Toward precision medicine in glioblastoma: the promise and the challenges


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Integrated sequencing strategies have provided a broader understanding of the genomic landscape and molecular classifications of multiple cancer types and have identified various therapeutic opportunities across cancer subsets. Despite pivotal advances in the characterization of genomic alterations in glioblastoma, targeted agents have shown minimal efficacy in clinical trials to date, and patient survival remains poor. In this review, we highlight potential reasons why targeting single alterations has yielded limited clinical efficacy in glioblastoma, focusing on issues of tumor heterogeneity and pharmacokinetic failure. We outline strategies to address these challenges in applying precision medicine to glioblastoma and the rationale for applying targeted combination therapy approaches that match genomic alterations with compounds accessible to the central nervous system.

Keywords: clinical trial, genomics, glioblastoma, precision medicine, targeted therapy.

Glioblastoma (GBM) is a molecularly heterogeneous malignancy that arises in the brain and is uniformly fatal. The median survival is ~15 months for patients who enroll in clinical trials. Population-based survival statistics are much worse, reflecting the majority of cases that are never referred or cannot qualify for such studies. It is estimated that fewer than 10% of patients are treated according to prospective clinical trials. Perhaps the bias against referral derives from the fact that despite decades of intense research into the biology and treatment of GBM, overall survival remains stagnant, with very few approved chemotherapeutic or biologic agents.

Surgical cure is not possible for GBM. Despite extensive surgical removal of what appears to be all gross macroscopic disease, either at initial diagnosis or at the time of relapse, all patients will continue to show tumor growth and progression because of rapidly proliferating infiltrative disease remaining after surgery. Based upon autopsy and historical surgical biopsy series, infiltrating tumor cells can be found far distant from even the gross imaging findings. It is this invasive, infiltrative disease component that is the ultimate cause of recurrence, resistance, and death. The current standard of care for newly diagnosed disease includes maximal safe resection, followed by 6 weeks of radiation and concurrent daily temozolomide (TMZ) chemotherapy, followed by at least 6 months of adjuvant TMZ. The addition of TMZ improves overall survival by ~2.5 months compared with radiation only. Despite attempts to improve outcome for newly diagnosed disease, effective treatment for glioblastoma remains an unmet need. Three large, placebo-controlled randomized phase III trials aimed at targeting the angiogenic phenotype of this disease were recently published. Two studies used bevacizumab, targeting vascular endothelial growth factor (RTOG 0825 and the AVAglio trial), and one used cilengitide, an integrin inhibitor.
None of these trials showed an improvement in overall survival compared with the current standard of care. Recurrence of disease is uniformly rapid, typically occurring within 6–9 months of initial diagnosis, and salvage therapies, if effective at all, are only able to control disease growth for another 4–6 months.10 The typical 6-month progression-free survival using older cytotoxic agents and many single-agent, molecularly targeted therapies is <20% when treated at the time of first or second relapse.11 Based upon several uncontrolled phase II clinical trials showing a high progression-free response rate compared with historical controls, the FDA granted accelerated approval for the use of bevacizumab for malignant glioma at the time of first or second relapse.12,13 At the time of this report, the only FDA approved agents for glioblastoma are TMZ and the nitrosoureas, with accelerated approval of bevacizumab. None of these therapies is curative.4,5,7–9,11–15

Biomarker-driven strategies that incorporate potential “actionable molecular targets” have been employed over the last several decades but, as mentioned above, have not yet proven effective in the clinical setting. In addition to clinical factors such as age, performance status, and extent of resection, only one specific molecular alteration is prognostic and to some degree predictive of treatment response: the presence or absence of methylation of the promoter region of the DNA repair enzyme methylguanine methyltransferase (MGMT).14 Approximately 30% of tumors have MGMT promoter methylation, and those patients respond better to treatment and live longer than patients with unmethylated MGMT.16 Unfortunately, patients without MGMT methylation only have about a one-month survival advantage when TMZ is given compared with radiation only.16 Intensifying TMZ to potentially deplete MGMT has not improved clinical outcome and produced only more toxicity.15

