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Actionable molecular biomarkers in primary brain tumors

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Abstract

Recent genome-wide studies of malignancies of the central nervous system (CNS) have revolutionized our understanding of the biology of these tumors. This newly gained knowledge provides a wealth of opportunity for biomarker driven clinical research. To date, however, only few of the available molecular markers truly influence clinical decision-making and treatment. The most widely validated markers in neuro-oncology presently are: 1) *MGMT promoter methylation* as a prognostic and predictive marker in glioblastoma, 2) *co-deletion of 1p and 19q* differentiating oligodendrogliomas from astrocytomas, 3) *IDH1/2* mutations, and 4) select pathway-associated mutations. This article focuses on currently impactful biomarkers in adult and pediatric brain cancers and it provides a perspective on the direction of research in this field.

Keywords

Biomarker; glioma; glioblastoma; medulloblastoma; co-deletion of 1p/19q; *MGMT* promoter methylation; *IDH* mutation; *BRAF* mutation

Clinical Biomarkers in Primary Brain Cancers: State-of-the-art Snapshot

The past two decades have provided a wealth of new knowledge on the biology and pathophysiology of primary tumors of the central nervous system (CNS), largely catalyzed by genome-wide studies that unveiled the genomic landscape of these malignancies. Some of these key alterations inferred the development of novel biomarkers that have been incorporated into the newly released World Health Organization (WHO) Classification of

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Tumors of the Central Nervous System, which for the first time classifies brain tumors not only based on their histological appearance but also on molecular parameters [1]. As a consequence, these molecular markers may overwrite the histologic phenotype in certain circumstances and thus, significantly impact treatment options and treatment selection for patients.

Many publications have focused on the potential utility of molecular biomarkers for prognostic and predictive purposes, with the goal of providing more personalized, tailored therapies for our patients. Traditionally, predictive markers are defined as a hallmark that is used to identify a subpopulation of patients that is most likely going to respond to a certain therapy while a prognostic biomarker provides information on the likely clinical course of the disease in an untreated individual. Not all of the molecular markers that are biologically meaningful fall into these two categories and are truly essential for clinical decision-making [2]. In fact, only a small number of markers are clinically relevant for diagnostic and therapeutic purposes and, thus, there is controversy of how much molecular testing should be done in patients. In the following sections, we will provide a year 2016 snapshot of the clinically most impactful and accepted molecular biomarkers in primary brain tumors, as well as perspectives of the research trends in this field (**Table 1**).

This article focuses on truly *actionable* biomarkers, defined as molecular hallmarks whose presence or absence has implication on clinical decision-making in standard clinical care. Many of the markers discussed are already used as a patient selection tool for clinical trials, but the article's emphasis is primarily on their utility in standard clinical practice based on currently available clinical evidence.

Diffuse Astrocytic Tumors

Diffuse astrocytic tumors are the most common malignant CNS neoplasms in adults. As defined by the WHO classification of 2016, these tumors are graded into three classes based on their histological features, i.e. diffuse astrocytomas (WHO grade II), anaplastic astrocytomas (AA, WHO grade III) and glioblastomas (GBM, WHO grade IV) and each class is further subdivided into *IDH mutant* and *IDH wildtype* [1].

As reflected by the addition to the WHO classification, *IDH* has received considerable attention since the discovery in human gliomas. Mutations in the genes coding for *isocitrate dehydrogenase 1 (IDH1)* or less frequently 2 (*IDH2*) occur in approximately 80% of grade II and III astrocytoma and oligodendrogliomas (see below), as well as secondary GBM, i.e. tumors that evolved from previously confirmed lower grade gliomas [3,4]. Studies have demonstrated that an isolated amino acid missense mutation in *IDH1/2* at arginine 132 (R132) or the analogous residue 172 (R172) results in metabolic reprogramming with the ability to convert α-ketoglutarate (α-KG) to the R(-)-2-hydroxyglutarate (2-HG) [5]. Presumaby, 2-HG alters the epigenetic machinery that contributes to gliomagenesis, chromatin modifications and dysregulation of gene expression. Significant prognostic differences have been identified between *IDH1/2* mutant and wildtype astrocytomas that have started to impact clinical treatment decisions, even outside of clinical trials. As *IDH-mutant* astrocytomas have a more favorable survival, they may require a less aggressive

treatment approach. By contrast, tumors lacking *IDH* mutations may feature genetic findings characteristic of GBM that predict an aggressive clinical course and require an intensified treatment protocol.

