Clinical Significance

1p/19q
FISH testing used for detecting loss of function 1p and 19q deletions in glial brain tumors has diagnostic and prognostic value. Because large or whole-arm deletions of chromosomes 1p and 19q are highly specific for oligodendroglioma, 1p/19q deletion testing is useful to distinguish oligodendrogliomas from astrocytomas, clear cell ependymomas, central or extra ventricular neurocytomas, and dysembryoplastic neuroepithelial tumors. The 1p/19q co-deletion is identified in 50-90% of oligodendrogliomas, whereas it is identified in less than 1% of astrocytomas. The presence of 1p/19q co-deletion was integrated as a defining feature of oligodendroglial tumors in the revised WHO classification. The 1p/19q co-deletion is mutually exclusive with TP53 mutation and EGFR amplification, frequently associated with MGMT promoter methylation, and always associated with an IDH1 or IDH2 mutation. Testing for 1p and 19q deletions in glial brain tumors, specifically oligodendrogliomas, also has prognostic value: co-deletion of both 1p and 19q regions in adult oligodendroglioma patients is associated with improved response and longer survival in patients receiving radiation and/or chemotherapy.1,5

AKT1
The AKT1 gene, on chromosome 14, encodes a serine-threonine protein kinase, which acts as a downstream effector of PI3K regulating cell proliferation and tumor growth. The PI3K/AKT/mTOR pathway is frequently activated in gliomas and medulloblastomas. While glioblastomas rarely have mutations in AKT1, mutations in AKT1 are often present in NF2-negative sporadic meningiomas. Overexpression of AKT1 is associated with resistance to chemotherapy or radiation therapy. Testing for AKT1 mutations can be useful for determining sensitivity to various drugs such as PI3K/AKT inhibitors or mTOR inhibitors including everolimus currently being tested for efficacy in AKT dysregulated tumors.6-11

ATRX
The ATRX gene encodes a protein involved in chromatin remodeling and telomere biology. Normally ATRX represses alternative lengthening of telomeres. Mutations are a loss of function, allowing cells to acquire an immortality hallmark. Mutations in ATRX gene have been reported in all types of cancers and particularly in brain tumors: 71% of grade II and III astrocytomas, 68% of oligoastrocytomas, and 57% of secondary glioblastomas. The presence of IDH1/2 mutations with ATRX mutations defines a specific subgroup of oligodendroglioma patients with significantly longer survival.12-16

BRAF
(BRAF V600E) mutations are detected in low-grade gliomas including approximately 20% of fibrillary astrocytomas, 50% of gangliogliomas, 75% of pleomorphic xanthoastrocytomas, and 5% of pilocytic astrocytomas. Mutations have been identified as sole drivers of the majority of papillary craniopharyngiomas. Use of BRAF inhibitors is being investigated.17,21

(BRAF- KIAA1549) fusions are common in low-grade astrocytomas. BRAF rearrangements with other partners have been observed and may also be detected by FISH. The BRAF-KIAA1549 fusion causes constitutive BRAF kinase activation and is found in about 70% of pilocytic astrocytomas and 15% of other low-grade gliomas in the differential diagnosis. The frequency of this fusion diminishes with patient age, from 80% up to age 10 and less than 10% in patients over 40. In gliomas, BRAF fusions have been reported primarily in pediatric low grade gliomas, and such fusions are noted as a diagnostic marker. Prognosis associated with BRAF fusions is generally positive. While fusion mutations with BRAF, like BRAF- KIAA1549, result in constitutive homodimers resistant to first generation BRAF inhibitors, such as vemurafenib, second generation BRAF inhibitors are being investigated.18-20,22,23

CDK6
The cell division protein kinase 6 (CDK6) gene, a member of the cyclin-dependent kinase family, is located on chromosome 7. The CDK6 protein contains a catalytic core composed of a serine/threonine domain. CDK6 is regulated by cyclin D proteins and cyclin-dependent kinase inhibitor proteins. CDK family members regulate cell cycle progression, while the CDK6 enzymatic complex phosphorylates the protein RB, RB releases its
binding partner E2F, which activates DNA replication. Additional functions of CDK6 include cell differentiation, cell cycle phases in neuron production, and dysregulation of cell metabolism. The protein is overexpressed in medulloblastoma (33%), glioblastoma, and meningioma, where it signals poor prognosis. Glioma malignancies overexpressing CDK6 tend to be resistant to temozolomide. CDK6 inhibitors in development include kinase inhibitors, CIP/KIPs that block the catalytic domain of assembled C-CDKs, mutated D-cyclin that binds to CDK6. The MicroRNA (miR)-124 has successfully controlled cancer progression in an in vitro setting for medulloblastoma and glioblastoma cells. Palbociclib, a CDK4/6 inhibitor, is approved by the FDA for ER+, HER2- advanced breast cancer.\textsuperscript{24,29}