Most molecularly informed clinical trials use a single “one size fits all” targeted treatment approach and have not taken into account the multitude of biologic differences found within individual patients. Fortunately, more comprehensive, in-depth molecular profiling of tumor tissue is increasingly available and has become much less expensive in recent years. Patients have accepted the notion and hope that individualized therapies may be helpful, but the proof of this concept has yet to be demonstrated in malignant glioma. The lack of precise tumor imaging combined with significant tumor heterogeneity, biologic complexity, and difficulty in drug delivery present unique challenges for the management of GBM.

**Molecular Profiling of Glioblastoma**

Molecular profiling has provided clinical benefit for patients with various advanced cancers.17,18 However, aside from MGMT promoter methylation and TMZ response, molecular biomarkers associated with therapeutic response are lacking in GBM. Over the last decade, glioblastoma has been characterized into several subtypes of disease using gene expression profiling. The recent application of integrated sequencing strategies has provided a broader understanding of the genomic landscape and molecular classifications of GBM.19–21

Sequencing analysis of GBM led to the identification of mutations in the isocitrate dehydrogenase 1 or 2 genes (IDH1, IDH2), typically found in younger patients and shown to independently confer a better prognosis.22 These cases are likely secondary glioblastoma, arising over time from lower-grade astrocytoma. The Cancer Genome Atlas (TCGA) analysis of primary glioblastomas confirmed earlier findings, subclassifying glioblastoma into at least 4 subtypes using an expression-based analysis.19,23 The proneural subtype shows improved survival and often harbors IDH mutations and other methylation abnormalities.24 The other 3 subtypes (mesenchymal, classical, and neural) segregate according to distinct gene expression patterns, but there is little to no survival difference among them. More extensive profiling has been completed that includes methylation profiles, which may lend themselves to better stratification factors and identify additional genetic abnormalities and pathway alterations. The field is only now starting to grasp the complicated and poorly understood epigenetic drivers and modifiers of certain key regulatory functions that define the more commonly altered pathways detected by the analyses by TCGA.

Whole-exome sequencing analysis from TCGA identified several significantly mutated genes, validating driver alterations from previous studies (EGFR, PDGFRα, PIK3CA, PTEN, NF1, RB1, TP53, etc) and identifying novel, significantly altered genes and pathways, including frequent alterations in genes involved in chromatin remodeling.20 The most frequent genomic gains and losses involved EGFR/MET/CDK6 (chromosome 7), CDK4/MDM2 (chromosome 12), PDGFRα (chromosome 4), and CDKN2A/CDKN2B (chromosome 9). Sequencing efforts also uncovered potentially targetable oncogenic RNA fusion events in GBM, such as the in-frame fusions involving fibroblast growth factor receptor/transforming acidic coiled-coil protein genes (FGFR1-TACC1, FGFR3-TACC3) or epidermal growth factor receptor/septin 14 (EGFR-SEPT14) that confer in vitro sensitivity to FGFR or EGFR inhibitors, respectively.4,19,25 Though knowledge of the genomic landscape of glioblastoma has increased, these findings have yet to be translated into improved outcomes for GBM patients.

**From Target to Treatment: The Challenges**

**Identifying Actionable Alterations**

Classifying and prioritizing variants identified through integrated genomic analysis is a major challenge in the application of precision medicine. In cases where an alteration has been previously reported and characterized, prior knowledge from the literature can be leveraged to map individual alterations and associated drug response relationships. However, most alterations identified by whole-genome or exome sequencing remain undefined in regard to the functional consequence and associated therapeutic implications.