GBM, the most common and most aggressive of these cancers, are among the best-described and the most studied primary brain cancers. Apart from the evolutionary mechanism, GBMs can be classified into four molecular subgroups with prognostic implications that are widely discussed in the literature [6]. Despite our increasing knowledge on the underlying biology of these tumors, their clinical management has not significantly changed over the past decade [7]. Treatment of newly-diagnosed GBM continues to be based on radiation and temozolomide as defined by the original European Organization for Research and Treatment of Cancer (EORTC) 26981/22981 National Cancer Institute of Canada (NCIC) trial showing a survival benefit from the addition of temozolomide overall and in all subgroups studied, an effect that was most dramatic in the *MGMT* (O6-methylguanine-DNA methyltransferase) promoter methylation positive cohort [8,9]. Subsequently, the methylation status of the promoter of the DNA repair enzyme *MGMT* evolved as an essential biomarker [10] that is detected in about 40% of patients with GBM. Methylation of the *MGMT* promoter region leads to epigenetic silencing of *MGMT*, thereby inhibiting DNA repair. Thus, *MGMT* promoter methylation is a strong predictor of prolonged survival, independent of other clinical factors or treatment and is associated with prolonged progression-free and overall survival in patients with GBM treated with chemotherapy and radiation therapy [10]. Of the 206 cases available of the original EORTC/NCIC study, 45% showed *MGMT* promoter methylation, which was associated with an independently favorable median overall survival (OS) of 21.7 months after radiochemotherapy, yet a smaller survival advantage of 15.3 months was also observed in patients with unmethylated *MGMT*. Thus, the majority of neuro-oncologists will still treat patients with temozolomide regardless of *MGMT* status but *MGMT* promoter methylation may be considered a tie-breaker in certain clinical scenarios, such as patients of older age, that account for a large fraction of GBM, and/or poor performance status for whom the addition of temozolomide to radiation may result in considerable toxicities [11]. This is supported by the results of the NOA-08 trial that illustrated how *MGMT* assessment can benefit the clinical decision-making indicating that temozolomide monotherapy was at least as effective as radiotherapy alone for ‘elderly patients’ with *MGMT*-methylated GBM [12].

Multiple recent randomized trials have confirmed the important prognostic and predictive roles of *MGMT* promoter methylation in patients with newly diagnosed GBM [12-14], including ‘elderly patients’ [11,12,15]. Moreover, in an analysis of long-term survivors with GBM, defined as OS >36 months, *MGMT* promoter methylation was the only enriched molecular marker detected [16]. Furthermore, *MGMT* promoter methylation has been associated with a higher rate of so-called pseudo-progression on imaging after chemoradiation, i.e. imaging findings suggestive of progressive disease that are caused by radiation-induced injury to the CNS [17]. Although not yet prospectively validated in studies, it is by some considered an adjunct decision maker in interpreting these imaging findings and distinguish pseudoprogression from truly progressive disease.

Even though the role of *MGMT* promoter methylation as a predictive and prognostic biomarker has been largely restricted to GBM, *MGMT* promoter methylation occurs with similar frequencies in grades II, III and IV diffuse astrocytomas and is thought to be an early event in gliomagenesis. Data in lower grade astrocytomas (WHO II and III), however, are less clear with regards to its clinical relevance. In the absence of these data, *MGMT* promoter methylation status is still frequently determined in anaplastic astrocytomas (WHO grade III) because a retrospective subgroup analysis has indicated a potential predictive role in that patient cohort, with a longer progression free survival observed in *MGMT* promoter methylated patients. However, those data are empirically extrapolated from other studies and dedicated prospective trials defining the role of the addition of temozolomide to radiation in these cancers are lacking [7].