**CDKN2A**

The CDKN2A gene, on chromosome 9, encodes the tumor suppressor protein isoforms, p16\textsuperscript{INK4a} and p14\textsuperscript{ARF}, which regulate cell division. Loss of CDKN2A function results from nonsense and missense mutations in ankyrin repeats, as well as promoter methylation, homozygous deletions, or loss of heterozygosity. Germ line mutations in CDKN2A are associated with increased risk of developing certain cancers. CDKN2A can become inactivated in various cancers through differing mechanisms: in CNS tumors 12.91% have point mutation and 32.39% have copy number loss, frame shifts have also been noted in grade III meningiomas, while the CDKN2A promoter is hyper-methylated in other cancers. Functional studies support a role of glioma-related BRAF mutants and activated Raf-1 in transformation and gliomagenesis, in particular in combination with CDKN2A inactivation. Loss of expression and promoter methylation of CDKN2A is generally a poor prognostic marker, and has been noted in high-grade glioma and oligodendrogliomas. Restoration of CDKN2A function can often be achieved through a combination of regulatory genes and small molecule epigenetic modifiers. Loss of CDK2A results in increased CDK4/6 driven signaling, so targeting with CDK4/6 inhibitors is another potential therapeutic approach for CDKN2A dysregulated tumors. Palbociclib is an FDA approved drug that may be sensitive to CDKN2A alternation.\textsuperscript{30-37}

**CIC**

Capicua transcriptional repressor (CIC), on chromosome 19, encodes a member of the HMG-box superfamily of transcriptional repressors involved in DNA binding, neuronal cell development, nuclear localization, cell cycle control, ATP-citrate lyase phosphorylation, reactive oxygen species homeostasis, bile acid homeostasis, and regulating EGFR, RAS/RAF, and MAPK pathways. CIC is also specifically expressed in cells of the developing central nervous system. Loss of CIC leads to overexpression of downstream members of the MAPK signaling cascade. CIC disruption is found in type I low-grade gliomas: oligodendrogliomas (46%) but rarely in astrocytomas or oligoastrocytomas (<10%). In oligodendrogliomas, TERT promoter mutations occurring concurrently with IDH mutations and 1p/19q loss results in the loss of CIC. CIC alteration is associated with poor prognosis. Drugs that block overactive MAPK signaling, including a class known as RAF-MEK-ERK inhibitors are already in development and preventing inactivation of CIC.\textsuperscript{38-46}

**CTNNB1**

The CTNNB1 gene, on chromosome 3, encodes beta-catenin in the WNT signaling pathway that plays critical roles in tissue homeostasis, cell renewal, and regeneration. Alterations in beta-catenin can promote oncogenesis and metastasis. CTNNB1 mutations have been identified as a principal abnormality in adamantinomatous craniopharyngiomas and occur often in medulloblastomas. While inhibitors that target beta-catenin are still in preclinical development, studies are also underway to evaluate the efficacy of inhibiting other components of the WNT signaling pathway. FDA approved drugs that may be sensitive to CTNNB1 gain of function mutations include sorafenib and regorafenib.

**EGFR**

The EGFR/ERBB1 gene, on chromosome 7, encodes a receptor tyrosine kinase that regulates cell proliferation, differentiation, motility, survival, and tissue development. EGFR alterations, including overexpression, amplification, and activating mutations, play a central role in development of numerous solid tumors. The EGFR\textit{vIII} mutation results in ligand independent activation. As per the COSMIC database, central nervous center tumors are found with point mutations 9.61% and copy number 25.71%. Alternations in the extracellular (EC) domain of EGFR, such as that encoded by the EGFR\textit{vIII} mutation, are predominantly associated with glioblastomas. Other large deletions involving multiple exons spanning parts of the extracellular or intracellular domains of EGFR or tandem duplication of portions of the EC or kinase domain have been reported in glioma cells. Diagnostically, EGFR amplification is associated with the classical subtype of glioma alone. The EGFR\textit{vIII} variant has been identified as a negative prognostic factor in glioblastoma. EGFR antibodies and kinase inhibitors have so far failed to demonstrate clinical relevance in EGFR amplified glioma. FDA approved drugs sensitive to EGFR include
osimertinib, lapatinib, vandetanib, gefitinib, erlotinib, cetuximab, panitumumab, necitumumab, and afatinib.

Acquired mutations, such as T790M, are known to be resistant to first generation EGFR kinase inhibitors. Second and third generation of EGFR kinase inhibitors such as afatinib and osimertinib have been developed to counter resistant variants. Another acquired mutation, C797S, may be responsible for resistance to third generation kinase inhibitors and inhibitors are in preclinical development to overcome this resistance. Cancer vaccines are being extensively explored to address EGFRvIII positive glioblastoma. RNA based therapies are now being explored preclinically in glioma. Antibodies such as nimotuzumab and matuzumab are in early phases of development in glioma.  

ERBB2 (HER2)

ERBB2, also known as HER2, on chromosome 17, encodes a growth factor receptor that regulates proliferation and survival. ERBB2 lacks an identified soluble ligand and can be activated upon dimerization with another ligand bound receptor, as occurs in cancers which overexpress ERBB2. Alterations in ERBB2 found in cancer include small insertions in the kinase domain or large deletions in the extracellular domain, and overexpression.

Overexpression and amplification of ERBB2 can be effectively evaluated by immunohistochemistry or FISH testing. ERBB2 overexpression is associated with poor prognosis in medulloblastoma. Testing for ERBB2 mutations can be useful in determining patient’s sensitivity to tyrosine kinase inhibitors or monoclonal antibody directed therapy. FDA approved drugs sensitive to ERBB2 include trastuzumab, afatinib, lapatinib, and pertuzumab. Specific mutations in ERBB2 (95HER2, Δ16HER2 L726, L755, P780) have been shown to be resistant to trastuzumab or lapatinib in preclinical and preliminary clinical studies. Kinase domain mutations have been suggested to be resistant to EGFR kinase inhibitors such as cetuximab, where irreversible kinase inhibitors, such as neratinib and afatinib, may be effective. Hsp90 function is required for stabilization of ERBB2, and is suggested as a therapeutic target.

