Contemporary Diagnosis of Gliomas using Biomarkers

Daniel J. Brat, MD, PhD

\(^1\)Department of Pathology and Laboratory Medicine, Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA

Address for Correspondence/Reprints:
Daniel J. Brat, MD, PhD
Department of Pathology and Laboratory Medicine
Emory University Hospital, H-195
1364 Clifton Rd. NE
Atlanta, GA 30322
Phone: 404-712-1266;
Fax: 404-727-3133;
e-mail: dbrat@emory.edu
Introduction:

Gliomas are the largest and most diverse group of primary brain tumors \(^1,2\) and are subdivided into distinct classes according to clinical, neuroimaging, histopathologic and molecular genetic characteristics\(^3,4\). The diffuse gliomas are a subset defined by widely infiltrative properties and their tendency toward biologically progression. Although they can occur throughout the neuraxis and at any age, they most frequently arise within the cerebral hemispheres of adults. The primary morphologic classes of diffuse gliomas are astrocytomas, oligodendrogliomas and oligoastrocytomas, and these are graded according to World Health Organization (WHO) criteria as grades II-IV\(^1\).

The diagnosis of glial neoplasms has been established primarily by histopathological examination since the early 1900s, when Bailey and Cushing first classified them based on their presumed histogenesis \(^5,6\). In these histologic schemes, diffuse astrocytomas are recognized by their irregular, elongated hyperchromatic nuclei and high degree of fibrillarity \(^1\). Mitotic activity is associated with a shorter survival and is used as a grading criterion to distinguish infiltrating astrocytoma, WHO grade II from anaplastic astrocytoma, WHO grade III. Likewise, necrosis and microvascular proliferation signal even more aggressive behavior and serve as criteria for GBM, WHO grade IV\(^7\). By contrast, oligodendrogliomas have round, regular nuclei, perinuclear halos and a delicate branching (“chicken-wire”) vasculature. Increased mitoses (≥ 6 per 10 high power fields), necrosis and microvascular proliferation are used as criteria to distinguish oligodendroglioma, WHO grade II from anaplastic oligodendroglioma, WHO grade III \(^8\).

The WHO has also recognized oligoastrocytomas, which show both astrocytic and
oligodendroglial morphology, and grading schemes for this class have largely followed
those of oligodendroglioma.

Many studies have demonstrated low reproducibility and interobserver concordance in
the diagnosis of diffuse gliomas, leading to confusion in clinical management. Similarly,
correlations of histologic class with molecular markers, clinical behavior and response to
therapies have been highly variable. Pilocytic astrocytomas, gangliogliomas and
PXAs can also occasionally pose diagnostic challenges, yet their proper recognition is
critical, since the treatment and prognosis differ from those of diffuse gliomas. Over the
past 20 years, investigations of genomic alterations in glial neoplasms have greatly
informed our understanding of molecular classes of disease and have led to our current
use of biomarker-driven glioma classification.

