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The medical necessity of advanced molecular testing in the diagnosis and treatment of brain tumor patients

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Abstract

Accurate pathologic diagnoses and molecularly informed treatment decisions for a wide variety of cancers depend on robust clinical molecular testing that uses genomic, epigenomic, and transcriptomic-based tools. Nowhere is this more essential than in the workup of brain tumors, as emphasized by the incorporation of molecular criteria into the 2016 World Health Organization classification of central nervous system tumors and the updated official guidelines of the National Comprehensive Cancer Network. Despite the medical necessity of molecular testing in brain tumors, access to and utilization of molecular diagnostics is still highly variable across institutions, and a lack of reimbursement for such testing remains a significant obstacle. The objectives of this review are (i) to identify barriers to adoption of molecular testing in brain tumors, (ii) to describe the current molecular tools recommended for the clinical evaluation of brain tumors, and (iii) to summarize how molecular data are interpreted to guide clinical care, so as to improve understanding and justification for their coverage in the routine workup of adult and pediatric brain tumor cases.

Overview and Barriers to Testing

Nervous system tumors are a unique and highly diverse group of neoplasms that develop from the central nervous system (CNS) and its coverings. This complexity makes clinical decision making and testing choices especially difficult for pathologists and treating physicians. Prognoses vary tremendously, ranging from highly malignant cancers with a median survival of less than one

year despite aggressive surgery, radiation, and chemotherapy, to indolent tumors that are usually curable with surgical resection alone. Accurate diagnosis is therefore critical, yet past experience has demonstrated that, because diagnoses and classifications relying solely on histology have an element of subjectivity, their interobserver reproducibility may be low. Benign tumors can resemble lethal malignancies under the microscope, and vice versa. And, unlike most neoplasms arising elsewhere, brain tumors are often difficult to biopsy, meaning that life-altering diagnoses are frequently being rendered on extremely small samples that might not contain all the required histologic features.

In addition to their clinical and histologic complexity, brain tumors have a highly diverse set of mutations and aberrations that directly impact their biology and response to treatment. Most brain tumors have a relatively low overall mutational burden, yet mutations in key genomic drivers are linked to tumor initiation and define tumor behavior. Like many other tumor types, copy number alterations can be diagnostic and/or define key therapeutic vulnerabilities for both low- and high-grade tumors, including focal (<10 megabases) and broad chromosome-level changes (arm or whole chromosomes) included in the new World Health Organization (WHO) classification criteria.[1] Gene fusions and rearrangements are important drivers in some brain tumors. Certain discrete methylation events, as well as global methylation patterns, are valuable as predictive and prognostic markers. Therefore, more than one molecular assay is usually needed to adequately evaluate a patient's brain tumor.

Because of recent advances in our knowledge of the clinically relevant genetic underpinnings of brain tumors, and of tumors arising elsewhere in the body, there is a critical need to apply that knowledge through molecular testing that captures important diagnostic, prognostic, and predictive information. In most cases, the main purpose of molecular testing in brain tumors is to ensure that the tumor is properly classified, so as to best inform the patient and treating physician about the true prognosis of the disease and the best treatment options. Molecular testing has already been guiding brain tumor therapy for more than 10 years, as 1p/19q codeletion and O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation have influenced the use of chemotherapies like procarbazine/lomustine/vincristine (PCV) and temozolomide (TMZ), respectively. New methods are now available—such as next-generation sequencing (NGS) and copy number profiling—that can determine the 1p/19q copy number status of a tumor, as well as provide a great deal more information about other aspects of the tumor's genome. Their use is therefore fully consistent with the goal of precision brain tumor diagnostics. The recent Food and Drug Administration approval of NGS,[2] and of tumor mutation burden (TMB) as a biomarker of sensitivity to immune checkpoint inhibitors,[3] provides further justification for such advanced molecular testing in all kinds of cancer, including brain tumors.

Details on the specific kinds of molecular tests required for the workup of brain tumors, including key strengths and drawbacks of each assay, are provided in the Supplementary text.

Why Molecular Testing Is Now Essential for the Workup of Brain Tumors

All the molecular panels, platforms, and assays routinely being used to improve the clinical care of brain tumor patients represent years of tremendous advancements in biotechnology, with decreasing costs to match. The first human whole-genome sequencing, officially completed in 2003, cost nearly $100 million. Now, it only costs several thousand dollars on most platforms, and targeted NGS panels are even less expensive.[4] Molecular data has fundamentally and permanently transformed the diagnosis and management of patients with gliomas and embryonal tumors, with similar changes forthcoming in sellar and meningeal tumors (Table 1, Figures 1 and 2). Even though the aggregate cost of this critical component of patient management is only a small fraction of the total cost of care (including imaging, surgery, and treatment), the reimbursement of these tests has fallen short, leading to a lack of implementation or full utilization at medical centers that treat brain tumor patients. Yet molecular testing is now required to make diagnoses according to the WHO 2016 scheme, and national practice guidelines, enumerated by the National Comprehensive Cancer Network and cIMPACT-NOW, have provided evidence and recommendations for their use in patient care.[5]

Glioma

Gliomas are the most common primary neoplasms arising within the brain parenchyma (meningiomas are more frequent overall but arise in the meninges covering the brain, not the brain itself).[6] In clinical usage, the term "glioma" usually implies an astrocytoma or oligodendroglioma that is infiltrative and ultimately fatal. But "glioma" encompasses a heterogeneous group of neoplasms arising from glial progenitor cells that, depending on driver mutations and other cues, develop into a wide range of tumors with markedly divergent outcomes. Because these outcomes are tightly linked with specific molecular alterations (Fig. 1), testing for these alterations enhances diagnostic and prognostic accuracy well beyond the capabilities of traditional light microscopy.