ERBB4 (HER4)

The ERBB4 gene, also known as HER4, on chromosome 2, encodes a receptor tyrosine kinase that signals the MAPK and PI3K pathways to induce mitogenesis and differentiation. ERBB4 acts as a cell surface receptor activated by binding to neuregulins, EGF family members, and other factors. ERBB4 activation via missense mutation, amplification, or overexpression has been observed in multiple tumors including medulloblastoma. Missense mutations in ERBB4 are observed across all domains including the extracellular domain, transmembrane domain, and the tyrosine kinase domain. ERBB4 normally regulates the timing of astrogenesis in the developing brain and is correlated with tumorigenesis. The expression of the JMa/Cyt-2 isoform of ERBB4 in glioma is correlated with significantly shortened survival. Four ERBB4 JM isoforms are seen in 28-54% of pediatric medulloblastomas. Aberrant ERBB4 expression in glioblastoma has been reported. ERBB4 variant isoforms are associated with an aggressive subtype of medulloblastoma. Testing for ERBB4 mutations can be useful in determining a patient’s sensitivity to tyrosine kinase inhibitors. Inhibition of ERBB4-activated melanoma cell lines has been observed after treatment with the pan-ERBB inhibitor, lapatinib. ERBB4 activation has been shown to play a role in mediating acquired resistance to ERBB2 inhibitors such as trastuzumab.

FGFR1

The FGFR1 gene, on chromosome 8, encodes a receptor tyrosine kinase involved in cell division, growth, maturation, angiogenesis, wound healing, and embryonic development. The most frequently occurring FGFR1 alterations in cancer are amplifications, gain of function mutations, and translocations. Type 2 FGFR1 fusions, found primarily in solid tumors, lead to constitutively active FGFR1. The activation of FGFR1 is associated with enhanced activation of both the MAPK and PI3K/AKT signaling pathways. As per the COSMIC database, point mutations in FGFR1 are found in 1.53% of central nervous system tumors. Inhibition of FGFR1 activated signaling is largely accomplished using specifically developed FGFR inhibitors or multi-tyrosine kinase inhibitors that also target FGFR1.

FGFR2

The FGFR2 gene, also known as CD332, on chromosome 10, encodes the membrane spanning FGFR2 protein is involved in wound healing, bone growth, embryonic development, proliferation, differentiation, survival, migration, and angiogenesis. The FGFR family receptor tyrosine kinases promotes activation of the RAS/MAPK and PI3K/AKT signaling pathways. Mutations, rearrangements, deletions, and amplifications in FGFR2 are associated with certain cancers. In glioblastoma and lower grade gliomas, FGFR2 is mutated about 3% of the time, usually as deletions and is amplified in 2.3% of central nervous systems cancer. FGFR2 is known to have two alternatively
spliced isoforms, \textit{FGFRIIIb} and \textit{IIIc}. FGFR2 amplification is associated with a poor disease outcome in gastric cancer. While there are no approved FGFR2 inhibitors, FGFR kinase inhibitors and FGF-ligand traps are being evaluated in clinical trials. Preclinical and early clinical studies have demonstrated potential in a variety of FGFR harboring cancers with FGFR-selective agents such as NVP-BGJ398, ARQ 087, JNJ-42756493, TAS-120, AZD4547, and CH5183284/Debio 1347. Four agents with varying degrees of FGFR selectivity, ponatinib, pazopanib, regorafenib, and lenvatinib, are FDA-approved for use in cancer, although the approval was not based on their activity against FGFR.\textsuperscript{73,85}

**FGFR3**

The \textit{FGFR3} gene, on chromosome 4, encodes a receptor tyrosine kinase that promotes activation of \textit{RAS/MAPK} and \textit{PI3K/AKT} pathways to regulate cell proliferation, survival, migration, and differentiation. The majority of the \textit{FGFR3} alterations in cancer are activating missense mutations that occur in the extracellular, the transmembrane, and intracellular tyrosine kinase domains. Gene fusions have also been observed in solid tumors. An \textit{FGFR3}-\textit{TACC3} fusion has been reported to have transforming effects in primary glioma and wild type \textit{FGFR} expression predicts favorable survival for glioma patients. Evidence for effective therapeutic targeting of \textit{FGFR3} activation by kinase inhibitors is preliminary and being explored in preclinical and early clinical settings. FDA approved drugs potentially sensitive to \textit{FGFR3} include pazopanib and ponatinib.\textsuperscript{96-101}

**FUBP1**

\textit{FUBP1}, on chromosome 1, encodes an ssDNA binding protein that stimulates expression of \textit{C-MYC} in undifferentiated cells and functions as an ATP-dependent DNA helicase. \textit{FUBP1} influences differentiation, cell proliferation, apoptosis, proliferation, and migration. \textit{FUBP1} acts as an oncogene in some cancers and as a tumor suppressor in oligodendroglialomas, astrocytomas, and oligoastrocytomas. \textit{FUBP1} mutations occur frequently in oligodendroglialomas (24%) but rarely in astrocytomas or oligoastrocytomas (<10%), and thus can be used to stratify patient subgroups. Absent \textit{FUBP1} expression is linked with unfavorable prognosis in brain tumors where it functions as a tumor suppressor.\textsuperscript{38,42,46,92-95}