**IDH Mutations Subdivide Infiltrating Gliomas in Adults into Distinct Subsets**

The mutational status of *isocitrate dehydrogenase* 1 and 2 (*IDH1* and *IDH2*) is now
recognized as a major discriminator of biological classes among the diffuse gliomas. When mutations occur in *IDH1* or *IDH2*, the mutant enzyme develops a preferential
affinity for alpha-ketoglutarate instead of isocitrate, which leads to the production and
accumulation of the oncometabolite 2-hydroxyglutarate. Both *IDH1* and *IDH2*
mutations occur in infiltrating gliomas, though *IDH2* mutations are much less common
than *IDH1* and *IDH1* mutations are found in approximately 70-80% of histologic grade II
and III infiltrating gliomas and secondary GBMs, yet are much less frequent in primary
GBMs (~ 5%) and secondary GBMs (~ 5%). The most frequent *IDH1* mutation is R132H, which occurs at
codon 132 and leads to exchange of the amino acid arginine for histidine.
Infiltrating gliomas occurring in young adults are more likely to harbor an IDH1 mutation
than those in the elderly \textsuperscript{15,21,23,24}, but this mutation is rarely found in gliomas arising in
young children \textsuperscript{18,25}. IDH mutant gliomas progress more slowly over time than those
lacking IDH mutations, suggesting that these are biologically distinct forms of disease.
Therefore, diffuse gliomas with IDH mutations are associated with a better prognosis,
grade for grade, than those without \textsuperscript{15,24}. While IDH1 mutations are frequent in diffuse
gliomas, they are rarely found (or absent) in other CNS neoplasms, including
ependymomas, pilocytic astrocytomas, gangliogliomas and PXAs \textsuperscript{15,21-23}. Thus, the
finding of an IDH mutation in a primary glial neoplasm strongly suggests that it is a
diffusely infiltrative glioma. Since IDH1 mutations are present in both diffuse gliomas
that show astrocytic differentiation and in those that show oligodendroglial
differentiation, they are thought to occur early in gliomagenesis, before those molecular
alterations that promote specific differentiation \textsuperscript{15,21,22}, such as TP53 and ATRX
mutations in astrocytomas, or 1p/19q co-deletion, CIC, FUBP1 and TERT promoter
mutations in oligodendrogliomas \textsuperscript{21,24}.

**IDH Mutant Astrocytomas have TP53 mutations and ATRX alterations**

Among diffuse gliomas that are IDH mutant, there is a relatively strict molecular
dichotomy: one subset, accounting for about 30-40\% of IDH mutant gliomas, will
demonstrate 1p/19q co-deletion; the other larger subset will be defined largely by TP53
mutations and loss of ATRX (Figure 1)\textsuperscript{16}. Studies based on morphologic class have
consistently shown that the large majority of grade II and III diffuse astrocytomas and
secondary glioblastomas have TP53 mutations \textsuperscript{15,21,26}. When restricted to IDH-mutated
infiltrating astrocytomas, an even higher percentage have TP53 mutations, since IDH wild-type diffuse gliomas have a lower frequencies of TP53 mutation. The Cancer Genome Atlas project demonstrated that 94% of IDH mutant grade II and III diffuse gliomas that lacked 1p/19q co-deletion had TP53 mutations\textsuperscript{16}.

The combination of IDH and TP53 mutations is strongly coupled to inactivating alterations in Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX), a gene that encodes a chromatin remodeling regulator. Nearly all diffuse gliomas with an ATRX mutation had an IDH1 mutation as well. In tumors with IDH and ATRX mutations present, 94% had a TP53 mutation. ATRX mutations are associated with the Alternative Lengthening of Telomeres (ALT) phenotype\textsuperscript{27}, an alternative mechanism for maintaining telomere length in tumors that do not have constitutive telomerase activity\textsuperscript{28}. In establishing the diagnosis of an IDH mutant diffuse glioma, loss of immunohistochemical staining for ATRX in neoplastic cells is an excellent surrogate for ATRX gene inactivation and supports the diagnosis of an astrocytoma\textsuperscript{29}. The evidence to date indicates that the molecular signature of IDH mutant astrocytoma includes TP53 mutation and ATRX alteration and is associated with ALT\textsuperscript{16,19,29}.

The grading of astrocytomas has traditionally been performed based on morphologic features, and the presence of mitotic activity has been use to stratify tumors as grade II and III. These histopathologic grading schemes have included both IDH mutant and IDH wild type astrocytomas that were classified based on morphology. Grading schemes may need to be reconsidered for IDH mutant astrocytomas that are classified based on TP53 mutations or ATRX loss. It is not entirely clear whether mitotic activity or other
molecular, morphologic or clinical feature will optimally stratifies IDH mutant astrocytomas.