Pilocytic Astrocytoma, Ganglioglioma, and Pleomorphic Xanthoastrocytoma

The most common form of glioma is astrocytoma, which is still histologically graded on a WHO scale of I to IV. Pilocytic astrocytomas (PAs) are the most frequent grade I astrocytomas, arising most often in the posterior fossa and, less commonly, the optic nerve, supratentorial region, and spinal cord, primarily in children but also sometimes in adults.[7–10] The majority of PAs are indolent and curable with surgery alone. However, some can be histologically mistaken for higher-grade infiltrative gliomas, and midline PAs that are not fully resectable may require adjuvant therapy.[8,9] The detection of a *BRAF* fusion strongly favors the diagnosis of PA, and the fusion is most characteristic of cerebellar PAs in children.[10] In young adults and older patients, tumor location shifts to the supratentorium, and

Table 1 Molecular testing for the accurate diagnosis and subclassification of brain tumors*

Glioma	Embryonal Tumors	Other Tumors
• 1p/19q codeletion[1] • *ATRX* mutation[4] • *BRAF* fusion[4] • *BRAF* mutations[2] • *CDK4* amplification[4] • *CDKN2A* deletion[2] • Chromosome 10 monosomy[2] • Chromosome 7 gain[2] • *EGFR* amplification[2] • *EGFR* mutation[4] • *FGFR1* gain[2] • *FGFR1* mutation[2] • *FGFR3* fusions[3] • *H3F3A* mutations[1] • *IDH1* and *IDH2* mutation[1] • Methylation profiling[4] • *MGMT* promoter methylation[3] • *MYB* or *MYBL1* rearrangement[2] • *NOTCH1* mutation[4] • *RB* mutation or deletion[4] • *RELA* fusion[1] • *TERT* promoter mutation[2] • *TP53* mutation[4]	• *APC* mutation[1] • *BRG1* mutation[1] • C19MC amplification[1] • *CTNNB1* mutation[1] • GAB1 expression[1] • *INI1* mutation[1] • Methylation profiling[4] • Monosomy 6[4] • *MYC* amplification[4] • *MYCN* amplification[4] • SHH activation[1] • *TP53* mutation[1] • *WNT* activation[1] • YAP1 expression[1] • β-catenin expression and localization[1]	Craniopharyngioma • BRAF V600E mutation[4] • *CTNNB1* mutation[4] • β-catenin nuclear expression[4] Meningioma • *BAP1* mutation or deletion[4] • Methylation profiling[4] • *TERT* promoter mutation[4]

*Each molecular marker and/or test is denoted by the main source recommending its use: [1]WHO 2016 classification scheme; [2]cIMPACT-NOW updates; [3]treatment stratification; [4]proposed with good evidence in the literature.

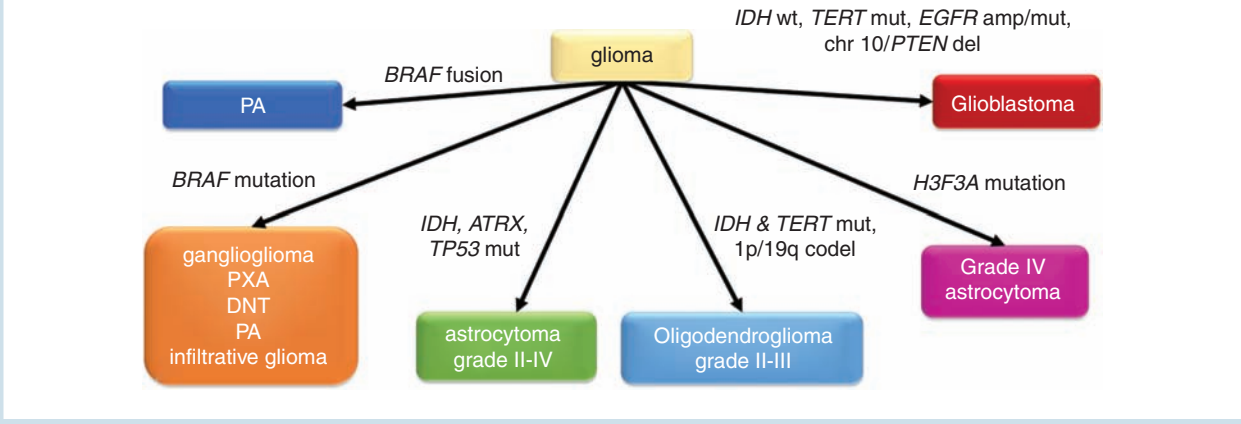

Fig. 1 Interpretation of the most common molecular test results in a glioma or glioneuronal tumor. Molecular data must always be interpreted in the appropriate clinical and histopathologic contexts. *MGMT* promoter methylation testing is recommended in all grades III–IV gliomas. PXA = pleomorphic xanthoastrocytoma; DNT = dysembryoplastic neuroepithelial tumor; amp = amplification; del = deletion; codel = codeletion.