In summary, multiple studies have shown that GBM can be separated into two biologically different entities based on *MGMT* promoter methylation status. However, due to the lack of alternative treatment options for patients with unmethylated GBM, *MGMT's* role in clinical decision-making remains limited and the direction-changing impact of routine *MGMT* analysis is restricted to only a few clinical scenarios. As there is currently no uniform *MGMT* testing methodology available and various testing methods, including methylation-specific polymerase chain reaction, pyrosequencing, methylation-specific multiplex ligation-dependent probe amplification and immunohistochemistry are being used, standardized procedures need to be created to allow inter-laboratory reproducibility, especially if future treatment decisions will be based on these results. It is of note that other molecular markers have been identified and contribute to a more fine-tuned subcategorization of diffuse astrocytomas. Recently, alterations of *TERT, ATRX* and *TP53* have been implicated in differential pathways in gliomagenesis (see also separate sections below), but so far neither of these markers has directly impacted clinical decision-making in astrocytomas (**Table 1**).

Diffuse Oligodendroglial Tumors

Historically, oligodendrogliomas and oligoastrocytomas (so-called ‘mixed’ gliomas) were considered variants of gliomas and were treated essentially identically to astrocytomas of the same histopathological grade [7]. Clinically, however, these cancers are associated with better survival and increased chemosensitivity. Fluorescence *in situ* (FISH) studies performed on patients enrolled in the two landmark anaplastic oligodendroglioma (AO) studies, RTOG 9402 and EORTC 26951, showed that patients whose tumors harbored a completed *co-deletion of chromosomes 1p and 19q* (co-deletion of 1p/19q) had significantly longer survival than patients without this marker, proving its prognostic relevance [18,19]. Follow-up analysis of patients carrying the co-deletion within these two studies compared the treatment with radiation plus chemotherapy with PCV (procarbazine, lomustine, vincristine) versus radiation alone (historical standard), showed significant separation of the survival curves after approximately 7 years, with striking differences in median survival between patients who received combination therapy versus radiation alone: Median survival in patients treated with PCV followed by RT in the RTOG 9402 study was 14.7 versus 7.3 in patients treated with RT alone; median survival in patients treated with RT followed by PCV in the EORTC 26951 study had not been reached yet [20,21]. These studies demonstrated that co-deletion of 1p/19q was not only a prognostic but also a predictive biomarker in these

cancers. Until publication of these data, patients with AOs had been commonly treated with radiation and temozolomide, modeled after standard treatment for GBM. Results of these two studies provided striking survival advantages of the addition of PCV that have been unparalleled in neuro-oncology to-date. The question of how radiation and temozolomide might compare to radiation and PCV has remained unknown but will eventually be answered by the currently ongoing CODEL study (NCT00887146) [22] that is comparing the two regimens in newly-diagnosed AO. Co-deletion of 1p/19 is therefore a truly impactful biomarker that, if present, leads to a change in therapy. A related study, RTOG9802, conducted in low-grade gliomas has yielded similar results [23]. However, 1p/19q co-deletion status was not determined in patients in trial, and the distinction between oligodendrogliomas versus astrocytomas was determined based on histopathology [23]. Regardless, the results suggested similar outcomes in classic low-grade oligodendrogliomas compared to the results of RTOG 9402 and EORTC 26951, and low-grade oligodendrogliomas are now also being included in the prospective CODEL trial. The question of appropriate molecular testing has been addressed because presence of co-deletion of 1p/19q (whole arm losses of 1p and 19q) as well as a mutation in *IDH1/2* are now a prerequisite for the diagnosis of oligodendrogliomas, based on the 2016 WHO classification. A potentially predictive role for IDH mutations in AO had also been suggested as part of a subgroup analysis of study RTOG 9402 in AO, in which IDH mutation status was found to be predictive of response to chemotherapy [24].

Mutational analysis of the breakpoint region in 1p/19q co-deleted oligodendrogliomas revealed frequent mutations of the *CIC* gene on chromosome 19q and of *FUBP1* on chromosome 1p. In addition, new data, integrating 1p/19q co-deletion status, mutations of *IDH*, *TP53*, *ATRX* and *TERT* have emerged. The clinical importance of these and other markers has however not yet impacted on clinical practice and the main ‘tie breaker’ for selection of treatment has remained presence or absence of co-deletion of 1p/19q (**Table 1**).

