BlueCross BlueShield of North Carolina

An independent licensee of the Blue Cross and Blue Shield Association

Corporate Medical Policy

Molecular Analysis for Gliomas AHS - M2139

File Name:	molecular_analysis_for_gliomas
Origination:	1/1/2019
Last CAP Review:	3/2021
Next CAP Review:	3/2022
Last Review:	10/2021

Description of Procedure or Service

Glioma refers to tumors resulting from metaplastic transformation of glial tissue of the nervous system. Tumors have historically been classified by the retained histologic features of the three types of glial cells: astrocytes, oligodendrocytes, and ependymal cells. Tumors of each type can vary widely in aggressiveness, response to treatment and prognosis (D. Louis, Schiff, & Batchelor, 2020).

Molecular genetic features were added to histopathologic appearance in the current WHO classification to yield more biologically homogeneous and narrowly defined diagnostic entities for greater diagnostic accuracy, improved patient management, more accurate determinations of prognosis, and better treatment response (D. Louis et al., 2016, D.N. Louis et al., 2021).

Related Policies:
Molecular Panel Testing of Cancers for Diagnosis, Prognosis, and Identification of Targeted Therapy AHS-M2109

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for molecular analysis for gliomas when it is determined to be medically necessary because the medical criteria and guidelines shown below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Molecular Analysis for Gliomas is covered

The use of the following tests for the prognosis of malignant gliomas is considered **medically necessary:**

a. *MGMT* promoter methylation testing;
b. *IDH1* and *IDH2* testing for prognosis of malignant gliomas;
c. *ATRX* mutation testing via EITHER immunohistochemistry OR gene sequencing;
d. Genetic sequencing of *TERT*;
e. H3F3A testing using a K27M histone antibody;
f. *H3F3A* and *HIST1H3B* gene sequencing for prognosis of suspected midline gliomas;
g. *BRAF* fusion and mutation testing, including *BRAF V600E* common variant;

Reimbursement is allowed for RELA fusion testing using either RNA sequencing analysis (RNA-Seq) or fluorescent in situ hybridization (FISH)

Reimbursement is allowed for testing for the co-deletion of 1p and 19q by either fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) for the characterization of gliomas and/or to guide treatment decisions;

Reimbursement is allowed for ATRX mutation testing via immunohistochemistry

When Molecular Analysis for Gliomas is not covered

Reimbursement is not allowed for ATRX mutation co-testing using BOTH immunohistochemistry and gene sequencing.

Policy Guidelines

According to the American Cancer Society, an estimated 24,530 adults in the United States will be diagnosed with primary cancerous tumors of the brain and spinal cord in 2021. Further, an additional 3,640 children under the age of 15 will be diagnosed. (American Cancer Society, 2021). As the 10th leading cause of death in both men and women, approximately 18,600 adults are estimated to die from primary cancerous brain and CNS tumors in 2021 (American Cancer Society, 2021).

Studies over the past two decades have clarified the genetic basis of tumorigenesis in the common and some rarer brain tumor entities (D. Louis et al., 2016), and identified clinically relevant molecular genetic characterizations which complement standard histologic analysis providing additional diagnostic and prognostic information to improve diagnostic accuracy, influence treatment selection and improve survival. Molecular and/or genetic characterization do not replace standard histologic assessment, but rather serve as a complimentary approach (NCCN, 2020).

Isocitrate dehydrogenase (IDH1/2) mutations

IDH 1 and *2* are metabolic enzymes which oxidize isocitrate to alpha-keoglutarate and are important in the mitigation of cellular oxidative damage (Horbinski, 2013b). Mutations in genes encoding these enzymes leads to the aberrant production of D-2 hydroxyglutarate (Dang et al., 2009), an oncometabolite that causes epigenetic modifications in affected cells (Horbinski, 2013b).

IDH mutations are a defining feature of WHO grade II and III astrocytomas and oligodendrogliomas (D.Louis et al., 2016). Their presence distinguishes lower grade gliomas from primary glioblastomas, which are *IDH* wild type. *IDH* mutations are commonly associated with *MGMT* promoter methylation and associated with a relatively favorable prognosis (Brat et al., 2015; Eckel-Passow et al., 2015).

O-6-methylguanine-DNA methyltransferase (MGMT) methylation

MGMT is a DNA repair enzyme that reverses the DNA damage caused by alkylating agents, resulting in tumor resistance to temozolomide and nitrosourea-based chemotherapy. Methylation of the *MGMT* promoter silences MGMT making the tumor more sensitive to treatment with alkylating agents (Esteller et al., 2000; Gusyatiner & Hegi, 2018).

MGMT promoter methylation is strongly associated with IDH mutation and genome wide epigenetic change (Eckel-Passow et al., 2015), and with longer survival in patients with glioblastoma who receive alkylating agents (Hegi et al., 2005; Zhao, Wang, Song, Zha, & Li, 2016). *MGMT* promoter methylation is particularly useful in treatment decisions for elderly patients with high grade gliomas (Malmstrom et al., 2012; Wick et al., 2012; Wick et al., 2014).

Codeletion of 1p and 19q

This codeletion represents an unbalanced translocation (1;19)(q10;p10) leading to whole arm deletion of 1p and 19q (Jenkins et al., 2006).

Codeletion of 1p and 19q a defining feature of oligodendroglial tumors, strongly associated with oligodendroglial histology, and helps confirm the oligodendroglial character of tumors with equivocal or mixed histologic features (Brat et al., 2015; Burger et al., 2001; Eckel-Passow et al., 2015). Combined loss involving chromosomes 1p and 19q is significantly associated with both favorable therapeutic response and longer recurrence-free survival after chemotherapy (Cairncross et al., 1998).