BRAF V600E becomes somewhat more common.[10] In isolation, either a *BRAF* fusion or V600E mutation tends to impart an especially favorable prognosis, but if additional alterations involving cell cycle regulators are present, such as deletion of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and alterations of thalassemia/mental retardation syndrome X-linked (*ATRX*), the tumor is more likely to recur and progress to a higher grade.[9,11,12] A small subset of PAs have activating alterations in fibroblast growth factor receptor (*FGFR1*) instead of *BRAF*.[13] Even though both sets of alterations lead to mitogen-activated protein kinase pathway activation, PAs with *FGFR1* mutations or copy number gains might behave more aggressively.[12,14,15] Even so, the overall prognosis of PAs is far better than for most other gliomas. Isolated BRAF V600E is more often found in other potentially curable grade I neoplasms, like gangliogliomas and dysembryoplastic neuroepithelial tumors.[16] Grades II and III pleomorphic xanthoastrocytomas typically have *BRAF* V600E mutations, as well as alterations in genes encoding cell cycle proteins.[16,17] The Braf inhibitor, vemurafenib, is active against V600E-driven gliomas[18] but does not work against gliomas with *BRAF* fusions.[19] In this context, defining the molecular alterations surrounding *BRAF* can be highly informative for accurate delineation of lower-grade brain tumors, and may be impactful in their therapeutic management.

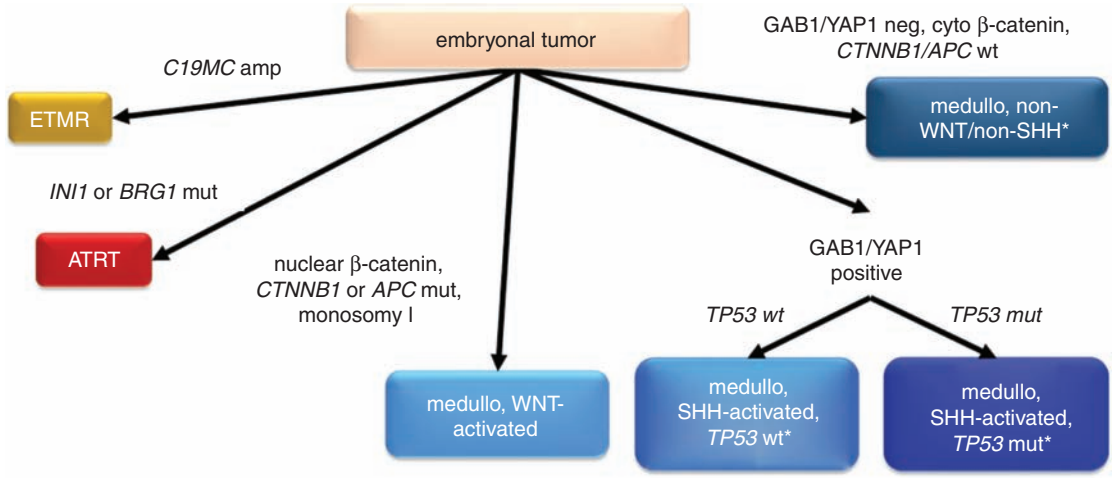

Fig. 2 Interpretation of the most common molecular test results in an embryonal tumor. Molecular data must always be interpreted in the appropriate clinical and histopathologic contexts. medullo = medulloblastoma; cyto = cytoplasmic. *MYC/MYCN amplification testing recommended.

Diffusely Infiltrative Astrocytomas

Most grades II and III astrocytomas in adults, and grade IV astrocytomas that arise from lower-grade tumors (previously known as secondary glioblastomas), are characterized by point mutations in isocitrate dehydrogenase 1 and 2 genes (hereafter referred to collectively as "IDH^{mut}").[20] While still ultimately fatal, IDH^{mut} astrocytomas tend to be less aggressive, and benefit more from chemotherapy (such as TMZ or PCV) than histologically similar tumors that are *IDH* wild-type (IDH^{wt}).[20,21] As might be expected from a mutation that causes global cytosine-phosphate-guanine hypermethylation, most IDH^{mut} gliomas have *MGMT* promoter methylation.[22] The majority of IDH^{mut} astrocytomas also contain mutations in *TP53* and *ATRX*, distinguishing them from IDH^{mut} oligodendrogliomas (see below).[23] Once an astrocytoma is identified as IDH^{mut}, mitotic index no longer has much prognostic relevance, meaning that there is little difference in overall survival between grades II and III IDH^{mut} astrocytomas that are diagnosed according to current histologic grading criteria.[24] Of far more prognostic importance is the presence of additional molecular alterations involving the cell cycle, including homozygous deletion of *CDKN2A*, deletion or mutation of *RB1*, and *CDK4* amplification, all of which are associated with shorter overall survival in grades II–III IDH^{mut} astrocytomas.[25] Because the CATNON trial showed that grade III anaplastic astrocytomas should be treated with radiotherapy (RT) and TMZ upfront,[21] and the histopathologic distinction between grades II and III astrocytomas is unclear, some neuro-oncologists prefer treating both grades II and III astrocytomas with RT/TMZ immediately. The presence of necrosis and/or microvascular proliferation is still important, as IDH^{mut} glioblastomas (GBMs) behave worse than IDH^{mut} grades II–III astrocytomas.[26] Furthermore, IDH^{mut} is not found in any grade I glioma, so its detection essentially precludes the diagnosis of grade I tumors like ganglioglioma.[27]