The landscape of pediatric brain tumors

Pediatric brain tumors differ fundamentally from their adult counterparts in terms of tumor types, locations, underlying biology and clinical course [25,26]. Based on data from CBTRUS, the most common malignant pediatric CNS tumors fall into three groups: 1) gliomas; 2) embryonal tumors, consisting predominately of medulloblastomas (MBs), primitive neuroectodermal tumors (PNETs), as well as atypical teratoid rhabdoid tumors; and 3) germ-cell tumors [27]. Despite our increased understanding of the complex biologic makeup of these tumors, only few genetic aberrations in a subset of these tumors have proven clinically relevant as molecular biomarkers. Those tumors include MBs and subgroups of gliomas, which we will discuss in detail in this section; for the remaining childhood CNS tumors, clinically relevant molecular markers remain elusive.

Medulloblastoma

Medulloblastoma (MB) is a small-cell embryonal brain cancer located in the cerebellum and the most common malignant brain tumor in children. Historically, clinicopathologic studies have supported a dramatic heterogeneity in this disease entity that with the rapid

advancements in our molecular understanding has been linked to inherent molecular differences and resulted in differentiation of at least four MB subtypes: wingless (WNT) activated, sonic hedgehog (SHH) activated, Group 3 that is associated with *MYC* and *OTX2* activation, and Group 4 that lacks a unifying pathway [28-30]. Each subgroup has a unique tumor cell histology, genetics, and clinical behavior, which, when identified at diagnosis, are strongly associated with clinical outcomes: WNT tumors have an excellent prognosis (>90% survival at 5 years), SHH and Group 4 carry an intermediate prognosis (approximately 75% survival at 5 years), and Group 3 tumors have a comparatively poor prognosis (50-60% survival at 5 years) [31,32].

Irrespective of these divergent biologic behaviors, current therapeutic decision-making is still largely based on the histology and clinical factors, particularly metastatic disease at presentation and residual disease after surgical resection, to determine the risk-adapted treatment protocol consisting of maximal surgical resection, craniospinal radiotherapy and chemotherapy [33,34]. Because some of these histological and genetic subtypes are associated with dramatic prognostic and therapeutic differences, such an approach carries a significant risk for over- or undertreatment, which may negatively impact disease control in patients with high-risk disease or long-term adverse effects in patients with favorable disease [35-37]. Biomarkers indicating the respective molecular subgroup have thus emerged in the clinical routine, aimed at preciser risk stratification and improved outcomes although testing methods vary and have not yet been standardized internationally.

More than 90% of WNT-MB harbor mutations in *CTNNB1*, a key gene that encodes β-catenin and renders the protein resistant to degradation, leading to its accumulation in the cell nucleus [38]. β-catenin gene expression profiling or β-catenin nucleopositivity on immunohistochemistry along with *CTNNB1* mutations and monosomy 6 characterize this molecular subgroup [34]. On the basis of the uniformly good prognosis of WNT-MB, a de-escalated treatment regimen with reduced-dose craniospinal radiation, reduced-intensity chemotherapy or a combination of both is considered for patients without metastatic disease.

Unlike WNT-MB, MBs characterized by activation of SHH signaling are heterogeneous and associated with a variety of genetic aberrations and outcomes [28]. *TP53* mutation status appears to be particularly critical and can segregate individuals with SHH-MB into favorable and poor survival groups [39,40]. Patients with SHH/*TP53* mutant MBs have profoundly worse outcome than those with SHH/*TP53* wild-type tumors because mutant *TP53* has been associated with catastrophic cellular events, a high rate of anaplasia and *MYCN* amplification [39,40]. Given its clinical impact, *TP53* mutation status has been incorporated into the 2016 WHO classification for CNS tumors and is now routinely assessed in all SHH activated MBs [1]. Nevertheless, patients with tumors carrying poor prognostic indicators are rarely cured even if treated with high-dose craniospinal radiation plus adjuvant chemotherapy and dedicated clinical trials should be utilized. Other markers that are subject to ongoing investigations include *GLI2* and the *MYC gene* family, both of which also infer a poor prognosis [41]. At the current time, those are not routinely assessed outside of clinical trials but may help to select patients for intensification of therapy in the future, for example arsenic treatment in SHH-MB with *GLI2* overexpression.