Oligodendrogliomas are Diffuse Gliomas with IDH Mutations and 1p/19q Co-deletion

A second form of diffuse glioma is characterized by IDH mutations and 1p/19q co-deletion, and shows a strong association with oligodendroglioma histology (Figure 1). While past studies based on morphologic classification of disease have emphasized the tight correlation of 1p/19q co-deletion and the oligodendroglioma morphology, more recent interpretations have emphasized that the combination of IDH mutation and 1p/19q co-deletion is the molecular signature of this disease rather than an association. IDH mutant diffuse gliomas with 1p/19q co-deletion show a slightly higher percentage of IDH2 mutations than those without co-deletion, yet the percentage is still small compared to IDH1 mutations. Deletion of the 1p and 19q chromosomal arms occurs through an unbalanced translocation that results in the formation of der(1;19) (q10;p10). Inactivating mutations of the tumor suppressor genes FUBP1 and CIC, on chromosomes 1p and 19q, respectively, occur secondary to this unbalanced translocation. Among diffuse gliomas, these mutations are specific for oligodendrogliomas with 1p/19q co-deletion, with a frequency ranging from 46 to 83% for CIC and 20-30% for FUBP1. They are rare or absent in infiltrating astrocytomas of all grades and are mutually exclusive with mutations in TP53 and ATRX. Other genes that are mutated in IDH mutant, 1p/19q co-deleted gliomas include Notch1, PIK3CA and PIK3R1.
Oligodendrogliomas also have a mechanism for maintaining telomere length. While the ALT serves this purpose in astrocytomas, it is rarely seen in oligodendrogliomas. Instead, activating mutations in the telomerase reverse transcriptase (TERT) promoter are present in nearly all oligodendrogliomas. In the TCGA study of diffuse grade II and III diffuse glioma, 96% of cases with IDH mutations and 1p/19q co-deletion showed TERT mutations, while only 4% of IDH mutant gliomas without 1p/19q co-deletion showed this mutation. Thus, the molecular signature of oligodendroglioma includes mutations of IDH and the TERT promoter, together with co-deletion of 1p/19q. With this emerging definition of disease, the optimal morphologic or molecular prognostic and predictive markers will need to be defined.

**Oligoastrocytoma**

Previous WHO classifications have recognized grade II, III and IV diffuse gliomas with mixed histology (oligoastrocytoma, grades II and III, and GBM with oligodendroglioma component, grade IV). However, numerous investigations have indicated that IDH-mutant gliomas are either 1p/19q co-deleted or TP53 mutant, with few gaps or overlaps, and reflect two distinct molecular lineages that are best represented by oligodendroglioma (TERT mutation and 1p/19q co-deletion) or astrocytoma (TP53 mutation and ATRX loss). In the TCGA analysis of grades II and III diffuse gliomas, the majority of tumors characterized morphologically as oligoastrocytomas were IDH mutant and had TP53 mutations, while smaller numbers were 1p/19q co-deleted or were IDH wild-type. Similarly, studies of GBM-O have concluded that these tumors are either IDH mutant, combined with either 1p/19q co-deletion or TP53
mutation, or alternatively, are \textit{IDH} wild-type and contain genetic alterations typical of classic GBM, such as \textit{EGFR} amplification and \textit{PTEN} loss.\textsuperscript{39-41} Combined, these studies have not provided evidence for a genetic signature specific for gliomas of mixed histology (oligoastrocytomas), but rather indicate that these mixed histology gliomas consist of discrete molecular entities.\textsuperscript{36,38,42} Since the diagnoses of oligoastrocytoma and GBM-O have been associated with a high degree of interobserver variation and molecular alterations and clinical outcomes have not been consistent, the use molecular signatures to define astrocytoma and oligodendroglioma lineage should improve interobserver concordance. Biomarker-driven diagnosis also seems likely to reduce the diagnosis of “oligoastrocytoma” and the confusion related to its clinical management.