Oligodendrogliomas

While less common than astrocytomas, oligodendrogliomas are the other major subset of diffusely infiltrative glioma. Grades II–III oligodendrogliomas (there are no grade I or IV oligodendrogliomas) are usually still lethal, but confer longer survival than even IDH^{mut} astrocytomas. The diagnosis of an oligodendroglioma now requires the presence of IDH^{mut} and 1p/19q codeletion.[1] Interestingly, telomerase reverse transcriptase (*TERT*) promoter mutations, which are found in most GBM IDH^{wt}, are also present in most oligodendrogliomas.[23] Astrocytomas and oligodendrogliomas can be difficult to reliably distinguish solely based on histologic criteria. Prior to molecular diagnostics, this caused a lack of consistency among neuropathologists—a situation complicated by the varying use of the hybrid (and nebulous) term "oligoastrocytoma" for gliomas with mixed or equivocal features.[28] Yet the distinction is important, because not only are oligodendrogliomas less aggressive than their astrocytic counterparts, they appear to specifically benefit from PCV chemotherapy.[29,30] Because oligodendrogliomas also show increased sensitivity to TMZ, some neuro-oncologists prefer TMZ as a frontline therapy. Others prefer delaying adjuvant treatment in grade II IDH^{mut}, 1p/19q codeleted oligodendrogliomas until the tumor shows progression to grade III. At that time, chemotherapy is sometimes given alone, thereby sparing patients the side effects of RT as long as possible.[31] Such an option is available only in oligodendrogliomas because of their less aggressive nature. In current practice, *ATRX*, *TP53*, *TERT*, and 1p/19q screenings reliably segregate gliomas with hybrid morphology into astrocytomas or oligodendrogliomas, thus eliminating "oligoastrocytoma" as an integrated diagnosis within the WHO classification.[23,32] Although IDH^{mut}–*TERT*^{mut}–1p/19q codeleted oligodendrogliomas do have much better prognoses overall, mutations in *NOTCH* are associated with more rapid progression in these tumors.[33] Likewise, polysomy of 1p

and 19q has been linked with shorter survival in oligodendrogliomas.[34,35]

Glioblastoma

The most common glioma, IDH^{wt} de novo or primary GBM (GBM IDH^{wt}), unfortunately is also highly malignant. If a diffusely infiltrative glioma lacks IDH^{mut} but has other molecular alterations associated with GBM, it should probably be treated like a GBM even if necrosis and microvascular proliferation cannot be detected microscopically. The third update from cIMPACT-NOW recommended diagnostic criteria for "diffuse astrocytic gliomas, IDH-wild type, with molecular features of GBM, WHO grade IV" for such tumors; these criteria include *TERT* promoter mutations, gain of chromosome 7 and loss of chromosome 10, and *EGFR* amplification.[36] Additional molecular hallmarks of GBM include amplification of other receptor tyrosine kinases like *PDGFRA* and *MET*, as well as mutations in *PTEN* and *NF1*.[37] Because the vast majority of GBMs will contain at least 2 of the aforementioned alterations, great care must be taken when dealing with a lesion that has only one alteration. For example, isolated *PTEN* mutations and deletions are characteristic of certain nonneoplastic dysplasias, malformations, and hamartomas that might rarely be mistaken for gliomas.[38]

Thorough molecular characterization of a suspected GBM not only contributes to a definitive diagnosis, it also helps guide treatment decisions. Small-molecule inhibitors of epidermal growth factor receptor (EGFR), and antitumor vaccines directed against EGFR variant (v)III, have thus far failed to provide significant survival benefit in definitive clinical trials of GBM patients.[39–41] However, there is renewed interest in detecting EGFR-driven GBMs, as they may be responsive to depatuxizumab mafodotin (ABT-414). This is an antibody–drug conjugate that delivers the anti-microtubule compound monomethyl auristatin F into cells with high expression of EGFR or mutant EGFRvIII.[42] Another recently discovered driver of some GBM, *FGFR3* fusions, may respond to FGFR inhibitors.[43]

Pediatric Low-Grade Gliomas

Low-grade infiltrative gliomas in children and adolescents are broadly associated with much better outcomes than in adults, even when they lack IDH^{mut}. Some of these have BRAFV600E mutations, as discussed above. Others have *FGFR1* alterations, including internal tandem duplications or mutations, or have rearrangements in *MYB* or *MYBL1*.[44] Although such tumors may resemble classic astrocytomas or oligodendrogliomas, anaplastic progression is uncommon and overall survival in patients is markedly prolonged. Consequently, this subset of infiltrating gliomas warrants separate classification.[44]

MGMT in Gliomas

MGMT encodes O^6-methylguanine-DNA methyltransferase, an enzyme that repairs the DNA damage inflicted by alkylating agents like TMZ and nitrosourea-based chemotherapies. *MGMT* promoter methylation is associated with much better response to TMZ.[45] In several phase III trials utilizing TMZ for newly diagnosed glioblastoma, including EORTC/NCIC 26981-22981/CE3, RTOG 0525, and RTOG 0825, the median overall survival of patients with *MGMT* promoter-methylated GBMs ranged from 21.2 to 23.2 months, compared with 14.0–15.3 months for patients with unmethylated tumors.[46–49] In conjunction with the biochemical role *MGMT* plays in TMZ resistance, these and other data support *MGMT* promoter methylation as a prognostic and predictive factor associated with favorable TMZ response in patients with previously untreated GBM.

