This is particularly true now for *CDKN2A/B* homozygous deletions, which are strongly associated with a poor prognosis in astrocytomas, *IDH*-mutant.[10,19] The WHO CNS5 now includes the presence of *CDKN2A/B* homozygous deletions as a criterion for a CNS WHO grade of 4, even in the absence of necrosis and microvascular proliferation.[2] However, less than half (28 of 67) of responding laboratories indicated that this testing was routinely performed, with 16 of 28 and 12 of 28 laboratories always or sometimes performing testing, respectively (Table 4). Because 12% to 20% of grade 2 and 3 gliomas harbor *CDKN2A/B* deletions, including this biomarker in routine clinical testing for gliomas provides essential tumor grading and prognostic information for the clinical management of these patients.

Generalized treatment strategies for glioma patients include surgical resection as the primary treatment, followed by varying combinations of radiotherapy and chemotherapy agents. Temozolomide is an oral DNA alkylating agent used to treat gliomas because it is capable of penetrating the blood-brain barrier and has demonstrated fewer myelosuppressive side effects. The most significant biomarker used to determine which patients will benefit from temozolomide therapy is *MGMT* promoter methylation. The significance of this predictive biomarker is evident in that more than 90% of clinical laboratories indicated that testing was performed for glioblastomas. It is important to note that the prognostic impact of *MGMT* promoter methylation currently is limited to glioblastoma because similar predictive value in grades 2 and 3 gliomas remains unclear.

Although this study highlights positive trends toward integrating molecular testing into clinical testing practices for diffuse gliomas, there are limitations that should be acknowledged. First the results that are reported are restricted to those laboratories that are performing clinical testing and are enrolled in the CAP PT program, thereby biasing the pool of respondents. Because only 113 laboratories are enrolled in the survey, we recognize this does not account for laboratories that may integrate molecular testing into their clinical practices but rely exclusively on send-out testing. The responses from this population of laboratories may be different from those that participated in this survey. Additionally, there are other biomarkers (eg, *EGFRvIII*, gain of entire chromosome 7, and loss of entire chromosome 10) and assays (eg, methylome profiling) that may be routinely tested in some laboratories, although not specifically assessed in the survey questions. Lastly, the survey used for this study focused on the assessment of specific biomarkers and did not assess test methodology.

Integration of molecular and morphologic features for glioma classification serves to improve precision and reproducibility between pathologists. Additionally, the molecular findings provide greater clinical significance and, in some settings, predictive value. Although the availability of molecular testing continues to expand and identify critical biomarkers, new diagnostic classifications provided by updated WHO editions require time to implement. The findings from this study provide a baseline for diffuse glioma testing practices in 2020, prior to the publication of the WHO CNS5. They also demonstrate how significant changes in testing practices will be required in order to become aligned with WHO CNS5.[2] As the 2021 WHO classification system relies heavily on genomic and molecular data, it will be incumbent upon laboratories to expand both the frequency of molecular testing as well as the spectrum of biomarkers being assessed. This will likely require increased use of targeted next-generation sequencing panels to be able to assess all relevant biomarkers in a single assay and a migration away from single-gene/biomarker assays. Given the heterogeneous nature of these tumors and the overlapping histologic features, it is recognized that integration of molecular biomarkers is critical to accurately diagnose gliomas. The information provided by an integrated diagnosis improves diagnostic accuracy and can help guide therapeutic decisions.

We acknowledge the members of the clinical laboratory community who provided responses to the survey questions.

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