The absence of markers indicating WNT or SHH pathway activation defines MBs of group 3 and 4 and are thus categorized as Non-SHH/WNT. They frequently show amplification of *MYC* or *MYCN* and chromosome 17 imbalance, which prospectively predict a poor prognosis [41,42]. Therapeutically, it is currently investigated whether the addition of gemcitabine, a nucleoside analog, and pemetrexed, a folate antimetabolite, can improve prognosis in these patient populations (NCT01878617) [43,44].

Pediatric diffuse gliomas

Pediatric diffuse gliomas can be clinically distinguished in low-grade (LGG, grade II) and high-grade (HGG, grades III and IV) tumors to reflect their anticipated biologic behavior and clinical course. In contrast to adults, high-grade gliomas only encompass about 8–12% of childhood primary CNS tumors [45] and traditionally include GBM, AA, AO and diffuse midline gliomas, previously known as diffuse intrinsic pontine glioma (DIPG). Furthermore, almost all pediatric HGG arise *de novo* and malignant transformation from a LGG (secondary HGG), as frequently observed in younger adults, is exceedingly rare [46].

While pediatric HGGs are characterized by a similar morphology, clinical behavior and prognosis as their adult counterparts, there are clear differences in location (predominately involving midline structures, e.g. pons, thalamus and cerebellum) and underlying genetic abnormalities [46]. Chromatin-remodeling defects are key in the pathogenesis of pediatric HGG, especially in diffuse midline glioma, but are virtually absent in adult tumors [47]. Of particular clinical relevance are mutations in histone *H3F3A*, with 78% of DIPG harboring the amino acid substitution lysine 27 to methionine (*K27M*) and up to approximately one-third of non-brainstem HGGs carrying glycine 34 to valine or arginine (*G34V/R*) or *K27M* mutations [48,49]. Tumors harboring these mutations carry a grim prognosis [50]. However, the identification of this molecularly defined subgroup provides a rationale for therapies directed against the effects of these mutations. Candidate drugs include the epigenetic modifier panobinostat and GSKJ4, an inhibitor of the Jumonji-domain demethylase *H3K27* [51,52]. At the moment, these pre-clinical findings await further validation and clinical translation while standard therapies with radiation and temozolomide largely failed to significantly improve survival [53]. It should be noted that *MGMT* promoter methylation status was also found to have a predictive and prognostic role in these tumors [53], however, testing is not routinely performed in the pediatric neuro-oncologic community due to unclear therapeutic relevance and lack of alternative options.

Pilocytic Astrocytoma

Oncogenic activation of *BRAF* was identified in a high proportion of pediatric LGGs. Tandem duplication at 7q34 creates the fusion gene *KIAA1549:BRAF* and results in abnormal activation of MAPK/ERK pathway and deregulation of cell growth, differentiation and apoptosis. The central role of this pathway is further supported by neurofibromatosis 1, a disorder associated with RAS overactivation in which about 15% of children develop a low-grade glioma. *KIAA1549:BRAF* is found in more than 50-70% of childhood pilocytic astrocytomas but also in other age groups [54,55].

The prognostic significance of the *KIAA1549:BRAF* fusion remains uncertain, although a few studies have found slight improvements in survival between tumors with and without BRAF duplication/fusion [56] while others found no difference [57,58]. Regardless, the high frequency of this *BRAF* alteration in PA may serve as a diagnostic tool for differentiating between PA and grade II astrocytoma [55]. In addition, it may serve as a novel therapeutic target for pharmacological inhibition of MAPK pathway, particularly for inoperable tumors [59]. Preclinical studies showed that *BRAF* influences the proliferative potential of cells and silencing of BRAF through shRNA lentiviral transduction and pharmacological inhibition with various MEK inhibitors, such as U0126, PD0325901 and AZD6244 blocked proliferation and arrested growth of glioma cells, whereas wild-type xenografts were insensitive to MEK inhibition [54,60,61]. Furthermore, therapeutic manipulation of the BRAF and MAPK pathway with sorafenib, a potent RAF1 inhibitor with action against BRAF, showed encouraging preclinical results although clinical investigations were prematurely discontinued due to the unexpected acceleration of tumor growth [62].