Several ongoing trials in North America and Europe are using *MGMT* unmethylated status as an inclusion criterion for studies that do not employ TMZ, thereby giving patients who are less likely to benefit from TMZ other options earlier in the course of disease.[50] Even if a clinical trial is not an option, TMZ is often withheld from patients with low Karnofsky performance status scores if their GBMs are *MGMT* unmethylated, since the benefit-to-toxicity ratio may be deemed too low. Conversely, *MGMT* promoter hypermethylation is used as an inclusion criterion for TMZ-sensitizing strategies in the Alliance A071102 clinical trial combining adjuvant TMZ and veliparib against GBM, as well as for several planned or ongoing National Cancer Therapeutic Network trials. Elderly patients, whose tolerance for TMZ is generally lower, can still be considered candidates for TMZ if their tumors are *MGMT*-methylated.[51–53] If a patient shows radiologic changes while on TMZ that may or may not indicate tumor progression, TMZ will usually be continued so long as the tumor has *MGMT* promoter methylation.

While not yet prospectively validated, the role of *MGMT* promoter methylation is also of growing importance in grades II–III IDH^{wt} astrocytomas that have GBM molecular profiles. Among IDH^{mut} grade II gliomas in the EORTC 22033 phase III trial, *MGMT* promoter methylation was predictive of response to TMZ, but not RT.[54] It is worth noting that since the *MGMT* gene is located on chromosome 10q, and 10q is usually retained in grades II–III gliomas but lost in GBM, there are 2 *MGMT* alleles in grades II–III tumors but only 1 in GBM. *MGMT* promoter methylation may therefore need to be more extensive in those lower-grade tumors to have the same suppressive effect on gene expression as in GBM. Regardless, any glioma in which TMZ therapy is being considered must be tested for *MGMT* promoter methylation.

Histone Mutations in Gliomas

Mutations in *H3F3A*, encoding a histone H3 variant, are present in some diffusely infiltrative astrocytomas (mutations may also be found in the related *HIST1H3B* gene in the brainstem). H3-K27M is characteristic of glioma arising in the pons (known as "diffuse intrinsic pontine glioma," or DIPG), as well as anywhere in the midline (basal ganglia, thalamus, midbrain, and brainstem) of children and adults; in contrast, H3-G34 mutations are more often found in hemispheric high-grade pediatric gliomas.[55] *H3F3A* K27M is mutually exclusive with IDH^{mut}, rarely has *MGMT* promoter methylation, and is associated with aggressive

behavior, such that the 2016 WHO classification scheme includes "diffuse midline gliomas with histone H3-K27M mutation" as a grade IV diagnosis, irrespective of whether or not the tumor shows classic grade IV histologic features like necrosis and microvascular proliferation.[1] Since biopsies of midline lesions tend to be small and difficult to interpret histologically, the detection of an *H3F3A* mutation is very helpful in supporting the clinical and pathologic suspicion of a high-grade infiltrative midline glioma. Of note, *H3F3A* K27M is also occasionally present in non-infiltrative gliomas and, in this context, does not automatically indicate grade IV behavior.[56]

Like astrocytomas and oligodendrogliomas, ependymomas arise from glial precursor cells, and hence are also classified as gliomas. Unlike astrocytomas and oligodendrogliomas, ependymomas do not contain IDH^{mut} or 1p/19q codeletion. There are multiple molecular subtypes of ependymomas,[57] but 2 that have been proposed to have unfavorable prognoses are (i) posterior fossa ependymomas group A (PF-EPN-A); and (ii) ependymomas with fusions involving v-rel avian reticuloendotheliosis viral oncogene homolog A (*RELA*). The PF-EPN-A subtype mostly occurs in the cerebellum of young children, and although it does not exhibit recurrent genomic alterations, it is readily identified by a characteristic hypermethylation signature and/or an absence of H3K27me3 immunostaining in tumor cell nuclei.[57,58] *RELA* fusions cause increased signaling of nuclear factor-kappaB, are often found in supratentorial ependymomas, and may associate with much more aggressive behavior, independent of histologic grade. The latter subset of ependymomas now have their own WHO designation as "ependymoma, *RELA*-fusion positive."[1,57] In addition to facilitating accurate diagnosis and guiding prognosis, molecular classification of ependymoma is beginning to influence choice of treatment, including extent of surgical resection and RT.[59]

Collectively, these data demonstrate that the molecular characterization of gliomas not only ensures accurate diagnoses, but also provides important prognostic information that can inform treatment decisions.