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutations

Mutations in the chromatin regulator gene, *ATRX*, enable alternative lengthening of telomeres (Abedalthagafi et al., 2013). *ATRX* is a switch/sucrose helicase that assists with H3.3 chromatin deposition in telomeric regions. Disruption of this gene leads to the alternative lengthening of telomeres stated above and is thought to represent an early event in gliomagenesis (Batchelor, 2020).

ATRX mutations in glioma are strongly associated with *IDH* and *TP53* mutations and are nearly always mutually exclusive with 1p19q codeletion (Reuss et al., 2015). *ATRX* deficiency, coupled with *IDH* mutation, is typical of astrocytoma (Brat et al., 2015).

Tumor protein 53 (TP53) mutation

TP53 is essential for regulating cell division and preventing tumor formation (Parikh et al., 2014). Missense mutations in the *TP53* gene are present in the clear majority of IDH-mutant astrocytomas (Brat et al., 2015). Immunopositivity for mutant p53 is not entirely sensitive or specific for *TP 53* mutation, however, and loss of *ATRX* expression may be a more reliable marker of astrocytic differentiation (Louis et al., 2019).

Telomerase reverse transcriptase (TERT) mutations

TERT encodes the catalytic active site of telomerase, the enzyme responsible for maintaining telomere length in dividing cells. *TERT* mutations in its noncoding promoter region cause increased expression of the *TERT* protein and are one of the major mechanisms of telomerase activation in gliomas (Arita et al., 2013). *TERT* mutations are strongly associated with 1p19q codeletion and are found in most glioblastomas. *TERT* mutation in combination with IDH mutation and 1p19q codeletion is characteristic of oligodendroglioma. Absence of *TERT* mutation, coupled with IDH mutation, designates astrocytoma(Eckel-Passow et al., 2015). In terms of survival, mutation in the *TERT* promoter is generally unfavorable in the absence of *IDH* mutation and favorable in the presence of *IDH* mutation and 1p/19q codeletion. *TERT* promoter mutation is associated with an older age of the patient at presentation, regardless of whether *IDH* mutation is present (Eckel-Passow et al., 2015).

Histone (H3FA) mutations

A lysine to methionine substitution in the *H3F3A* gene *(H3K27M)* is the most common histone mutation in brain tumors and inhibits the trimethylation of H3.3 histone (Sturm et al., 2012) arresting cells in a primitive state refractory to differentiation induction(Weinberg, Allis, & Lu, 2017). *G34R/G34V* mutations are more common in cortical gliomas in children (Schwartzentruber et al., 2012). *H3FA* mutations can be useful in the diagnosis of infiltrative glioma (Sturm et al., 2012). The *H3K27M* mutation is an adverse prognostic marker in children and adults (Meyronet et al., 2017). The G34 mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established (Sturm et al., 2012).

A similar mutation to *H3K27M* may also occur in the *HIST1H3B/C* gene, which encodes the histone H3.1 variant. However, the mutation at *HIST1H3B/C* is about one third as common as *H3F3A* and often confers a better prognosis than its *H3F3A* counterpart (Louis et al., 2019).

B-Raf proto-oncogene (BRAF) mutations

BRAF is a serine-threonine protein kinase involved in cell survival, proliferation, and differentiation(Davies et al., 2002). Activating mutations in BRAF, most often V600E have been discovered in most pediatric and some adult gliomas (Chappe et al., 2013; Horbinski, 2013a) including approximately 80% of pleomorphic xanthoastrocytomas, 20% of gangliogliomas, 10% of pilocytic astrocytomas and occasionally diffuse gliomas (Chi et al., 2013). Tandem duplication of chromosome 7q34 resulting in an activating fusion of the *BRAF* and *KIAA1549* genes occur in 60-80% of pilocytic astrocytoma (Jones et al., 2008).

The presence of a *BRAF* fusion is reliable evidence that the tumor is a pilocytic astrocytoma and predicts better clinical outcome (Hawkins et al., 2011). *BRAF* mutation is more complicated, as it can occur in a variety of tumors and requires integration with histology. Tumors with *BRAF* mutations may respond to *BRAF* inhibitors, however in pediatric gliomas, *BRAFV600E* indicates poor prognosis when treated with current adjuvant therapy, especially in combination with *CDKN2A* mutation (Lassaletta et al., 2017).

v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA, p65, NFKB3) fusion

Fusion between the *C11orf95* and *RELA* genes defines approximately 70 percent of all childhood supratentorial ependymomas (D. Louis et al., 2020). These fusions are associated with increased NF-kappa-B signaling and poor outcome (Malgulwar et al., 2018). Normally, NFkB is an inactive transcription factor in the cytoplasm. When its inhibitor degrades, it activates transcription of certain genes, *RELA* among them (NCBI, 2011).
New research supports the hypothesis that the status of *RELA* fusion and p53 overexpression are significantly associated with the prognosis of supratentorial extraventricular ependymomas (Wang et al., 2019).

New Tests

Assessment of gliomas is incredibly difficult, and new methods of molecular analyses for gliomas are consistently being developed. For example, Miller et al. (2019) devised a liquid-biopsy based method to evaluate cerebrospinal fluid from 42 (of 85) patients. The genomic profile developed from the cerebrospinal fluid (CSF) samples closely matched established profiles, such as the characteristic 1p/19q codeletion and *IDH1/2* mutations. The authors stated that the ability to monitor the glioma genome in real time could be useful in management of this condition (Miller et al., 2019). Other researchers report that "A cerebrospinal fluid ct-DNA liquid biopsy approach may virtually support all the stages of glioma management, from facilitating molecular diagnosis when surgery is not feasible, to monitoring tumor response, identifying early recurrence, tracking longitudinal genomic evolution, providing a new molecular characterization at recurrence and allowing patient selection for targeted therapies" (Simonelli et al., 2020).