**H3F3A**

The \textit{H3F3A} gene is one of two genes that encodes histone H3.3, a member of the histone H3 family. Mutations in \textit{H3F3A} have been reported in 31% of pediatric glioblastomas. There are two critical single-point mutations (K27M and G34R/V) found in pediatric astrocytic gliomas that effect gene expression, cell differentiation, and telomere maintenance. The K27M mutation is observed in 78% of diffuse intrinsic pontine gliomas and 22% of non-brain-stem gliomas. \textit{H3F3A} mutations have been reported to have 100% specificity for pediatric astrocytic gliomas, and are not found in pediatric low-grade gliomas, embryonal tumors, ependymomas, or in adult glioblastoma. A K27M mutation in \textit{H3F3A} in diffuse intrinsic pontine glioma is associated with a shorter clinical survival. Histone demethylase inhibitors tested in glioblastoma cultures were shown to have suppressive effects. HDAC inhibitors, including panobinostat and vorinostat, have so far failed to translate into effective therapies in clinical trials, possibly because of their role as substrates for efflux transporters at the blood–brain barrier. Drug screening assays have identified menin as an H3.3 K27M inhibitor and testing is ongoing.\textsuperscript{96-104}

**HRAS**

The \textit{HRAS} gene, on chromosome 11, is one of the three human \textit{RAS} oncogenes, and encodes the HRAS GTPase that acts as an intracellular molecular switch downstream of receptor tyrosine kinases and impacts proliferation, migration, and survival. Activating mutations in \textit{HRAS} lock the protein in a constitutively activate state, thereby resulting in the activation of \textit{RAS/RAF/MEK/ERK} and \textit{PI3K/AKT/mTOR} pathways. The most frequently mutated residues are located within the phosphate binding loop/G1 motif (residues 10-17) and the switch II region (residues 59-67). \textit{HRAS} is not as frequently mutated as its counterparts \textit{NRAS} and \textit{KRAS}, but is the predominant isoform to be mutated in certain cancers. \textit{HRAS} induces neuronal differentiation of neural stem cells and appears to be involved with tumorigenesis in brain cancer through transcriptional down-regulation. While only 1% of glioblastomas have a \textit{RAS} mutation or amplification, 10% of glioblastomas contain \textit{NFI} alterations that lead to hyperactive \textit{RAS} activity. Over-expression of \textit{HRAS} correlates poor prognosis in gastric cancer. Screening for \textit{HRAS} mutations can be useful in determining sensitivity to tyrosine kinase inhibitors, such as MEK inhibitors. Resistance to anti-EGFR therapy appears to be correlated with the presence of activating mutations in \textit{HRAS}. FDA approved drugs sensitive to \textit{HRAS} include cobiimetinib and trametinib.\textsuperscript{105-112}

**IDH1/IDH2**

The \textit{IDH1} gene, on chromosome 2, encodes an isocitrate dehydrogenase that plays an important role in oxidative respiration, and lipid synthesis. \textit{IDH1} and \textit{IDH2} mutations have been detected in various solid tumors. Though the most frequent alteration in \textit{IDH1} is a missense mutation at residue R132, the \textit{IDH2} R172 mutation occurs in

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tumors lacking IDH1 mutations. As per the COSMIC database, point mutations in IDH1 are found in 34.31% of central nervous system cancers, but copy number gain in IDH1 are rare. IDH1 or IDH2 mutations are detected in more than 70% of grade II or III brain gliomas. IDH1/2 mutational status can distinguish between primary glioblastoma and other brain cancers. IDH1 mutations are characteristic of the proneural subtype of glioblastomas and are a positive prognostic marker, associated with better overall survival in secondary glioblastoma. Long-term survival after aggressive tumor resection has been reported for patients with IDH1-positive astrocytomas. Several therapeutic interventions such as inhibitors of mutant-IDH1, peptide vaccines, and hypomethylating drugs are under evaluation. Recent studies in IDH1 mutant-AML have suggested a synthetic lethal interaction between BCL2 and IDH1, thus raising the possibility of benefit from BCL2 inhibitors in IDH1-mutant patients.113-122

**KRAS**

The KRAS gene, on chromosome 12, is one of three human RAS genes, and encodes the KRAS GTPase regulating proliferation, migration, and cell survival. KRAS alterations are gain of function mutations that result in constitutive activation of the RAS/RAF/MEK/ERK pathways. The majority of KRAS missense mutations are found in the phosphate binding loop (residues 10-17), the switch II domain (residues 57-67), and the G5 motif (residues 145-147). Point mutations in KRAS are seen in pediatric gliomas (5-10%) and pilocytic astrocytomas (1-3%). KRAS mutations are generally associated with poor prognosis. Several inhibitors of the RAF/MEK pathway, including selumetinib, are under clinical evaluation for the treatment of patients with KRAS-mutated cancers. Clinical studies in colon cancer with KRAS alterations indicate sensitivity to chemotherapy with oxaliplatin. Patients with mutant KRAS have been shown to be resistant to anti-EGFR antibody therapies such as cetuximab, panitumumab, and erlotinib. FDA approved drugs sensitive to KRAS mutations include sorafenib, regorafenib, palbociclib, cobimetinib, and trametinib,123-126