Embryonal Tumors

Brain tumors with a more primitive histologic appearance, including small nuclei with dense chromatin and sparse cytoplasm, are grouped under the category of "embryonal tumors" (Fig. 2). Embryonal tumors are rare and occur mostly in children. Some of them have only recently been codified as discrete entities by the WHO classification system, and are often very difficult to distinguish from each other histologically. Molecular studies are therefore an indispensable way to ensure that they are not misdiagnosed.

Medulloblastomas

The archetypal embryonal neoplasm is medulloblastoma, a cerebellar malignancy arising most often in children and young adults. While all medulloblastomas are grade IV, there are actually 4 major molecular subtypes, with widely varying behavior, that have warranted their own separate designations in the updated WHO classification system.[1,60,61]

Wingless (WNT)-driven medulloblastomas are by far the least aggressive (and the least common), and are characterized by mutations in catenin beta-1 (*CTNNB1*) or, less frequently, adenomatous polyposis coli (*APC*), as well as monosomy 6. Immunostaining for β-catenin shows nuclear localization in such tumors but can be equivocal and requires molecular confirmation.

Mutations in *PTCH1*, *SMO*, and/or *SUFU* characterize sonic hedgehog (SHH) medulloblastomas, which as a group have a worse prognosis compared with WNT tumors, but are usually less aggressive than non-WNT/non-SHH medulloblastomas. Immunopositivity for both growth factor receptor bound protein 2 associated–binding protein 1 (GAB1) and Yes-associated protein 1 (YAP1) is characteristic of SHH-driven medulloblastomas. SHH medulloblastomas may respond to hedgehog pathway inhibitors, such as vismodegib.[62] SHH-driven tumors with *TP53* mutations fare much worse than those without, as do SHH tumors with *MYC* or *MYCN* amplification and large cell/anaplastic histology.[60,63]

For diagnostic purposes, the last 2 molecular subtypes of medulloblastoma, Group 3 and Group 4, are combined by the WHO scheme into a single "non-WNT/non-SHH medulloblastoma" entity.[1] Tumors in this group are often the most aggressive and metastatic, especially if they have large cell/anaplastic features, contain isochromosome 17q, and have amplification of *MYC* or *MYCN*.[61] Such molecular data can help physicians determine whether to offer adjuvant chemotherapy after RT in patients who are otherwise considered only average risk (eg, no metastases, no histologic anaplasia).[64,65]

Atypical Teratoid/Rhabdoid Tumors

Another highly aggressive embryonal tumor, atypical teratoid/rhabdoid tumor, is defined by alterations of either *INI1* or, rarely, *BRG1*.[66] Loss of normal integrase interactor 1 (INI1) expression in tumor cell nuclei, demonstrable by immunohistochemistry, is a good surrogate marker of *INI1* mutations.[67]

Primitive Neuroectodermal Tumors

For a long time, the term "primitive neuroectodermal tumor" (PNET) was used to diagnose CNS neoplasms that had embryonal features. But recent molecular studies, including methylation profiling, showed that most PNETs are actually other well-known kinds of tumor, such as GBM.[68] Furthermore, new distinct entities have emerged among tumors previously grouped together as PNETs. "Embryonal tumor with abundant neuropil and true rosettes," "ependymoblastoma," and "medulloepithelioma" all have similarly poor prognoses, and are all characterized by amplification of the microRNA cluster C19MC. Accordingly, these terms have been merged into a new WHO entity, "embryonal tumor with multilayered rosettes" (ETMR), defined by C19MC amplification.[1,69] Molecular diagnostics have defined other tumors formerly termed

PNETs, including "CNS neuroblastoma with *FOXR2* activation," "CNS Ewing sarcoma family tumor with *CIC* alteration," "CNS high-grade neuroepithelial tumor with *MN1* alteration," and "CNS high-grade neuroepithelial tumor with *BCOR* alteration."[68]

Other Tumors

Craniopharyngiomas

The sella turcica is a depression in the sphenoid bone that accommodates the pituitary gland. Pituitary adenomas are the most common sellar neoplasm, but a surprising variety of lesions and tumors can arise in this region, including craniopharyngiomas. Locally destructive and recurrent tumors that arise from embryonic pituitary tissue, craniopharyngiomas are divided into adamantinomatous and papillary subtypes, which are usually easy to distinguish from each other but sometimes histologically overlap. Although these subtypes have similar prognoses, molecular diagnostics are still very useful in their workup. Adamantinomatous craniopharyngiomas contain *CTNNB1* mutations and/or nuclear localization of β-catenin, both of which help differentiate these tumors from papillary craniopharyngiomas, which are characterized by *BRAF* V600E.[70,71] Several case reports have demonstrated that patients with BRAF-positive papillary craniopharyngiomas may respond to targeted Braf inhibitors[71–75]; this is currently being investigated in an Alliance cooperative group clinical trial (NCT02114767). *CTNNB1* and *BRAF* screening also help distinguish craniopharyngioma from a nonneoplastic cystic lesion in the sella, Rathke's cleft cyst.