Clinical Validity and Utility

Nikiforova et al. (2016) validated GlioSeq, a commercial next generation sequencing (NGS) panel of 30 genes, in 54 patients with CNS tumors against fluorescence in-situ hybridization (FISH), Sanger sequencing, and reverse transcription polymerase chain reaction (PCR). GlioSeq correctly identified 71/71 (100%) genetic alterations known to be present by conventional techniques. The assay sensitivity was 3%-5% for mutant alleles of single nucleotide variants (SNVs), and 1%-5% for gene fusions. Likewise, Zacher et al. (2017) developed an NGS panel of 20 genes that allowed for molecular classification of 121 gliomas. The researchers conclude that gene panel NGS is a promising diagnostic technique that may facilitate integrated histological and molecular glioma classification.

Ramkissoon et al. (2017) used OncoPanel and OncoCopy to identify targetable alterations in tumors for the establishment of best practices in routine clinical pediatric oncology. They analyzed 117 samples by OncoPanel and 146 by OncoCopy; further, 60 tumors were subjected to both methodologies. OncoPanel revealed clinically relevant alterations in 56% of patients (44 cancer mutations and 20 rearrangements), including *BRAF* alterations that directed the use of targeted inhibitors. Rearrangements in *MYB-QKI, MYBL1, BRAF*, and *FGFR1* were also detected. Furthermore, while copy number profiles differed across histologies, the combined use of OncoPanel and OncoCopy identified subgroup-specific alterations in 89% (17/19) of medulloblastomas.

Ryall et al. (2016) evaluated the prognostic impact of H3K27M and MAPK pathway aberrations in 64 gliomas (44 low grade, 22 high grade). Tumors are designated as low-grade if the cells are well differentiated, are less aggressive overall, and suggest a better prognosis for the patient. Five low grade gliomas contained the *H3F3A/HIST1H3B K27M* (*H3K27M*) mutation, and 11 high grade gliomas contained the *H3K27M* mutation. Survival analysis evaluated the median survival at 9.12 years for wildtype H3 patients compared to 1.02 years for patients with the *H3K27M* mutation. MAPK pathway mutations (through *BRAF* or *FGFR1* mutation) were associated with long-term survival in absence of *H3K27M* mutations. Further, H3K27M status and high-grade histology were found to be the most significant independent predictors of poor overall survival with hazard ratios of 6.945 and 7.721 respectively. MAPK pathway activation is considered as a predictor of "favorable patient outcome," but dependent on other factors (Ryall et al., 2016).

Houdova Megova et al. (2017) evaluated the prognostic value of the *IDH1/2* mutation in glioblastomas. A total of 37 *IDH* mutations were examined and studied. The authors found that *IDH1* mutations were positively associated with *MGMT* methylation (odds ratio [OR]: 3.08), 1p/19q co-loss (OR: 8.85), and negatively associated with *EGFR* amplification. IDH-mutant patients had an overall survival of 25 months compared to only 9 months for IDH-wildtype gliomas (Houdova Megova et al., 2017).

Johnson et al. (2017) performed comprehensive genomic profiling of 282 pediatric gliomas:157 high-grade and 125 low-grade. The investigators used a 315 gene panel and calculated the tumor mutational burden (TMB). In low grade gliomas, *BRAF* was the most frequent mutation found (48%), followed by *FGFR* missense (17.6%), *NF1* loss of function (8.8%), and *TP53* (5.6%). Rearrangements were found in 35% of low-grade gliomas. In high-grade gliomas, *TP53* was the most frequent mutation found (49%), followed by *H3F3A* (37.6%), *ATRX* (24.2%), *NF1* (22.2%), and *PDGFRA* (21.7%). *H3F3A* mutations were found to be the K28M variant. Approximately 6% of the high-grade gliomas were found have a TMB of >20 mutations/Mb ("hypermutated") (Johnson et al., 2017).

Back et al. (2020) studied the pattern of failure in anaplastic glioma (AG) patients with an *IDH1/2* mutation. A total of 156 patients participated in the study, with data collected from 2008 to 2014; the median follow-up time was 5.1 years. Of all 156 patients, 75% were found to have an *IDH1 or IDH2* mutation. The authors concluded that "patients with *IDH*-mutated AG have improved outcomes"; however, this population also had a greater number of distant relapses approximately two years after intensity-modulated radiation therapy compared to individuals with *IDH* wild type mutations (Back et al., 2020).

Ji et al. (2021) studied the clinical utility of comprehensive genomic profiling to detect CNS tumors in children and young adults using the OncoKids next-generation sequencing panel, chromosomal microarray analysis, and germline testing. NGS was performed on 222 samples and CMA was performed on 146 of the 222 samples. The OncoKids NGS panel identified diagnostic biomarkers in 138/222 samples (62%), prognostic information in 49/222 cases (22%), and targetable genomic alterations in 41/222 samples (18%). Additionally, CMA revealed prognostic copy number alterations (CNA) in 101/146 cases (69%). Further, germline cancer predisposition testing was performed in 57 of 212 patients which identified 20 patients which a confirmed germline pathogenic/likely pathogenic variant of genes *TP53, NF1, SMARCB1, NF2, MSH6, PMS2*, and a patient with Klinefelter syndrome. Overall, the authors conclude that there is "significant clinical utility of integrating genomic profiling into routine clinical testing for pediatric and young adult patients with CNS tumors" (Ji et al., 2021).