**MET**

The MET gene, on chromosome 7, encodes a transmembrane receptor tyrosine kinase that regulates morphogenesis, regeneration, survival, and possibly the maintenance of cancer stem cells. The most frequently observed validated gain of function mutation in MET is Y1253D. Other key gain of function variants in MET result disruption of juxtamembrane (JM) domain. Occasional missense mutations in MET have been noted. Translocations of MET gene are rare but have been noted in low-grade glioma. As per the COSMIC database, copy number gain in MET is found in 2.74% of central nervous system cancers. MET overexpression and amplification is identified by published guidelines as a targetable abnormality and is generally associated with poor prognosis. The gain of function involving disruption of juxtamembrane domain is known to indicate response to MET inhibitors. MET gene amplification, as detected by FISH, is a known mechanism of acquired resistance to several receptor tyrosine kinase inhibitors such as ERBB2, EGFR, and FGFR inhibitors. FDA approved drugs sensitive to MET include cabozantinib and crizotinib. Other inhibitors and antibodies targeting MET, such as such as volitinib and AMG337, are under investigation.127-133

**MGMT**

The MGMT gene, on chromosome 10, encodes a DNA alkyltransferase crucial for genome stability that repairs the mutagenic guanine nicks, prevents DNA mismatch and replication errors. Methylation of the MGMT gene promoter down-regulates normal DNA-repair function MGMT, which can make tumors more susceptible to radiation or alkylating agent-based therapy. The majority of sporadic cancers with a DNA repair deficiency have epigenetic alterations, including methylation leading to MGMT expression. MGMT methylation is seen in 40-59% of glioblastomas, and MGMT promoter methylation is more often found in secondary glioblastomas (75%). MGMT methylation status helps to distinguish true progression and pseudo-progression in patients with newly diagnosed glioblastoma treated with surgery followed by radiochemotherapy. Prolonged survival is associated with methylated MGMT promoter in glioblastoma and anaplastic gliomas. Methylation of MGMT is associated with more sensitivity to temozolomide and radiation in glioblastoma. IDH mutations in low-grade gliomas predict the presence of MGMT promoter methylation (84%) and are associated with better overall survival with temozolomide treatment, independent of histologic phenotype.134-143

**MYC**

MYC (C-MYC or MYCC), on chromosome 8, codes for a transcription factor that plays a role in cell cycle progression, apoptosis, proliferation, growth, apoptosis, differentiation, stem cell renewal, cellular transformation, and B-cell proliferation. The MYC protein belongs to MYC family that includes MYCN and MYCL. MYC regulates 10-15% of all human genes. MYC also facilitates cellular glucose uptake by inducing the expression of glucose transporter 1 (GLUT1), and activates the Warburg effect. MYC may also regulate CD47 and PD-L1 expression. MYC overexpression can initiate chromosomal instability. MYC is a proto-oncogene and found to be upregulated in many types of cancers. MYC is deregulated in various malignant brain tumors in children and adults, including medulloblastoma, diffuse intrinsic pontine, grade II and grade III, and pediatric high grade glioma. One of the
marked differences between pediatric and adult glioblastoma mutational landscapes is an increased focal amplification rate of MYC. Deregulated expression MYC, particularly MYC amplification (~16%-17%), is correlated with poor prognosis. MYC rearrangement is also associated with inferior prognosis across multiple tumor types. BET inhibitors have been used to successfully block MYC function in pre-clinical cancer models and are currently being evaluated in clinical trials.144-159

**MYCN**

MYCN (N-MYC) is proto-oncogene that encodes MYCN that controls proliferation, growth, metabolism, regeneration, cell cycle, apoptosis, differentiation, replication, transcription, and splicing. MYCN is highly expressed in the fetal brain and is critical for normal brain development. MYCN is also overexpressed, as detected by FISH, in neuroblastoma, medulloblastoma, and astrocytoma, and this amplification is a poor prognostic marker. High-risk neuroblastoma is defined by presence of tumor MYCN amplification or children with metastatic disease older than age 18 months at diagnosis. TERT rearrangements (23%), ATRX deletions (11%), and MYCN amplifications (37%) characterize three subgroups of high-risk neuroblastoma, each associated with very poor prognosis. For patients with MYCN amplified neuroblastoma, BET inhibitors can induce cell death by interfering with MYCN transcription. Other drugs that have effects on MYCN stability (aurora kinase A and mTOR inhibitors) as well as those that target MYC-dependent metabolic changes (difluoromethylornithine) are being studied.157,160-167

**NF1**

NF1, on chromosome 17, encodes a tumor suppressor that regulates RAS signaling and neurofibromin expression in nerve cells, oligodendrocytes, and Schwann cells. NF1 mutations are associated with resistance to therapy and adverse outcomes in several tumor types. MEK inhibitors have been found to be effective in treating NF1 deficient glioblastoma cell lines in mouse models.168-171

**NF2**

NF2, on chromosome 22, encodes a tumor suppressor, merlin (schwannomin), in Schwann cells that regulate cell shape, growth, and adhesion. NF2 mutations are associated with meningioma and glioblastoma. Merlin expression is reduced or absent in 32-61% of glioblastomas. NF2 mutations are associated with resistance to therapy and adverse outcomes in several tumor types. NF2 mutations in multiple cell lines are resistant to BRAF inhibitors, dihydrofolate reductase inhibitors, and JNK inhibitors, but are sensitive to drugs such as dasatinib.75,172-174