Meningiomas

Meningiomas are actually more common than gliomas.[6] But because they are often treatable with surgical resection and/or RT, they are widely regarded as less problematic than gliomas. Thus, they have received proportionately far less attention and research efforts. But some meningiomas repeatedly grow back, invade the underlying brain and venous sinuses, and require multiple surgical resections and high doses of RT. Such aggressive meningiomas cause serious long-term cognitive decline, and can even be lethal. Despite its relative accessibility in most cases, lack of single-cell infiltration, and nonexistent blood–brain barrier, no effective adjuvant therapy besides RT has yet been discovered for meningiomas.

While histologic grading remains the standard for prognostic stratification of meningiomas, the current WHO scheme fails to accurately predict tumor recurrence, systemic treatment options, or overall prognosis in a large proportion of cases. Massive sequencing endeavors have now identified potentially actionable targets in meningiomas, including oncogenic mutations in *SMO*, *AKT1*, and *PIK3CA*.[76–78] *SMO* mutations are characteristic of olfactory groove meningiomas, *AKT1* mutations are seen in skull base tumors, and *NF2* inactivation is most common in tumors of the cerebral convexities and is present in about half of all RT-induced meningiomas.[79] Certain mutations correlate with specific subtypes of meningioma, as *BAP1* alterations are often found in rhabdoid tumors, and *KLF4* mutations are specific for secretory meningiomas.[80,81] A national precision medicine trial is under way to explore the role of targeted therapies in meningioma (Alliance A071401; NCT02523014). *TERT* promoter mutation and *DMD* deletion suggest a worse prognosis.[82,83] Although high TMB is rare in meningiomas, such tumors may respond to immune checkpoint inhibitors.[84]

Methylation array-based profiling of meningiomas may ultimately prove superior to light microscopy at determining which meningiomas are more likely to behave aggressively.[85,86] Specific copy number alterations have also been proposed as adverse prognostic markers.[87] As in gliomas, molecular testing will likely soon become necessary for optimal prognostication and treatment decisions in meningiomas.

Conclusion and Recommendations

Thorough molecular testing is now essential for the care of patients with brain tumors. Appropriate application of these testing strategies provides enhanced diagnostic and prognostic accuracy, defines targetable alterations, and improves outcomes (Fig. 3).

The combination of an NGS panel plus genome-wide scan for copy number variations (CNVs) is the ideal approach for the majority of gliomas and embryonal tumors, as well as for cases suspected of being metastatic tumors. Immunostains linked with specific molecular alterations are also helpful. In the next few years, the methylation 850K array and classifier might replace genome-wide CNV platforms, since methylation 850K generates genomic CNV data plus an entirely new dimension of epigenomic information on tumor subtype and tissue of origin (see Supplementary text). *MGMT* promoter methylation is still required for high-grade gliomas, but traditional *MGMT*-specific testing could be replaced with broader methylation profiling. When tissue and tumor cellularity are sparse, single-target molecular testing like fluorescence in situ hybridization (FISH), Sanger sequencing, and pyrosequencing are the next best options. Since these tissues are precious resources for patient care, the choice of tests and immunostains should be determined by highly experienced neuropathologists.

As we have demonstrated, advanced molecular diagnostics are now required to accurately guide complex, multimodal integrated brain tumor therapy. The aggregate cost of NGS and CNV or methylation arrays is less than a comparable series of older tests, including single-gene sequencing, FISH, and immunohistochemical analyses. It is also far less expensive than surgery, RT, or even a single MRI scan. Yet, while insurers routinely cover older methods of testing and all the other required elements of patient care, they are often reluctant to cover advanced molecular diagnostics. But these new molecular tools produce more accurate diagnostic and prognostic information, reduce the use of inappropriate treatment regimens, and minimize the