\textbf{\textit{IDH} Wild-type Diffuse Gliomas in Adults are Biologically Aggressive}

The majority of infiltrating gliomas that are \textit{IDH} wild-type are primary GBMs\textsuperscript{12,15}. Approximately 95\% of primary GBMs lack an \textit{IDH} mutation, compared with only 20-30\% of grade II and III infiltrating gliomas\textsuperscript{15}. However, the subset of diffuse gliomas that are \textit{IDH} wild-type and grade II-III by histological criteria (lacking necrosis and microvascular hyperplasia) represent a clinically important subset, because their genetic alterations and clinical behavior are similar to those of \textit{IDH} wild type (primary) GBM \textsuperscript{27}. For example, \textit{IDH} wild-type grade II and III diffuse gliomas have a much lower frequency of \textit{TP53} mutations and do not demonstrate 1p/19q co-deletion \textsuperscript{24}. \textit{TP53} mutations are present in only 15-25\% of \textit{IDH} wild type grade II-III astrocytomas as compared to 94\% of \textit{IDH} mutant astrocytomas \textsuperscript{15,21}. Furthermore, diffuse grade II-III gliomas that are \textit{IDH} wild-type more frequently show molecular genetic alterations similar to GBM, such as
EGFR, PTEN, NF1, RB1, CDKN2A alterations, than those tumors that have an IDH mutation. TERT promoter mutations are frequent in IDH wild type grade II-III gliomas and GBMs (83%), but are rare in IDH1-mutant infiltrating astrocytomas. Since IDH-mutated and IDH wild-type astrocytomas have disimilar sets of mutations and clinical behaviors, they appear to arise by distinct oncogenic pathways and represent separate diseases, despite their histopathologic similarity.

**Primary vs Secondary Glioblastoma**

The clinical presentation of GBM may be primary, with a de novo grade IV neoplasm, or secondary, in which the GBM arises over time from a lower grade infiltrating glioma. The large majority of GBMs are primary (90-95%) while secondary GBMs are less common (5-10%) . These two clinical presentations of GBM have molecular correlates as well, with most primary GBMs being IDH wild-type and most secondary GBMs being IDH-mutant. Primary, IDH wild-type GBMs typically demonstrate an accumulation of molecular alterations within three dominant genetic families: 1) receptor tyrosine kinase (RTK); 2) Retinoblastoma (RB1); and 3) p53. EGFR amplification, PTEN deletion and mutations of the PI3K subunits are common alterations in the RTK family that are commonly associated with primary GBMs. Approximately 40-50% of GBMs show amplification of EGFR and about half of these have the variant III (vIII) mutation. The vIII mutation causes truncation of the receptor, which leads to constitutive tyrosine kinase activity. PTEN deletion or mutation occurs in about 80% of GBMs. Other members of this pathway that are commonly altered in primary GBMs include: PDGFRA, which is amplified in 18%; phosphatidylinositol-3-OH kinase (PI(3)K), which is mutated in 15%;
and NF1, with 18% showing mutations and/or deletions. Among the p53 family of genetic alterations, the most frequent are mutations and deletions of CDKN2A/ARF (49%), amplification of MDM2 (14%) and mutations and deletions of TP53 (35%). Alterations in the RB1 pathway include mutations and deletions of CDKN2A/p16 (52%), amplification of CDK4 (18%) and mutations and deletions of RB1 (11%)12. In the evolution of IDH wild-type, primary GBM, the precise sequence of oncogenic events involving RTK, RB1 and p53 has not been determined.

In contrast, secondary tumors show a high frequency of IDH1 and TP53 mutations, together with ATRX inactivation43. Approximately 85% of secondary GBMs harbor an IDH1 mutation while 62% have a TP53 mutation 15. Since TP53 mutation is thought to occur early in IDH mutant astrocytoma development, additional alterations are likely associated with biological progression45.