Mutations of interest but yet unclear clinical significance

The continuous identification of novel molecular signatures in brain neoplasms has started to transform the clinical neuro-oncological practice. Apart from the already mentioned ones, additional biomarkers, including *BRAFV600E*, *EGFRvIII*, *TERT*, *ATRX*, *TP53* and micro-satellite instability (MSI)/mismatch repair deficiency genes have also gained attention in the neuro-oncological field but their ability to predict clinical behavior, response to therapy and outcome appears limited or unclear.

BRAFV600E mutations were found in a variety of tumors including PAs, pediatric diffusely infiltrating gliomas (WHO grades II-IV), gangliogliomas as well as pleomorphic xanthoastrocytomas (PXA) [63,64]. In contrast to the *BRAF* fusion, the diagnostic value of *BRAFV600E* is limited and the predictive and prognostic significance have yet to be determined [64]. Likewise, clinical responses to *BRAF* inhibitors, such as dabrafenib and vemurafenib, are currently unknown except for few isolated case reports or case series and are the subject to clinical trial investigations [65,66].

EGFRvIII is the most common mutated receptor tyrosine kinase receptor in approximately 20-30% of GBM cases leading to constitutive activation of the EGFR-PI3K pathway [67]. As *EGFRvIII* is tumor-specific and absent in normal tissues, it represents a rare molecular target with promising potential for therapeutic, diagnostic and prognostic purposes. However, these high hopes have mostly been disappointed. At the current time, *EGFRvIII's* predictive and prognostic relevance remains unclear despite a strong association with a more invasive phenotype [67,68]. Furthermore, *EGFRvIII* targeting therapies have not yet shown clinical benefit [69]. A prominent example is the *EGFRvIII* peptide vaccine, rindopepimut, which, despite encouraging data in the early clinical investigational stages, did not reach the OS endpoints in a phase III multicenter trial for patients with newly diagnosed GBM with minimal residual disease according to the company's announcement [70].

TERT promoter mutations, which result in enhanced telomerase activity and lengthened telomeres are strongly associated with 1p19q and *IDH.* The combination of these genetic

markers is diagnostic of an oligodendroglioma and predicts greater benefit from adjuvant chemotherapy and radiation with longer survival [71]. By contrast, isolated *TERT* mutations predict poor survival, suggesting the need for early adjuvant therapies [71].

Mutations in *ATRX* and *TP53* are frequent in adult diffuse gliomas (WHO grade II and III) and are strongly associated with astrocytic tumors carrying *IDH1/2* mutations [72]. As such, they are of diagnostic relevance and if present may obviate the need to perform more time consuming and expensive testing for 1p/19q codeletion; however, the clinical significance is limited and the prognostic value is unclear [73].

Mismatch repair–deficiency has been observed in a small fraction of brain neoplasms leading to a higher mutational load. Recently, it was shown that tumors with a high number of somatic mutations secondary to mismatch-repair defects are more susceptible to immune checkpoint blockade [74]. Its importance in cancers of the CNS as a biomarker, however, has yet to be defined although early data are encouraging [75-77]. If these data can be reproduced in further clinical trials, MSI testing may evolve into routine in the future.

Concluding remarks

Development of clinically useful biomarkers is an increasingly important topic in neuro-oncology. This is largely due to the recently gained wealth of information from the large genome wide studies of CNS cancers as well as due to more targeted clinical research in oncology that is now also applied to neuro-oncology. High-level evidence-based treatments options, however, are still scarce and only very few markers are currently fully developed to impact on standard clinical practice (see outstanding question box). New marker discovery and, most importantly, more effective treatments are necessary to move this field forward.