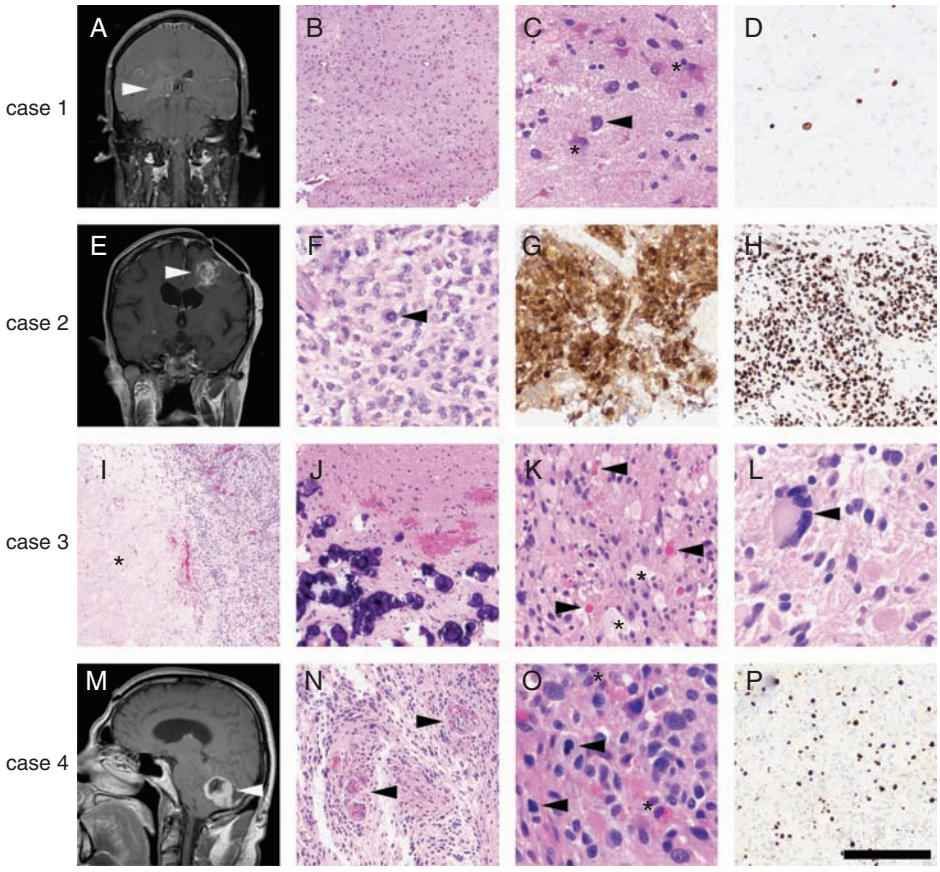

Fig. 3. Examples of the impact of advanced molecular testing in brain tumors. Case 1 was a 44-year-old man with an ill-defined enhancing lesion in the right deep temporal lobe (A, arrowhead). A stereotactic biopsy contained moderately hypercellular brain tissue (B), composed mostly of reactive astrocytes (C, asterisks) and cells with highly atypical nuclei (C, arrowhead). No mitoses, necrosis, or microvascular proliferation was present in the biopsied material. Ki67 immunostain (D) showed only scattered positive cells. NGS detected mutations in *TP53*, *PTEN*, and the *TERT* promoter, leading to the diagnosis of GBM and sparing the patient a repeat surgery to obtain histologically diagnostic tissue. The patient was treated with RT/TMZ and survived less than a year after diagnosis. Case 2 was a 50-year-old woman in whom was histologically diagnosed a left frontal WHO grade II diffuse astrocytoma 10 years ago at an outside hospital (OSH), who now presented with recurrence and new enhancement (E, arrowhead). Histologically, the recurrent tumor was high grade with necrosis and microvascular proliferation (not shown), as well as mitoses (F, arrowhead) and nuclei that appeared more astrocytic than oligodendroglial. An immunostain for IDH1 R132H was positive (G), yet nuclear ATRX expression was retained (H). NGS confirmed the IDH1 R132H mutation and detected a *TERT* promoter mutation, as well as 1p/19q codeletion. The tumor was therefore reclassified as anaplastic oligodendroglioma, WHO grade III. The patient responded well to RT and PCV, with no recurrences 1 year later. Case 3 was a pathology consult from an OSH, consisting of a right parietal mass in a 25-year-old man (no radiology available). Histologically the tumor had necrosis (I, asterisk) and mitoses (not shown), but also had abundant mineralization (J), eosinophilic granular bodies (K, arrowheads), foamy lipidized cells (K, asterisks), and highly atypical cells, including multinucleated giant cells (L, arrowhead). The OSH diagnosed the tumor as a GBM, because the screening for BRAF V600E was negative. However, NGS detected a rare oncogenic variable lymphocyte *receptor* insertion between codons 506 and 507 of *BRAF*, previously described in Langerhans cell histiocytosis.[88] No other alterations were detected, so the tumor was reclassified as anaplastic pleomorphic xanthoastrocytoma, WHO grade III. The patient had already been treated at the OSH with RT/TMZ based on the original GBM diagnosis, but was still alive 2 years later with no recurrences. Case 4 was a 50-year-old man with an enhancing, well-circumscribed cerebellar mass (M, arrowhead) containing microvascular proliferation (N, arrowheads), numerous mitoses (O, arrowheads), eosinophilic granular bodies (O, asterisks), and a high Ki67 proliferation index (P), but no necrosis or Rosenthal fibers. This tumor was originally diagnosed at the OSH as a GBM and treated with RT/TMZ, but NGS (performed several years later) detected an *FGFR1* mutation and *CDKN2A* deletion, changing the diagnosis to an anaplastic pilocytic astrocytoma.[12–15] The patient survived 7 years before the tumor recurred, and is still alive 2 years after re-resection (9 years total). Scale bar = 500 microns in I; 200 microns in B, G, H, J, N, P; 100 microns in C, D, K; 50 microns in F, L, O.

exposure of patients to unnecessary life-altering morbidity (and sometimes even mortality) from such treatment. They are also indispensable for discovering targetable mutations and fusions, especially in uncommon variants of common CNS tumors (eg, *BRAF* mutation in GBM). Molecular diagnostics help stimulate new drug development, and identify patients likely to benefit from new pan-cancer clinical trials aimed at specific genetic lesions, rather than at tumor histology or tissue of origin.

Advanced molecular testing can directly lead to tangible improvements in the diagnosis, prognosis, and prediction of therapeutic response for patients with brain

tumors. Leaders in the brain tumor community, including physicians and patient advocacy groups, can help by petitioning the National Cancer Institute, the Food and Drug Administration, and insurance payors to provide better coverage for these powerful new molecular diagnostic tests.

Supplementary Material

Supplementary data are available at *Neuro-Oncology* online.

Keywords

embryona | ependymoma | glioma | meningioma | molecular

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