Muralidharan et al. (2021) studied the diagnostic utility of a novel digital droplet PCR (ddPCR) assay for detection of two TERT promoter mutations (*C228T* and *C250T*) and monitoring of gliomas. In comparison with the gold-standard tumor tissue-based detection of *TERT* mutations, the ddPCR assay had an overall sensitivity of 62.5% and a specificity of 90%. Longitudinal monitoring of five patients demonstrated that the peripheral *TERT* mutant allele frequency reflects the clinical course of the disease. *TERT* mutant alleles decreased after surgical intervention and pharmacotherapy but increased with tumor progression. The authors conclude that the ddPCR assay has feasibility in "detecting circulating cfDNA TERT promoter mutations in patients with glioma with clinically relevant sensitivity and specificity" (Muralidharan et al., 2021).

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN, 2020, 2021)

The NCCN published Clinical Practice Guidelines in Oncology (2020) for Central Nervous System Cancers which recommend:

- ***IDH1* and *IDH2* mutation**

Recommendation: *IDH* mutation testing is required for the workup of glioma.
"The most common *IDH1* mutation (R132H) is reliably screened by mutation specific immunohistochemistry, which is recommended for all glioma patients. If the R132H immunostain result is negative, in the appropriate clinical context, sequencing of *IDH1* and *IDH2* is highly recommended to detect less common *IDH1* and *IDH2* mutations. Prior to age 55 years, sequencing of *IDH1* and *IDH2* is required if the R132H immunostain result is negative. Standard sequencing methods include Sanger sequencing, pyrosequencing, and next-generation sequencing, and should be performed on formalin fixed, paraffin embedded tissue (NCCN, 2020)."

- ***MGMT* promoter methylation**

Recommendation: *MGMT* promoter methylation is an essential part of molecular diagnostics for all high-grade gliomas (grade III and IV). The NCCN also notes that "*MGMT* promoter methylation is strongly associated with IDH mutations and other genome-wide epigenetic changes (G-CIMP phenotype)" (NCCN, 2020, 2021).
"There are multiple ways to test for *MGMT* promoter methylation, including methylation-specific PCR, methylation-specific high-resolution melting, pyrosequencing, and droplet-digital PCR" (NCCN, 2021)."

- **Codeletion of 1p and 19q**

Recommendation: 1p19q testing is an essential part of molecular diagnostics for oligodendroglioma.
"The codeletion of 1p and 19q is detectable by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR). Additional methods, including array based genomic copy number testing and next generation sequencing may also be employed… IDH mutated gliomas that do not show loss of *ATRX* (for example, by IHC) should be strongly considered for 1p19q testing even if not clearly oligodendroglial by histology. Conversely, *IDH1* wild type gliomas do not contain true whole-arm 1p19q codeletion. Therefore, 1p19q testing is unnecessary if a glioma is not *IDH*-mutant, and a glioma should not be regarded as 1p19q codeleted without an accompanying *IDH* mutation, regardless of the test results (NCCN, 2020, 2021)."

- ***ATRX* mutation**

"Recommendation: *ATRX* mutation testing is strongly recommended but not required for glioma."
"*ATRX* mutations can be detected by IHC for wild-type *ATRX* (loss of wild type expression) and/or sequencing. ATRX mutations in glioma are strongly associated with *IDH* mutations and are nearly always mutually exclusive with 1p/19q codeletion. *ATRX* deficiency, coupled with IDH mutation, is typical of astrocytoma. A lack of *ATRX* immunostaining in glioblastoma should trigger *IDH1/2* sequencing if *IDH1* R132H immunostaining is negative, due to frequent co-occurrence of *ATRX* and *IDH* mutations (NCCN, 2020)."

- ***TERT* mutation**

"Recommendation: *TERT* mutation testing is recommended but not required for gliomas. *TERT* mutation can be detected by sequencing of the promoter region (NCCN, 2020, 2021)."

- ***H3F3A* and *HIST1H3B* mutation**

"Recommendation: *H3F3A* and *HIST1H3B* mutation testing is recommended in the appropriate clinical context."
"Although a K27M histone antibody is available, it is not 100% specific and interpretation can be difficult for non-experts. Therefore, screening by *H3F3A* and *HIST1H3B* sequencing is a viable alternative and preferred approach, especially since it will also detect mutations in G34 (NCCN, 2020)."

"Histone mutations most commonly occur in pediatric midline gliomas (eg, diffuse intrinsic pontine gliomas [DIPG]), although midline gliomas in adults can also contain histone modifications. Their presence can be considered solid evidence of an infiltrative glioma, which is often helpful in small biopsies of midline lesions that may not be fully diagnostic with light microscopy or do not fully resemble infiltrative gliomas. The *K27M* gliomas typically do not have MGMT promoter methylation, and the mutation is an adverse prognostic marker in children and adults. The *G34* mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established (NCCN, 2021)."

- ***BRAF* mutation**

"Recommendation: *BRAF* fusion and/or mutation testing is recommended in the appropriate clinical context."
"*BRAF* V600E is best detected by sequencing, and *BRAF* fusions can be detected with RNA-Seq or other PCR-based breakpoint methods that capture the main 16-9, 15-9, and 16-11 breakpoints between *BRAF* and its main fusion partner, *KIAA1549*. FISH is too unreliable to detect *BRAF* fusions" (NCCN, 2020, 2021).
"The presence of a *BRAF* fusion is reliable evidence that the tumor is a pilocytic astrocytoma, provided the histology is compatible. *BRAF* V600E is more complicated, as it can occur in a variety of tumors over all four WHO grades and requires integration with histology. Tumors with *BRAF* fusions tend to be indolent, with occasional recurrence but only rare progression to lethality. *BRAF* V600E tumors show a much greater range of outcomes and need to be considered in context with other mutations and clinicopathologic findings (eg, CDKN2A/B deletion) (NCCN, 2020)."