**MGMT Promoter Methylation**

Standard therapy for GBM includes radiation and chemotherapy with temozolomide, which acts by crosslinking DNA by alkylating multiple sites including the O6 position of guanine 46. DNA crosslinking is reversed by the DNA repair enzyme MGMT (O6-methylguanine-DNA methyltransferase). Therefore, low levels of MGMT would be expected to be enhanced response to alkylating agents. The expression level of MGMT is determined in large part by the methylation status of the gene’s promoter. Epigenetic silencing of MGMT occurs in 40% to 50% of GBMs and can be assessed by its promoter methylation status on PCR-based tests of genomic DNA. MGMT promoter methylation is a strong predictor of prolonged survival, independent of other clinical
factors or treatment \(^{47}\) and \(\text{MGMT}\) promoter methylation is associated with prolonged progression-free and overall survival in patients with GBM treated with chemotherapy and radiation therapy \(^{47}\).

Like \(\text{IDH}\) mutations, \(\text{MGMT}\) promoter methylation occurs with similar frequencies in grades II, III and IV diffuse astrocytomas and is thought to be an early event in gliomagenesis \(^{48-50\,49,51\,47,49-51}\). Secondary GBMs show a particularly high frequency of \(\text{MGMT}\) promoter methylation \(^{49,50}\). In low grade diffuse astrocytomas and secondary GBMs, \(\text{MGMT}\) promoter methylation is tightly correlated with \(\text{TP53}\) mutation and overexpression of the p53 protein \(^{48,50}\). In one study, 92% of grade II diffuse astrocytomas with \(\text{MGMT}\) promoter methylation harbored a \(\text{TP53}\) mutation, compared to only 39% of those that were unmethylated. Similarly, secondary GBMs with \(\text{TP53}\) mutations had a greater percentage with \(\text{MGMT}\) promoter methylation that those without (92% vs 25%, respectively) \(^{50}\).

**Pediatric Gliomas**

High grade infiltrating astrocytomas in pediatric patients are vastly different than those found in adult patients.\(^{52,53}\) Though histologically similar, these two groups can be distinguished by their locations, clinical behavior, and mutation and gene expression profiles. Pediatric GBMs, for example, nearly always form \textit{de novo} and rarely progress from a lower grade glioma \(^{54}\). \(\text{IDH1}\) mutations are uncommon in pediatric GBMs, occurring in less than 10% \(^{54}\), and are typically absent in high grade gliomas of young children \(^{55}\). Conversely, mutations in \(\text{H3F3A, ATRX}\) and \(\text{DAXX}\) (\textit{death-domain associated protein}) are more typical of pediatric GBMs \(^{52,54,56}\). These genes encode
proteins with roles in chromatin remodeling. H3F3A encodes for H3.3, a replication-independent histone variant that is normally recruited to DNA via the ATRX-DAXX heterodimer. Mutations in H3.3 lead to decreased methylation of H3 histones and usually involve amino acid substitutions at K27 and G34. Approximately 40% of pediatric GBMs harbor H3F3A mutations and nearly all (95%) also carry an ATRX mutation. A smaller percent of these (4.2%) had a DAXX mutation. In this same study, approximately half (54%) of the GBMs had a TP53 mutation and there was significant overlap between TP53 mutations and ATRX, H3F3A or DAXX mutations. Alternative lengthening of telomeres (ALT) was associated with loss of ATRX expression and was most frequent in tumors that had combined mutations in ATRX, H3F3A and TP53. The site of H3F3A mutations in pediatric high grade glioma is associated with patient age and tumor location: those with mutations at K27 tend to occur in young children (median age 11 yrs) and in midline locations (pons and thalamus); those with mutations at G34 occur in teenagers and young adults and arose from the cerebral hemispheres. A mutual exclusivity of H3F3A and IDH1 mutations has been described in pediatric high grade gliomas.

Low grade gliomas of childhood are also distinct from those of adults. Among those pediatric gliomas that appear diffusely infiltrative, a lower percentage contain IDH mutations. However, the IDH wild-type status does not necessarily have the same implications for biologically aggressive behavior in low grade diffuse gliomas of childhood. A recent study of oligodendrogliomas in children found that only 18% of those cases tested had IDH R132H mutations and only 25% of cases had 1p/19q co-deletion. With the definition of oligodendrogliomas of adults emphasizing IDH
mutation and 1p/19q co-deletion as a definitive signature, the neuropathologic diagnosis of pediatric oligodendroglioma will not likely follow the same paradigm.