The subset of significantly mutated genes identified in the glioblastoma dataset of TCGA includes several genes that represent direct targets of an FDA-approved therapy (EGFR, PDGFRα, BRAF) or that map to a pathway that is targeted by an approved drug or investigational agent (PTEN, PIK3CA, NF1, TP53) (Fig. 1A). However, these gene alterations range from known gene variants with well-described clinical implications to novel alterations with unknown functional and therapeutic
relevance. Various strategies have been and are being developed to aid in variant annotation and prioritization (reviewed by Dienstmann et al). Computational tools and functional predictive models can aid in mutation prioritization, incorporating evidence on mutation type (frameshift, nonsense, missense), mutation domain (kinase domain), mutant allele fraction, corresponding mutant expression data from RNA sequencing, and whether the mutation has been previously reported in a cancer database (TCGA, COSMIC [Catalogue of Somatic Mutations in Cancer]). To identify clinically relevant, potentially actionable alterations, these prioritized alterations can be mapped to potential therapies using an evidence-driven knowledge base that captures variant-drug associations from the literature. As predictive markers are lacking in GBM, it will be valuable to include drug–gene relationships from other tumor types. These therapeutic associations can be further characterized based on the strength of evidence surrounding the alteration–drug relationship, including whether the variant is an FDA-defined pharmacogenomic biomarker in GBM or another tumor type and whether the alteration is associated with drug response based on clinical or preclinical data.

As an example, we applied a basic strength of evidence grading scale to classify the identified somatic variants from the glioblastoma TCGA dataset based on therapeutic association. In this example, the variant classification scheme ranges from 0 to 5, as outlined in Table 1. Several alterations identified

![Fig. 1. Potential therapeutic implications of significantly mutated genes identified in the primary glioblastoma TCGA dataset. (A) Pathway representation of frequently altered pathways and selected potential therapeutic agents. (B) Table of frequently mutated genes from TCGA mapped to potential FDA-approved therapeutic agents. (Right) Bar chart of level of evidence for the association between an alteration and the therapeutic implication using the levels of evidence defined in Table 1.](image-url)
Table 1. Variant classification scheme

<table>
<thead>
<tr>
<th>Classification Level</th>
<th>Classification Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>FDA pharmacogenomic biomarkers with established evidence for an associated drug response in the same cancer type</td>
<td>BRAF V600E mutation and vemurafenib response in melanoma</td>
</tr>
<tr>
<td>1</td>
<td>FDA pharmacogenomic biomarkers with established evidence for an associated drug response in a different cancer type</td>
<td>BRAF V600E mutation and vemurafenib response in glioblastoma</td>
</tr>
<tr>
<td>2</td>
<td>Alteration associated with drug response based on clinical or preclinical data</td>
<td>PIK3CA H1047R mutation and everolimus response in breast cancer patients</td>
</tr>
<tr>
<td>3</td>
<td>Novel mutations in genes where literature evidence supports a strong pathway relationship to a therapeutic target</td>
<td>Random point mutations and insertion/deletion mutations mostly seen among tumor suppressor genes such as PTEN and CDKN2A</td>
</tr>
<tr>
<td>4</td>
<td>Alterations that impact the same amino acid or affect an important protein domain (ie, kinase domain) as those with known clinical implications but may not be validated for functional consequence and relationship to therapeutic response</td>
<td>FGFR3 kinase domain mutation not previously linked to drug response (ie, FGFR3 P6715)</td>
</tr>
<tr>
<td>5</td>
<td>Variant previously described as somatic in cancer but not directly associated with therapeutic response</td>
<td>EGFR extracellular domain mutation previously reported in cancer but not yet linked to therapeutic response (ie, EGFR S229C)</td>
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in GBM have evidence for a therapeutic association in cancer (Fig. 1B). BRAF V600E mutation, an FDA pharmacogenomic biomarker for vemurafenib, dabrafenib, and trametinib in BRAF V600E mutant melanoma, is present in a subset of GBM and represents the only level 1 alteration detected. Level 2 and 3 alterations, representing mutations that have been previously characterized and linked to drug response in clinical and/or preclinical studies, are also present, including the EGFR A289V mutation, PIK3CA E545K and H1047R mutations, and nonsense mutations in NF1 and PTEN.27–30 An additional subset of alterations map to key functional domains or have been previously identified in cancer, yet are currently functionally uncharacterized and lack direct literature evidence for a drug–response relationship. Refining methods to annotate these variants and catalog drug–alteration relationships will be critical to applying these integrative strategies to glioblastoma.