- ***RELA* fusion**

"Recommendation: *RELA* fusion testing is recommended in the appropriate clinical context."
"The most common *RELA* fusion partner is C11orf95. This can be detected with RNASeq or a break apart FISH probe set. Detection of *RELA* fusion is not required for the diagnosis of ependymoma, as this entity is still diagnosed by light microscopy. *RELA* fusion-positive ependymomas are now a distinct entity in the WHO classification of CAN tumors, as this subset of ependymomas tends to be far more aggressive than other supratentorial ependymomas (NCCN, 2020)."

- **Miscellaneous**

Other markers have been suggested for various uses in evaluating gliomas. For example, other markers for subtyping grade II-III gliomas include assessment *of PTEN, TP53, NOTCH1, CIC, FUBP1, EGFR*, chromosome 7 gain, and chromosome 10 loss. However, these markers are not currently widely accepted as markers for gliomas.
Finally, the NCCN states there are no identified targeted agents with demonstrated efficacy in glioblastoma (NCCN, 2020, 2021).

National Institute for Health and Care Excellence (NICE, 2021)
NICE recommends the following molecular markers for investigation of gliomas: *IDH1/2* mutations, *ATRX* mutations, 1p/19q co-deletion, histone H3.3 K27M, *BRAF* mutation, and *MGMT* promoter methylation (for prognosis). NICE also notes that testing *IDH* wild type gliomas for *TERT* promoter mutations may be considered (NICE, 2021).

European Society for Medical Oncology (ESMO, 2014)
The ESMO has published clinical practice guidelines for the diagnosis, treatment, and follow-up of high-grade gliomas. They state that *MGMT* promoter methylation status, *IDH1/2* mutation status, and 1p/19q codeletions are "commonly determined" for assessment of gliomas (ESMO, 2014).

World Health Organization (WHO) (Wen & Huse, 2016)
In 2016, the WHO published guidelines on the classification of central nervous system tumors. These WHO guidelines, for the first time, incorporated molecular testing in the diagnosis of gliomas and medulloblastomas. The following key points were given by the WHO regarding molecular testing:

- "*IDH1* R132H, which accounts for approximately 90% of *IDH* mutations, can be detected immunohistochemically. If this testing is negative, sequencing of *IDH1* and *IDH2* is necessary to ensure that no other *IDH* mutations are present.

- Given the importance of *IDH* mutational status in the diagnosis or gliomas, at a minimum, it will be important that most institutions have the capacity to both stain tumor specimens for *IDH1* R132H by immunohistochemistry and, ideally, sequence those tumors that are negative for both *IDH1* and *IDH2* mutations" (Wen & Huse, 2016).
- "Because of the growing importance of molecular information in CNS tumor classification, diagnoses and diagnostic reports need to combine different data types into a single, 'integrated' diagnosis. To display the full range of diagnostic information available, the use of layered (or tiered) diagnostic reports is strongly encouraged. Such reports feature an integrated diagnosis at the top, followed by layers that display histological, molecular, and other key types of information" (D. N. Louis et al., 2021).
- Certain tumors (Diffuse astrocytoma, MYB- or MYBL1-altered; Angiocentric glioma; Polymorphous low-grade neuroepithelial tumor of the young; and Diffuse low-grade glioma, MAPK pathway-altered) "require molecular characterization and the integration of histopathological and molecular information in a tiered diagnostic format as molecular work-up helps to characterize the lesion as one type or the other" (D. N. Louis et al., 2021).
- For other tumors such as Myxopapillary ependymoma and Subependymoma, "although these can be identified with methylome studies, molecular classification does not provide added clinicopathological utility for these 2 tumors" (D. N. Louis et al., 2021).
- "Several molecular biomarkers are also associated with classification and grading of meningiomas, including SMARCE1 (clear cell subtype), BAP1 (rhabdoid and papillary subtypes), and KLF4/TRAF7 (secretory subtype) mutations, TERT promoter mutation and/or homozygous deletion of CDKN2A/B62, H3K27me3 loss of nuclear expression (potentially worse prognosis), and methylome profiling (prognostic subtyping)" (D. N. Louis et al., 2021).

European Association of Neuro-Oncology (EANO) (Weller et al., 2021)

In 2021, the EANO published guidelines regarding diagnosis and management of adult patients with diffuse gliomas. The following recommendations were made on molecular testing:

- "Patients with relevant germline variants or suspected hereditary cancer syndromes should receive genetic counselling and might subsequently be referred for molecular genetic testing.
- Immunohistochemistry for mutant *IDH1* R132H protein and nuclear expression of *ATRX* should be performed routinely in the diagnostic assessment of diffuse gliomas.
- If immunohistochemistry for *IDH1* R132H is negative, sequencing of *IDH1* codon 132 and *IDH2* codon 172 should be conducted in all WHO grade 2 and 3 diffuse astrocytic and oligodendroglial gliomas as well as in all glioblastomas of patients aged < 55 years to enable integrated diagnoses according to the WHO classification and to guide treatment decisions.
- 1p/19q codeletion status should be determined in all *IDH*-mutant gliomas with retained nuclear expression of *ATRX*.
- *MGMT* promoter methylation status should be determined in glioblastoma, notably in elderly or frail patients, to aid in decision-making for the use of temozolomide.
- *CDKN2A/B* homozygous deletions should be explored in *IDH*-mutant astrocytomas.
- Combined chromosome 7 gain and chromosome 10 loss (+7/–10 signature), *EGFR* amplification and *TERT* promoter mutation should be tested in *IDH*-wild-type diffuse gliomas lacking microvascular proliferation and necrosis as histological features of WHO grade 4 to allow for a diagnosis of *IDH*-wild-type glioblastoma" (Weller et al., 2021).