**NRAS**

The NRAS gene, on chromosome 1, is one of the three human RAS genes, which encodes the NRAS GTPase that impacts proliferation, migration, and cell survival. The majority of the NRAS gain of function missense mutations are found within the phosphate binding loop/G1 motif (residues 10-17), the switch II region (residues 59-67), and the G5 motif (residues 145-147). Activating mutations in NRAS result in activation of the RAS/RAF/MEK/ERK pathway. Point mutations in NRAS are found in meningeal cancer (7.2%), while aberrant NRAS activation is associated with glioblastoma and neurocutaneous melanosis. NRAS is down-regulated in long term survivors relative to short term survivors in glioblastoma. NRAS mutations are associated with resistance to anti-EGFR and anti-BRAF therapies in some cancers, such as cetuximab, panitumumab, vemurafenib and dabrafenib. Inhibitors of the RAS/RAF/MEK/ERK pathway, such as selumetinib, are under clinical evaluation for the treatment of patients with NRAS mutated cancers. Emerging clinical literature suggests an association between NRAS mutation status and benefit from immune therapies in melanoma patients. FDA approved drugs sensitive to NRAS include cobimetinib and trametinib.175,176

**PD-L1**

PD-L1 (B7-H1) is a transmembrane protein encoded by the CD274 gene that suppresses the immune system. PD-L1 binds to its receptor, PD-1 or B7.1, on T-cells inhibiting activation of IL-2 and CD8+ T-cell proliferation and stimulating apoptosis. Multiple solid tumor types overcome immune protection by aberrantly expressing PD-L1. In tumors that respond to anti-PD-1 agents, the level of PD-L1 expression, as monitored by IHC, ranges widely from 14% to 100%. A large percentage (61%) of brain tumors express PD-L1. PD-L1 expression is associated with negative prognosis in multiple tumor types. Multiple tumor types have demonstrated durable responses to immune checkpoint inhibition, notably PD1/PD-L1 inhibitors. Several PD-1/PD-L1 therapeutics are approved to treat a variety of cancers including PD1 inhibitors pembrolizumab and nivolumab, and PD-L1 inhibitors atezolizumab, durvalumab, and avelumab.177-183
**PDGFRA**
Platelet-derived growth factor receptor alpha (PDGFRA), on chromosome 4, is a member of the subfamily of type III receptor tyrosine kinases, which includes KIT, PDGFRB, FLT3, and CSF-1. PDGFRA encodes the alpha isoform of the receptor tyrosine kinase activated upon ligand binding. PDGFR receptors play important roles during embryonal development and tissue homeostasis in adults. Constitutive activation of PDGFRA results in oncogenic activation of signaling pathways that promote cell growth, proliferation, and survival. As per the COSMIC database, copy number gains are found in 6.77% of central nervous system cancers. Mutations in PDGFRA are commonly observed in a variety of cancers. PDGFRA amplification, fusions, and missense mutations are observed in glioblastoma. Testing for PDGFRA alterations by FISH may be useful in determining sensitivity to tyrosine kinase inhibitors, such as imatinib, sunitinib, and nilotinib. Whereas PDGFRA gain of function mutations, fusions, and rearrangements are responsive to imatinib, specific PDGFRA mutants are resistant to imatinib, sorafenib, and nilotinib. Concomitant activation of Kras, Nras, or Braf in PDGFRA activated tumors is associated with imatinib resistance. Other FDA approved drugs sensitive to PDGFRA include olaratumab and pazopanib.\textsuperscript{31,184-187}

**PIK3CA**
The PIK3CA gene, on chromosome 3, encodes the p110 alpha catalytic subunit of PI3K enzymes. PIK3CA is a member of the PI3K/AKT/mTOR pathway, important in regulation of growth, proliferation, survival, differentiation, adhesion, and motility. The most frequent PIK3CA alterations observed in cancer are missense mutations; notably H1047R, E542K, and E545K. PIK3CA gain of function mutations occur within the kinase, alpha-helical, C-, and adaptor domains domains.\textsuperscript{293,294} Amplifications are also observed in certain cancers and result in constitutively active PI3K. Alterations in PIK3CA are implicated in tumorigenesis in glioblastomas, medulloblastomas, and anaplastic astrocytomas. The PI3K/AKT/mTOR pathway is frequently activated by PTEN loss in glioblastoma. PI3K mutations are generally associated with more aggressive disease and poorer prognosis across a variety of cancer types, including glioblastoma. Activation of the PI3K/Akt/mTOR pathway leads to temozolomide resistance in glioblastoma as well as resistance to ERBB-targeted therapies such as cetuximab and trastuzumab and chemotherapy in other cancers. Idelalisib is a PI3K inhibitor with FDA approval for leukemia and lymphoma. Direct PI3K inhibitors under development include buparlisib, pilaralisib, pictilisib, alpelisib, tazalisib, CAL-101, and GDC-0941.\textsuperscript{75,188-191}