A large and important subset of pediatric low grade gliomas are better-circumscribed and more indolent in clinical behavior, including pilocytic astrocytomas, WHO grade I, gangliogliomas, WHO grade I, and pleomorphic xanthoastrocytomas (PXAs), WHO grade II. In addition to their general lack of infiltration, pilocytic astrocytomas, gangliogliomas and PXAs also differ from diffuse gliomas in their mutational profiles. They typically lack \textit{IDH} mutations, \textit{TP53} mutations and 1p/19q co-deletions \cite{21,23,60}. In contrast, these tumors are most often defined genetically by the presence of activating alterations of \textit{BRAF}, an oncogene that regulates cell growth, proliferation and survival and is a member of the ERK/MAPK pathway \cite{61}. A subset of pilocytic astrocytomas (60-70\%) demonstrate a specific fusion event between \textit{BRAF} and \textit{KIAA1549} (\textit{BRAF:KIAA1549}) that results from a tandem duplication at 7q34 and leads to BRAF activation \cite{60,62}. This fusion event occurs more commonly in tumors that are midline and infratentorial in location. \textit{BRAF} V600E missense mutations also occur in pilocytic astrocytomas, but are not as common (9\%) \cite{61,63}. While BRAF alterations are most common in sporadic pilocytic astrocytomas, they are not characteristic of pilocytic astrocytomas that arise in the setting of neurofibromatosis 1, since ERK/MAPK pathway activation in these tumors is directly due to the loss of proper NF1 function \cite{11}.

\textit{BRAF} (V600E) mutations occur frequently in PXAs (60\%) and gangliogliomas (50\%), and like \textit{BRAF} fusions, result in activation of the ERK/MAPK pathway \cite{61,63,64}. Although the histologic diagnosis of these tumors is straightforward in classic cases, the finding of \textit{BRAF} mutations may be helpful in distinguishing these low grade tumors from
diffusely infiltrative gliomas in diagnostically challenging cases, and could be clinically important given the contrast in prognosis and treatment between these groups\textsuperscript{60,61}.

**Ancillary Studies Used in the Diagnosis of Gliomas**

Ancillary studies such as immunohistochemistry, and molecular and cytogenetic testing are now used routinely in the diagnosis of gliomas\textsuperscript{10}. Immunostains commonly used to determine lineage and prognostic subsets of the diffuse gliomas include those that recognize the IDH1 mutant protein, ATRX and the p53 protein\textsuperscript{29}. Immunohistochemistry for IDH1 mutant protein is positive in those gliomas that have the R132H mutation, which accounts for over 90% of all $IDH$ mutations in diffuse gliomas\textsuperscript{65}. Because immunohistochemistry can detect rare positive cells among abundant non-neoplastic ones, this method has comparable sensitivity to PCR or sequencing\textsuperscript{23,66-68}. Since immunohistochemistry is less expensive and widely available, it is often used in favor of other methods. However, this antibody only recognizes the R132H IDH1 mutation, so that other less common $IDH1$ mutations and all $IDH2$ mutations, albeit rare, will be missed with this test. Since the designation of a diffuse glioma in adults as $IDH$ wild type has taken on heightened clinical and therapeutic significance, a negative immunohistochemical test for the IDH mutant protein should be followed by mutational analysis of $IDH1$ and $IDH2$.