In addition, EANO published a table to summarize the molecular markers used for the diagnosis and management of gliomas.

Table 1: Molecular Markers for the Diagnosis and Management of Gliomas (Weller et al., 2021)

Molecular Marker	Diagnostic Roles
IDH1 R132 or IDH2 R172 mutation	"Distinguishes diffuse gliomas with IDH mutation from IDH-wild-type glioblastomas and other IDH-wild-type gliomas
1p/19q codeletion	Distinguishes oligodendroglioma, IDH-mutant and 1p/19q-codeleted from astrocytoma, IDH-mutant

Loss of nuclear ATRX	Loss of nuclear ATRX in an IDH-mutant glioma is diagnostic for astrocytic lineage tumours
Histone H3 K27M mutation	Defining molecular feature of diffuse midline glioma, H3 K27M-mutant
Histone H3.3 G34R/V mutation	Defining molecular feature of diffuse hemispheric glioma, H3.3 G34-mutant
MGMT promoter methylation	None, but is a predictive biomarker of benefit from alkylating chemotherapy in patients with IDH-wild-type glioblastoma
Homozygous deletion of CDKN2A/CDKN2B	A marker of poor outcome and WHO grade 4 disease in IDH-mutant astrocytomas

State and Federal Regulations

No diagnostic tests have been specifically approved for use in detecting mutations in gliomas as of August 7, 2021. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 81120, 81121, 81210, 81287, 81345, 81479, 88341, 88342. 88374, 88377

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

Abedalthagafi, M., Phillips, J. J., Kim, G. E., Mueller, S., Haas-Kogen, D. A., Marshall, R. E., Perry, A. (2013). The alternative lengthening of telomere phenotype is significantly associated with loss of ATRX expression in high-grade pediatric and adult astrocytomas: a multi-institutional study of 214 astrocytomas. Mod Pathol, 26(11), 1425-1432. doi:10.1038/modpathol.2013.90

American_Cancer_Society. (2021). Cancer Facts & Figures 2021. Retrieved from https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf

Arita, H., Narita, Y., Takami, H., Fukushima, S., Matsushita, Y., Yoshida, A., Ichimura, K. (2013). TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. Acta Neuropathol, 126(6), 939-941. doi:10.1007/s00401-013-1203-9

Back, M., Jayamanne, D., Brazier, D., Newey, A., Bailey, D., Schembri, G., . . . Wheeler, H. (2020). Pattern of failure in anaplastic glioma patients with an IDH1/2 mutation. *Strahlenther Onkol, 196*(1), 31-39. doi:10.1007/s00066-019-01467-0

Batchelor, T., Louis, David. (2020). Molecular pathogenesis of diffuse gliomas. Retrieved from https://www.uptodate.com/contents/molecular-pathogenesis-of-diffuse-gliomas?sectionName=ATRX%20gene&search=medulloblastoma%20glioma&topicRef=5211&anchor=H67101883&source=see_link#H67101883

Brat, D. J., Verhaak, R. G., Aldape, K. D., Yung, W. K., Salama, S. R., Cooper, L. A., Radenbaugh, A. J. (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. N Engl J Med, 372(26), 2481-2498. doi:10.1056/NEJMoa1402121

Burger, P. C., Minn, A. Y., Smith, J. S., Borell, T. J., Jedlicka, A. E., Huntley, B. K.,

Cairncross, J. G., Ueki, K., Zlatescu, M. C., Lisle, D. K., Finkelstein, D. M., Hammond, R. R., Louis, D. N. (1998). Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst, 90(19), 1473-1479.

Chappe, C., Padovani, L., Scavarda, D., Forest, F., Nanni-Metellus, I., Loundou, A., Figarella-Branger, D. (2013). Dysembryoplastic neuroepithelial tumors share with pleomorphic xanthoastrocytomas and gangliogliomas BRAF(V600E) mutation and expression. Brain Pathol, 23(5), 574-583. doi:10.1111/bpa.12048

Chi, A. S., Batchelor, T. T., Yang, D., Dias-Santagata, D., Borger, D. R., Ellisen, L. W., Louis, D. N. (2013). BRAF V600E mutation identifies a subset of low-grade diffusely infiltrating gliomas in adults. J Clin Oncol, 31(14), e233-236. doi:10.1200/jco.2012.46.0220

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Su, S. M. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 462(7274), 739-744. doi:10.1038/nature08617

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. Nature, 417(6892), 949-954. doi:10.1038/nature00766

Eckel-Passow, J. E., Lachance, D. H., Molinaro, A. M., Walsh, K. M., Decker, P. A., Sicotte, H., Jenkins, R. B. (2015). Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. N Engl J Med, 372(26), 2499-2508. doi:10.1056/NEJMoa1407279

ESMO. (2014). High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Retrieved from https://www.annalsofoncology.org/article/S0923-7534(19)34077-3/fulltext

Esteller, M., Garcia-Foncillas, J., Andion, E., Goodman, S. N., Hidalgo, O. F., Vanaclocha, V., Herman, J. G. (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med, 343(19), 1350-1354. doi:10.1056/nejm200011093431901

Gusyatiner, O., & Hegi, M. E. (2018). Glioma epigenetics: From subclassification to novel treatment options. Semin Cancer Biol, 51, 50-58. doi:10.1016/j.semcancer.2017.11.010