**PTCH1**
PTCH1, a tumor suppressor, encodes for a serine/threonine phosphatase that binds to sonic hedgehog, regulating the release of smoothened protein and resulting cell proliferation. The canonical hedgehog (HH) pathway plays a pivotal role during embryonic development through activation of downstream effectors glioma-associated oncogene homolog 1 (GLI1), GLI2, and GLI3. Mutations of PTCH1 are found in 20% of medulloblastomas. Mutations and reduced expression of PTCH1 are associated with poorer prognosis across multiple tumor lines. Although targeting the hedgehog pathway with SMO inhibitors is a reasonable approach for PTCH1 mutant cancers, it has had limited clinical success. Molecular therapy targeted to GLT1, WNT, and FZD are being studied and show some promise.\textsuperscript{192-194}

**PTEN**
The PTEN gene, on chromosome 10, is a member of the targetable PI3K/AKT/mTOR pathway, and encodes a tumor suppressor that plays a role in growth, migration, survival, cell cycle progression, chromosome stability, DNA repair, and apoptosis. Nonsense mutations span the length of the protein, while most missense mutations cluster in the catalytic loops. PTEN loss of function due to substitution mutations, deletion, and epigenetic or post-translational modifications may result in constitutive activation of the PI3K/AKT/mTOR signaling pathway. PTEN is one of the most commonly mutated tumor suppressors in human cancer. As per the COSMIC database, 12.81% of central nervous system cancers harbor point mutations, while 4.94% display copy number loss in PTEN. PTEN mutations are found in 31–44% of glioblastoma. While traditionally glioblastoma patients have shorter survival with PTEN mutations, temozolomide treatment has equalized overall survival in PTEN mutated and wild type glioblastoma patients. Testing for PTEN mutations may be useful in identifying patients sensitive to PI3K/AKT and FRAP/mTOR inhibitors, as well as agents that target double stranded break repair pathway. It has also been suggested that activation of the PI3K/AKT/mTOR pathway as a result of PTEN loss may play a role in resistance to ERBB-targeted therapies such as cetuximab and trastuzumab.\textsuperscript{188,195,196}

**RB1**
The RB1 gene, on chromosome, encodes the RB tumor suppressor protein represses the E2F transcription factor and plays an important role in arresting cell cycle. RB repressor function is inactivated by phosphorylation by cyclin D-CDK4/6. Alterations in RB1 include large and small deletions, nonsense/misssense mutations, splice mutations, intragenic mutations, or loss of expression by methylation and chromosomal deletions, often disrupting the function of the pocket domain, composed of two highly conserved subdomains A and B, involved in interaction with E2F protein and cyclin D as well as stabilization of the protein. Missense mutations affecting
SETD2

SETD2, on chromosome 3, encodes a histone methyltransferase and tumor suppressor that plays a role in chromatin structure, DNA mismatch repair, transcription activation, and genomic stability. SETD2 is mutated in gliomas: 15% of pediatric high grade gliomas and 8% of adult high grade gliomas. SETD2 mutations are specific to high-grade tumors HGG with no SETD2 mutations in low-grade diffuse gliomas. Decreased expression of SETD2 is associated with poor prognosis in some tumors. Patients with SETD2 mutations tend to develop resistance to temozolomide.\textsuperscript{208-211}

SMAD4

The SMAD4 gene, on chromosome 18, encodes an intracellular co-activator involved in mediating TGF-β signaling, gene expression, cell growth, and proliferation. Loss of SMAD4 tumor suppressor function results from homozygous deletions involving the entire or parts of the coding region due to nonsense/frame shift/splice site mutations, as well as by loss of heterozygosity, disruption or loss of one or more of its functionally important domains, the MAD homology 1 (MH1) domain, the linker domain, and the MAD homology 2 (MH2) domain. Missense mutations in SMAD4 are known to occur predominantly in the MH2 domain, such as D351 and R361 involved in heteromerization. The expression level of SMAD4 is reduced in gliomas. SMAD4 loss in tumors results in poor overall survival in a number of cancer lines. Loss of SMAD4 is associated with increased expression of VEGF and increased angiogenesis, suggesting a possible therapeutic use of anti-angiogenic agents.\textsuperscript{212}

SMO

The SMO gene, on chromosome 7, encodes the G-protein-coupled smoothened receptor that functions as a transducer of the hedgehog (Hh) signaling pathway. The hedgehog pathway is essential for embryonic development, as well as regeneration and maintenance of adult tissues. Activating alterations in SMO result in dysregulation of the Hh pathway and accumulation of glioma-associated (GLI) transcription factors that stimulate transcription of Hh target genes. The majority activating missense mutations in SMO occur in the frizzled domain, particularly residues 412 and 535. Alterations in SMO including missense mutations, overexpression, and amplification have been observed in multiple cancers, including glioblastoma, medulloblastoma, and meningioma. As per the COSMIC database, point mutations in SMO are found in 3.48% of meninges cancers, while copy number gain is associated with 1.83% of central nervous system cancers. Testing for SMO mutations can be useful as a prognostic or therapeutic indicator. SMO inhibitors such as erismodegib and vismodegib are approved for the treatment of basal cell carcinoma, which usually harbors a dysregulated hedgehog signaling axis.\textsuperscript{341,342} Benefit with vismodegib is sometimes limited due to reactivation of hedgehog pathway, and next generation inhibitors are being developed, such as Itraconazole, and other agents that target SMO and GLI.\textsuperscript{213-221}