Before doing so, testing methods and conditions that are currently not well-established for the majority of these markers, must be standardized to avoid doubts regarding the quality of testing. A variety of RNA– and DNA-based methods are currently evaluated and cut-off values are being established to increase the robustness of these procedures for daily routine clinical use.

In addition to tissue-based prognostic and predictive markers that are discussed in this review, there have been efforts to develop minimally invasive dynamic markers to assess disease status and dynamic changes in tumor burden. In particular, circulating biomarkers in cerebrospinal fluid (CSF) and blood have been studied in CNS cancers similarly to other malignancies [22,78-80]. The advantages of so-called ‘liquid biopsies’ are that testing can be done without the need for repeated surgery; that highly sensitive detection methods exist that are both quantitative and qualitative; and that multiple specimens can be taken overtime. There has been extensive research on circulating markers, including nucleic acids (circulating tumor DNA, RNA, miRNA), proteins, as well as circulating tumor-derived microvesicles (exosomes) and circulating tumor cells. Compared to other cancers, their translation into clinical practice however has been hampered by low detectability rates, which has been attributed largely by the presence of the blood-brain barrier in CNS tumors.

Efforts to measure marker levels in CSF appear more promising than markers in peripheral blood and are currently underway [81].

Similarly, there has been significant interest in developing imaging technologies that can noninvasively determine molecular characteristics of the tumor. An example is magnet resonance spectroscopy to detect 2-hydroxybutyrate, the onco-metabolite of *IDH1/2* mutations [82].

Novel clinical trial designs that aim at selecting patients based on presence of targetable molecular alterations rather than tumor type, such as the so-called “basket trials” and large marker driven studies such as the NIH MATCH trial, are expected to significantly expedite the development of new targeted drugs and propel biomarker-driven clinical research, as highlighted in BRAFV600E mutated non-melanomatous cancers treated with vemurafenib [83].

In summary, for standard clinical practice, a restricted panel containing *IDH1/2*, *MGMT* promoter methylation, 1p/19q, *KIAA1549:BRAF* and *H3F3A,* depending on tumor type and age group, would cover the majority of diagnostic and clinically actionable markers in adult and pediatric glioma patients (**Table 1**; see also outstanding question box). In contrast, there is still no standard practice role for testing for *EGFRvIII* as well as *BRAFV600E, TERT* and *TP53* mutations, although these markers may be useful as selection criteria for specific clinical trials. In addition, the presence or absence of aberrations in the WNT and SHH pathway in MB can clarify the underlying mechanism and guide treatment decisions while testing of TP53, MYC, MYCN and GLI2 could identify certain high-risk subpopulations in the future.

We are cautiously optimistic that clinically actionable markers will become increasingly available for patients with brain tumors over the next ten years and that biomarker-driven patient selection will continue to be increasingly important in clinical research as well as in standard clinical practice.

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Glossary

Actionable biomarker	Molecular hallmarks whose presence or absence has implication on clinical decision-making in standard clinical care.
Basket trial	Biomarker-driven trials that include patients whose tumors have a specific molecular feature (e.g., *BRAF* mutation), testing targeted therapies in diverse populations of cancers. This trial design is well suited for the study of rare cancers with potentially actionable molecular alterations.

Prognostic markers	Presence of the marker is associated with better (or worse) outcome independent of a therapeutic intervention.
Predictive markers	Presence of the marker is associated with better (or worse) outcome dependent on a specific therapeutic intervention.

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Outstanding Questions Box

- How much testing should be done in individual brain cancer patients that are undergoing the standard of care treatment, outside of a clinical trial: Is genome wide testing appropriate for all patients with brain cancers or should only established markers be tested?
- Brain cancer patients frequently undergo more than one neurosurgical resection or biopsy tissues from different time points during their treatment course are available for potential testing. Excluding strictly diagnostic biomarkers, at what time point should molecular testing be done - at diagnosis (initial tissue) or at tumor recurrence (tissue from repeat resection)?
- The key question that needs to be addressed for each individual marker: Does the presence of the respective marker truly change the clinical outcome for patients?