Hawkins, C., Walker, E., Mohamed, N., Zhang, C., Jacob, K., Shirinian, M., Tabori, U. (2011). BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. Clin Cancer Res, 17(14), 4790-4798. doi:10.1158/1078-0432.ccr-11-0034

Hegi, M. E., Diserens, A. C., Gorlia, T., Hamou, M. F., de Tribolet, N., Weller, M., Stupp, R. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med, 352(10), 997-1003. doi:10.1056/NEJMoa043331

Horbinski, C. (2013a). To BRAF or not to BRAF: is that even a question anymore? J Neuropathol Exp Neurol, 72(1), 2-7. doi:10.1097/NEN.0b013e318279f3db

Horbinski, C. (2013b). What do we know about IDH1/2 mutations so far, and how do we use it? Acta Neuropathol, 125(5), 621-636. doi:10.1007/s00401-013-1106-9

Houdova Megova, M., Drabek, J., Dwight, Z., Trojanec, R., Koudelakova, V., Vrbkova, J., . . . Hajduch, M. (2017). Isocitrate Dehydrogenase Mutations are Better Prognostic Marker than O6-methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastomas - a Retrospective, Single-centre Molecular Genetics Study of Gliomas. *Klin Onkol, 30*(5), 361-371. doi:10.14735/amko2017361

Jenkins, R. B., Blair, H., Ballman, K. V., Giannini, C., Arusell, R. M., Law, M., Buckner, J. C. (2006). A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res, 66(20), 9852-9861. doi:10.1158/0008-5472.can-06-1796

Ji, J., Kaneva, K., Hiemenz, M. C., Dhall, G., Davidson, T. B., Erdreich-Epstein, A., . . . Biegel, J. A. (2021). Clinical utility of comprehensive genomic profiling in central nervous system tumors of children and young adults. *Neuro-Oncology Advances, 3*(1). doi:10.1093/noajnl/vdab037

Johnson, A., Severson, E., Gay, L., Vergilio, J. A., Elvin, J., Suh, J., . . . Ramkissoon, S. H. (2017). Comprehensive Genomic Profiling of 282 Pediatric Low- and High-Grade Gliomas Reveals Genomic Drivers, Tumor Mutational Burden, and Hypermutation Signatures. *Oncologist, 22*(12), 1478-1490. doi:10.1634/theoncologist.2017-0242

Jones, D. T., Kocialkowski, S., Liu, L., Pearson, D. M., Backlund, L. M., Ichimura, K., & Collins, V. P. (2008). Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res, 68(21), 8673-8677. doi:10.1158/0008-5472.can-08-2097

Lassaletta, A., Zapotocky, M., Mistry, M., Ramaswamy, V., Honnorat, M., Krishnatry, R., Tabori, U. (2017). Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas. J Clin Oncol, 35(25), 2934-2941. doi:10.1200/jco.2016.71.8726

Louis, D., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol, 131(6), 803-820. doi:10.1007/s00401-016-1545-1

Louis, D., Schiff, D., & Batchelor, T. (2018). Classification and pathologic diagnosis of gliomas - UpToDate. In A. Eichler (Ed.), UpToDate. Waltham, MA: UpToDate, Inc. Retrieved from https://www.uptodate.com/contents/classification-and-pathologic-diagnosis-of-gliomas?search=gliomas&source=search_result&selectedTitle=1~142&usage_type=default&display_rank=1.

Malgulwar, P. B., Nambirajan, A., Pathak, P., Faruq, M., Rajeshwari, M., Singh, M., Sharma, M. C. (2018). C11orf95-RELA fusions and upregulated NF-KB signalling characterise a subset of aggressive supratentorial ependymomas that express L1CAM and nestin. J Neurooncol, 138(1), 29-39. doi:10.1007/s11060-018-2767-y

Malmstrom, A., Gronberg, B. H., Marosi, C., Stupp, R., Frappaz, D., Schultz, H., Henriksson, R. (2012). Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol, 13(9), 916-926. doi:10.1016/s1470-2045(12)70265-6

Meyronet, D., Esteban-Mader, M., Bonnet, C., Joly, M. O., Uro-Coste, E., Amiel-Benouaich, A., Ducray, F. (2017). Characteristics of H3 K27M-mutant gliomas in adults. Neuro Oncol, 19(8), 1127-1134. doi:10.1093/neuonc/now274

Miller, A. M., Shah, R. H., Pentsova, E. I., Pourmaleki, M., Briggs, S., Distefano, N., . . . Mellinghoff, I. K. (2019). Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid.

Muralidharan, K., Yekula, A., Small, J. L., Rosh, Z. S., Kang, K. M., Wang, L., . . . Carter, B. S. (2021). TERT Promoter Mutation Analysis for Blood-Based Diagnosis and Monitoring of Gliomas. *Clinical Cancer Research, 27*(1), 169-178. doi:10.1158/1078-0432.Ccr-20-308

NCBI. (2011). RELA - RELA proto-oncogene, NF-kB subunit. Retrieved from https://www.ncbi.nlm.nih.gov/gene/5970/ortholog/?scope=117570

NCCN. (2020, 03/05/2020). NCCN Clinical Practice Guidelines in Oncology; Central Nervous System Cancers v2.2020. Retrieved from https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf

NCCN. (2021, 03/05/2021). NCCN Clinical Practice Guidelines in Oncology; Central Nervous System Cancers v1.2021. Retrieved from https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf

NICE. (2021). Brain tumours (primary) and brain metastases in adults. Retrieved from https://www.nice.org.uk/guidance/ng99/chapter/Recommendations