Tumor Heterogeneity

Even with the identification of potential therapeutic targets in GBM, several unique challenges exist for translating these discoveries into clinical practice, as exemplified by the disappointing efficacy of EGFR inhibitors in GBM clinical trials. EGFR is altered in about half of GBM, resulting from gene mutation, amplification, and gene fusion.21,28 However, EGFR tyrosine kinase inhibitors have thus far shown minimal clinical efficacy in GBM, even within predefined glioblastomas positive for EGFR and PTEN.35 The lack of efficacy with EGFR inhibitors illustrates several of the challenges with implementing precision medicine in GBM, including tumor heterogeneity and pharmacodynamic/pharmacokinetic failure.

Intratumor heterogeneity is a critical influence in treatment failure. There is spatial heterogeneity within the tumor: some tumor regions are hypoxic and necrotic and others more normoxic; some regions are more proliferative, with others very quiescent; some regions are more vascularized, whereas some are more infiltrative. These phenotypic features are accompanied by genotypic differences.16 Mosaic amplification, where amplification of key oncogenes occurs in a mutually exclusive pattern within neighboring subpopulations of tumor cells, occurs in a subset of glioblastomas.37,38 In GBM cell lines with mosaic amplification of EGFR and PDGFRA, simultaneous inhibition of both targets was required for pathway inhibition in the heterogeneous cell population.39 The complexity of intratumor heterogeneity extends beyond the pattern of receptor tyrosine kinase amplification in GBM subpopulations, as demonstrated by the extensive tumor heterogeneity detected by genome-wide copy number analysis in spatially distinct glioblastoma samples.40 Treatment for specific regional genotypic differences is problematic, particularly when one cannot identify those differences by routine imaging, and because multiple surgeries and/or biopsies are not done to confirm the changes that exist or that arise over time.

In addition to the issue of intratumor heterogeneity, there is clear evidence that treatment can drive clonal evolution, through either generation of de novo subclonal driver events or selection of preexisting subclones with genotypes associated with a drug–resistant phenotype. Indeed, TMZ has been associated with a mutator phenotype that drives a TMZ-associated glioma-to-glioblastoma evolutionary path through TMZ-driven mutations in the retinoblastoma and Akt–mammalian target of rapamycin (mTOR) pathways.40 Secondary mutations also contribute to clinical resistance to various molecularly targeted agents. For example, secondary mutations in EGFR, particularly the presence of the EGFR T790M mutation, are associated with resistance to EGFR inhibitors in non–small cell lung cancer. These mutations have been detected at low frequency prior to EGFR inhibitor treatment, suggesting that the T790M mutation may drive subclonal expansion.41 In contrast, a recent study in BRAF-mutant melanoma found that the resistant mutations were not present in the pretreatment tumor, suggesting that they were acquired de novo with BRAF/mitogen-activated...
protein kinase kinase (MEK) inhibitor therapy. Thus, another biopsy of the recurrent tumor will likely be required for adequate target evaluation and genomic-guided therapeutic selection at the time of tumor recurrence.

The pattern of intratumor heterogeneity and inherent molecular complexity of GBM will likely necessitate cotargeting of multiple alterations using combination therapy. Using single cell-based clonal analysis, Meyer and colleagues recently identified preexistent TMZ-resistant subclones within treatment-naïve primary GBM tumors. The authors further demonstrated the differences in genomic alterations and response to a panel of chemotherapeutic agents within clones isolated from the same primary tumor, illustrating the need for multiple agents to target the diverse driver events within clinically aggressive clones. Strategies to target parallel and/or redundant kinase pathways, such as cotargeting of EGFR and phosphatidylinositol-3 kinase (PI3K)/mTOR pathways, have shown preclinical efficacy in GBM cell lines. However, a phase I/II study combining the EGFR inhibitor erlotinib and the mTOR inhibitor temsirolimus encountered dose-limiting toxicity for this combination, and there were no responses reported among glioblastoma patients. Pharmacological considerations, including managing overlapping toxicities and drug–drug interactions, remain critical determinants of combination therapy design and efficacy.