Trends Box

- Genome-wide studies unveiled a plethora of cancer specific genetic alterations. The recently updated World Health Organization classification of brain tumors (2016) for the first time includes molecular markers to determine subclasses of gliomas and medulloblastomas. However, thus far only few markers are sufficiently characterized to impact the clinical practice in patients with CNS cancers. *MGMT* promoter methylation in high-grade astrocytomas and co-deletion of 1p/19q in oligodendrogliomas are proven prognostic and predictive markers that play a role in standard practice, and mutations of IDH1 or IDH2 are of strong prognostic value in gliomas. The true clinical impact of other markers in gliomas has yet to be determined.
- Cancer-specific markers are of increasing importance in patient selection for clinical trials and marker- rather than diagnosis-driven studies. Some of these markers enable us to pair available targeted drugs with subpopulations of tumors based on a biological rationale and a true drug target (e.g., *BRAF* inhibitors in *V600E* mutated tumors, ‘basket trials’).
- Further prospective research is needed to formally validate individual markers. This needs to also include the methodological standardization of testing to allow for reliable inter-laboratory concordance rates.

Table 1

Molecular markers in CNS tumors and their current clinical implications

Marker	Diseases for which these markers are currently of clinical relevance	Prognostic	Predictive	Diagnostic	Standard therapy available (relevant if marker present)	Investigational drugs and approaches (selection)	Comments	References
Co-deletion 1p/19q	Oligodendrogliomas	Yes	Yes	Yes	PCV, temozolomide		Tie-breaker' to distinquish oligo- from astrocytomas; favorable prognosis, associated with chemosensitivity	[18-21]
MGMT promoter methylation	Glioblastomas, anaplastic astrocytomas (less clear)	Yes	Yes	No	Temozolomide		Positive prognostic marker, predictive of benefit from alkylating chemotherapy	[10, 12]
IDH 1/2 mutation	Oligo- and astrocytomas WHO grade II and III, secondary GBM	Yes	To be determined	Yes	No	IDH1/2 inhibitors	Positive prognostic marker; in GBM associated with secondary GBM	[3,4]
EGFRvIII	Glioblastomas	Unclear	No	Yes	No	Immunotherapeutic approaches: vaccines, CAR T-cells	Tumor specific surface marker, widely used for targeted immunotherapy approaches	[6,67,69]
TERT mutation	Gliomas	Unclear	No	Yes	No		Associated with worse survival	[71]
ATRX mutations	Gliomas	Unclear	No	Yes	No		Closely correlated with mutations of TP53 and IDH 1/2 and mutually exclusive with co-deletion of 1p/19q	
TP53 mutations	Astrocytomas	Unclear	No	Yes	No			
BRAF V600 mutation	PXA, pilocytic astrocytomas, gangliogliomas	Unclear	To be determined	Yes	No	BRAF inhibitors	Found in supratentorial PA, PXA and GG	[83]
BRAF/KIAA1549 fusion	Pilocytic astrocytomas	Unclear	No	Yes	No	MEK inhibitors	Positive prognostic and diagnostic marker for PA and WHO grade II astrocytomas.	[56-58]
H3.3 histones	Pediatric HGG	Yes	No	Yes	No	Epigenetic inhibitors, JMJD3 inhibitor	Associated with worse survival in DIPG, can be targeted with epigenetic modifiers	[48,49]

Marker	Diseases for which these markers are currently of clinical relevance	Prognostic	Predictive	Diagnostic	Standard therapy available (relevant if marker present)	Investigational drugs and approaches (selection)	Comments	References
SHH pathway mutations	Medulloblastomas	Yes	No	Yes	No	SMO inhibitors	Heterogenous outcome based on acquired mutations	[34,38-42]
- *MYCN* amplification		Yes	No				High-risk mutations; associated with reduced survival	
- *GLI2* amplification		Yes	No					
- *TP53* mutations		Yes	No					
WNT pathway mutations		Yes	To be determined	Yes	Reduced dose of RT, chemotherapy or combination		Favorable prognosis	
MYC		Yes	No	No	No	Gemcitabine and pemetrexed; BET bromodomain inhibitors	Poor prognosis	