Nikiforova, M. N., Wald, A. I., Melan, M. A., Roy, S., Zhong, S., Hamilton, R. L., Horbinski, C. (2016). Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. Neuro Oncol, 18(3), 379-387. doi:10.1093/neuonc/nov289

Parikh, N., Hilsenbeck, S., Creighton, C. J., Dayaram, T., Shuck, R., Shinbrot, E., Donehower, L. A. (2014). Effects of TP53 Mutational Status on Gene Expression Patterns Across Ten Human Cancer Types. J Pathol, 232(5), 522-533. doi:10.1002/path.4321

Ramkissoon, S. H., Bandopadhayay, P., Hwang, J., Ramkissoon, L. A., Greenwald, N. F., Schumacher, S. E., Ligon, K. L. (2017). Clinical targeted exome-based sequencing in combination with genome-wide copy number profiling: precision medicine analysis of 203 pediatric brain tumors. Neuro Oncol, 19(7), 986-996. doi:10.1093/neuonc/now294

Reuss, D. E., Sahm, F., Schrimpf, D., Wiestler, B., Capper, D., Koelsche, C., von Deimling, A. (2015). ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta Neuropathol, 129(1), 133-146. doi:10.1007/s00401-014-1370-3

Ryall, S., Krishnatry, R., Arnoldo, A., Buczkowicz, P., Mistry, M., Siddaway, R., . . . Hawkins, C. (2016). Targeted detection of genetic alterations reveal the prognostic impact of H3K27M and MAPK pathway aberrations in paediatric thalamic glioma. *Acta Neuropathol Commun, 4*(1), 93. doi:10.1186/s40478-016-0353-0

Schwartzentruber, J., Korshunov, A., Liu, X. Y., Jones, D. T., Pfaff, E., Jacob, K., Jabado, N. (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature, 482(7384), 226-231. doi:10.1038/nature10833

Simonelli, M., Dipasquale, A., Orzan, F., Lorenzi, E., Persico, P., Navarria, P., . . . Boccaccio, C. (2020). Cerebrospinal fluid tumor DNA for liquid biopsy in glioma patients' management: Close to the clinic? *Crit Rev Oncol Hematol, 146*, 102879. doi:10.1016/j.critrevonc.2020.102879

Sturm, D., Witt, H., Hovestadt, V., Khuong-Quang, D. A., Jones, D. T., Konermann, C., Pfister, S. M. (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell, 22(4), 425-437. doi:10.1016/j.ccr.2012.08.024

Wang, L., Liu, L., Li, H., Wang, P., Hu, Z., Wei, Y., . . . Teng, L. (2019). RELA Fusion in Supratentorial Extraventricular Ependymomas: A Morphologic, Immunohistochemical, and Molecular Study of 43 Cases. *Am J Surg Pathol, 43*(12), 1674-1681. doi:10.1097/pas.0000000000001342

Weinberg, D. N., Allis, C. D., & Lu, C. (2017). Oncogenic Mechanisms of Histone H3 Mutations. *Cold Spring Harb Perspect Med, 7*(1). doi:10.1101/cshperspect.a026443

Weller, M., van den Bent, M., Preusser, M., Le Rhun, E., Tonn, J. C., Minniti, G., . . . Wick, W. (2021). EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nature Reviews Clinical Oncology, 18*(3), 170-186. doi:10.1038/s41571-020-00447-z

Wen, P. Y., & Huse, J. T. (2016). 2016 World Health Organization Classification of Central Nervous System Tumors. *Continuum (Minneap Minn), 23*(6, Neuro-oncology), 1531-1547. doi:10.1212/con.0000000000000536

Wick, W., Weller, M., van den Bent, M., Sanson, M., Weiler, M., von Deimling, A., Reifenberger, G. (2014). MGMT testing--the challenges for biomarker-based glioma treatment. Nat Rev Neurol, 10(7), 372-385. doi:10.1038/nrneurol.2014.100

Zacher, A., Kaulich, K., Stepanow, S., Wolter, M., Kohrer, K., Felsberg, J., Reifenberger, G. (2017). Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel. Brain Pathol, 27(2), 146-159. doi:10.1111/bpa.12367

Zhao, H., Wang, S., Song, C., Zha, Y., & Li, L. (2016). The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: a meta-analysis. *World J Surg Oncol, 14*(1), 261. doi:10.1186/s12957-016-1012-4

Specialty Matched Consultant Advisory Panel 4/2020

Medical Director review 4/2020

Medical Director review 10/2020

Specialty Matched Consultant Advisory Panel 3/2021

Medical Director review 3/2021

Medical Director review 10/2021

Policy Implementation/Update Information

1/1/2019	New policy developed. BCBSNC will provide coverage for molecular analysis for gliomas when it is determined to be medically necessary and criteria are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (lpr)
10/29/19	Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (gm)
12/31/19	Reviewed by Avalon 3rd Quarter 2019 CAB. Under Billing/Coding section: CPT codes 88364, 88365 removed and 88374, 88377 added. Medical Director review 11/2019. (lpr)
5/12/20	Specialty Matched Consultant Advisory Panel review 4/15/2020. No change to policy statement. (lpr)
11/10/20	Reviewed by Avalon 3rd Quarter 2020 CAB. Under "When Covered" section added gene HIST1H3B to item e. Updated references and policy guidelines section. Literature review and Medical Director review 10/2020. (lpr)

4/6/21 Specialty Matched Consultant Advisory Panel review 3/17/2021. No change to policy statement. (lpr)

11/16/21 Reviewed by Avalon 3rd Quarter 2021 CAB. Under "When Covered" section added item e. H3F3A testing using a K27M histone antibody as medically necessary. Updated policy guidelines and references. Medical Director review 10/2021. (lpr)