SRC

The SRC gene, a proto-oncogene on chromosome 20, encodes a non-receptor tyrosine kinase that plays a role in the regulation embryonic development, cell growth, immune response, cell adhesion, cell cycle progression, apoptosis, migration, and transformation. Overexpression and amplification of SRC is frequently observed, whereas activating mutations are rare.\textsuperscript{354} Alterations in SRC lead to increased proliferation via the upregulation of multiple downstream pathways including the RAS/MAPK, PI3K/AKT, and STAT signaling pathways. SRC activation in cancers manifests as increased levels of phosphorylated SRC. Overexpression and phosphorylation of SRC has also been observed in glioblastoma. SRC inhibition may inhibit invasion by disrupting crosstalk between SRC and FAK in glioblastoma. Activation of SRC has been linked to poor prognosis. FDA approved drugs sensitive to SRC include dasatinib and bosutinib. Inhibit SRC phosphorylation in addition to combinatorial therapeutic approaches may aid in SRC inhibition across multiple tumor lines.\textsuperscript{222-228}

TERT

The telomerase reverse transcriptase (TERT) gene, on chromosome 5, encodes a catalytic subunit of telomerase. Telomerase lengthens telomers, potentially allowing senescent cells to become immortal. The telomerase complex is composed of telomerase RNA, the catalytic TERT protein subunit, and associated proteins. In addition to telomere maintenance, telomerase is involved in proliferation, apoptosis, adhesion, and migration. Transcription factors that activate TERT include oncogenes (MYC, SP1, HIF-1, AP2), while tumor suppressors (TP53, WT1, and Menin) suppress TERT. TERT is often up-regulated in cells that divide rapidly, including both embryonic and adult stem cells. Overexpression of TERT is associated with cancer and 80-90% of cancers.
are characterized by increased telomerase activity. TERT is mutated in 43%-51% of CNS tumors. Aberrant TERT promoter hypermethylation correlates with elevated TERT gene expression in medulloblastoma tumors, whereas TERT promoter methylation correlates with reduced TERT gene expression in other cancers. Two consistent and mutually exclusive TERT promoter point mutations have been described in gliomas. Increased TERT transcription is characteristic of oligodendroglioma. TERT promoter mutations are negative prognostic biomarkers in the IDH wild-type grade II-III diffuse gliomas and in glioblastoma. In vitro, telomerase activity can be inhibited by phytochemicals such as isoprenoids, genistein, and curcumin, while other telomerase inhibitors being tested include nucleoside analogues, retinoic acid derivatives, quinolone antibiotics, catechin derivatives, immunotherapy, and adenovirus infection with PTEN.13,44,45,229-239

TP53

The TP53 gene, on chromosome 17, encodes the tumor suppressor P53 that has been termed as the “guardian of the genome” due to its central role in maintaining genome integrity. The most frequently reported alterations in TP53 are missense and frame shift mutations, though deletions are also reported. Missense mutations in TP53 are usually clustered in the DNA binding domain and are important for DNA and zinc ion binding. Six hotspots in the DNA domain constitute the majority of the missense mutations: R175, G245, R248, R249, R273 and R282. In addition to the loss of wild-type protein function, mutations at certain residues also confer oncogenic properties, or gain of function mutations, that promote survival, proliferation, and migration. TP53 mutations are detected in at least 50% of all adult tumors. TP53 mutations, especially mutations at residues that are involved in DNA contact, are associated with poor prognosis in multiple cancers. Gain of function mutations in TP53 are associated with poor prognosis with chemotherapy across multiple tumor lines. While TP53 alterations have largely remained non-actionable, development of P53-based therapeutics is an area of active research. WEE1 or ATR/CHEK1 inhibitors have been validated in preclinical studies in various cancers and may sensitize TP53 deficient cells to DNA damaging agents. Decreasing the levels of mutant P53 in tumor cells by targeting HSP90 and histone deacetylases (HDACs), has also been explored. The approved HDAC inhibitor, vorinostat has been shown to mediate cell death in P53 mutant tumors.240-251

Tumor Mutation Burden (optional)

Checkpoint proteins, such as PD-1, PD-L1, CTLA-4, and LAG-3, can be coopted by cancer to suppress autoimmunity and evade apoptosis. Immunotherapies, designed to restore the immune surveillance system to fight their cancer have received regulatory approval across a number of cancer types. While these checkpoint based therapies can be remarkably effective in some patients, only a limited subset of patients will benefit (e.g. 20-40% of NSCLC). Tumors with a large number of somatic mutations may be particularly responsive to these immunotherapies, whether or not they also overexpress checkpoint proteins. Tumor mutational burden (TMB) is defined as the total number of mutations per coding area of a tumor genome and measuring this biomarker is clinically actionable. A subset of patients exhibit high TMB across almost all types of cancer, while each individual type of cancer has a characteristic TMB. Melanoma and NSCLC represent some of the highest TMB indications, which also show the best response to immunotherapies. Co-occurrence of specific mutations with TMB, particularly in DNA repair genes, are correlated with higher TMB, and presumably better response to immunotherapies. High TMB predicts cancer patients who will benefit from a durable clinical benefit from immunotherapy as well as chemotherapy. For example, high TMB is associated with better clinical benefit from pembrolizumab, a PD-1 inhibitor, in NSCLC. Monoclonal antibodies that target immune checkpoints are FDA approved for multiple disease types, including NSCLC, and include blinatumomab, nivolumab, pembrolizumab, avelumab, ipilimumab, and durvalumab.252-262

Please see our website neogenomics.com for a complete test description and printable specimen requirements.

References