The p53 immunostain is targeted against the normal p53 protein. $TP53$ mutation leads to decreased degradation of p53 protein oligomers, which includes mutant and wild-type gene products, allowing their immunohistochemical detection within the nucleus. $TP53$ mutations are frequent in grade II and grade III astrocytomas, but not in
oligodendrogliomas and are nearly mutually exclusive with 1p/19 co-deletion. The p53 immunostain is therefore useful as a marker for astrocytic differentiation, with strong, diffuse staining favoring the diagnosis of astrocytoma over oligodendroglioma. Although this immunostain can be a useful aid in determining lineage, it is not entirely specific for TP53 mutations or for neoplastic disease, since the p53 protein can be upregulated by other mechanisms$^{69,70}$. Mutations and inactivating deletions of ATRX are a marker of astrocytic lineage among the IDH mutant gliomas and are mutually exclusive with 1p/19q co-deletion. Immunohistochemistry for ATRX demonstrates a loss of protein expression in neoplastic nuclei that harbor mutations, while expression is retained in non-neoplastic cells within the sample (e.g., endothelial cells).$^{29}$ Among the pediatric gliomas, antibodies to specific histone marks (H3K27me3) for high grade gliomas and to mutant BRAF (V600E) are useful for accurately subtyping disease.

Promoter methylation analysis of MGMT is performed routinely for high grade astrocytomas, typically by methylation-specific PCR$^{47}$. While immunohistochemistry for MGMT protein expression is also available, correlations with PCR results, response to therapy and patient survival have not been strong$^{71-75}$. Fluorescence in situ hybridization (FISH) is commonly used to assess copy number alterations of single loci, including EGFR and PDGFRA amplification, PTEN and CDKN2A deletions in high grade astrocytomas and 1p/19q co-deletion in oligodendrogliomas. As molecular techniques become more advanced and the number of clinically relevant molecular alterations increases, molecular diagnostic labs will continue to transition towards a panel-based assessment of mutations and high
resolution, whole genome analysis of copy number alterations. Genes that will need to be considered for inclusion in a diagnostic panel approach for gliomas include \textit{IDH1}, \textit{IDH2}, \textit{TP53}, \textit{ATRX}, \textit{CIC}, \textit{FUBP1}, \textit{TERT} promoter, \textit{NOTCH1}, \textit{PIK3CA}, \textit{PIK3R1}, \textit{EGFR}, \textit{PDGFRA}, \textit{PTEN}, \textit{NF1}, \textit{RB1}, \textit{H3F3A}, \textit{DAXX} and \textit{BRAF}. Whole genome assessment of copy number alterations is capable of detecting 1p/19q status; chromosome 7 gains and chromosome 10 losses; amplifications of \textit{EGFR}, \textit{PDGFRA} and \textit{c-MET}; deletions of \textit{PTEN} and \textit{CDKN2A/B}; alterations of \textit{BRAF} and other less frequent gains, losses and unbalanced translocations.

**Future Direction of Diagnosis and Management of Gliomas**

Because gliomas are increasingly being defined by their molecular profiles, biomarkers will be relied on to a greater extent to establish neuropathologic diagnoses. For example in the guidelines established by an international group of neuropathologists for the 4\textsuperscript{th} Edition of the WHO Classification of Central Nervous System Tumors, it was concluded that histology alone may be sufficient for the diagnosis of some tumors, while others will require a more “integrated” diagnosis, which would include the histological classification, the WHO grade and the results of molecular studies.\textsuperscript{76} In this layered format, the “Integrated Diagnosis” would form the top line, and while underneath would be categories for histological classification, WHO grade and molecular data. For each tumor entity, it will be determined whether molecular information would be necessary, suggested or not needed for the diagnosis. Furthermore, if molecular testing is needed or suggested, entity-specific guidelines will provide a list of appropriate molecular tests and a format for reporting results. Another important recommendation was that
selected pediatric tumors be separated from their histologically identical adult counterparts, on the basis of their distinct molecular profiles. It is hoped that these recommendations, once in practice, will lead to more objective, reproducible diagnoses that reflect the biological nature of each tumor.
References:


**Figure Legend**

**Figure 1.** Overview of altered genes in specific types of gliomas. AA = anaplastic astrocytoma, GBM = glioblastoma, PXA = pleomorphic xanthoastrocytoma.