Due to the inherent therapeutic resistance of GBM, combinations with chemosensitizing agents are of particular interest. Autophagy inhibitors, such as chloroquine, have been shown to increase the chemosensitivity to TMZ in xenograft models and have demonstrated clinical activity in combination with TMZ and radiation in patients with GBM. However, the related compound, hydroxychloroquine, was associated with dose-limiting toxicity and inconsistent autophagy inhibition in a phase I/II trial combining hydroxychloroquine with standard of care radiation and TMZ in newly diagnosed GBM. While this is a promising strategy, follow-up studies evaluating novel autophagy inhibitors and further optimization of the combination therapy regimen are needed. Immunotherapy has also shown promising results in early clinical trials, and multiple immunotherapeutic strategies are actively being evaluated in glioblastoma. Though the role of immunotherapy in combination therapy remains to be delineated, evidence suggests potential synergism with standard of care radiation and chemotherapy (reviewed by Patel et al).

Pharmacodynamic and Pharmacokinetic Failures

Pharmacodynamic and pharmacokinetic parameters are key influences on clinical efficacy. In contrast to other EGFR-driven tumor types, EGFR mutations in glioblastoma are typically located in the extracellular domain. The most frequent constitutively active oncogenic variant, termed EGFRvIII, arises from deletion of exons 2–7, resulting in an in-frame deletion of 267 amino acids in the extracellular domain. EGFRvIII is expressed in ~30% of glioblastomas, including about half of the glioblastomas with EGFR gene amplification. However, EGFRvIII is relatively resistant to erlotinib and gefitinib, EGFR tyrosine kinase inhibitors that target the active kinase conformation. Neither agent significantly inhibited EGFRvIII phosphorylation in BS153 cells, a GBM-derived cell line with EGFR amplification and EGFRvIII expression. Another EGFR inhibitor, lapatinib, has been shown to inhibit EGFRvIII in vitro by preferentially binding the inactive conformation of the kinase. However, lapatinib failed to achieve sufficient intratumor concentrations in glioblastoma patients. Clinical trials testing next-generation EGFR inhibitors or EGFRvIII-targeted vaccines, such as rindopepimut, are under way. Rindopepimut provided encouraging results in early-phase clinical trials and is currently being evaluated in combination with bevacizumab in a phase II clinical trial for EGFRvIII+ relapsed glioblastoma (NCT01498328) and in combination with TMZ in a phase III clinical trial for EGFRvIII+ newly diagnosed glioblastoma (NCT01480479).

Natural exclusion of agents due to the blood–brain barrier (BBB) coupled with the poor distribution of therapies within the brain and throughout the tumor region make adequate drug delivery a critical challenge in advancing glioblastoma treatment. The multiple published reports of in vivo animal and human clinical studies evaluating drug levels or target inhibition in the brain provide an initial resource for evaluating potential CNS activity of a compound. In addition, various in silico predictive models have been developed to aid in predicting BBB penetration of multiple alterations using combination therapy. Using predictive models with literature-derived evidence for BBB penetration can assist in prioritizing agents for therapeutic selection. Clinical trials that attempt to address the issue of drug delivery will typically treat a patient prior to surgery and then operate on the most surgically accessible disease, usually the enhancing disease based upon contrast MRI. A small sample is acquired in one small region and the drug concentration measured. The literature is fairly robust in terms of such trials that document what should be sufficient drug concentrations based upon preclinical testing in rodents, yet these trials are routinely negative, almost certainly because most of the disease remaining after surgery is nonenhancing and infiltrative, likely with different genomic changes and a more intact BBB. Together, these findings reinforce the need to consider BBB penetration of the selected therapy as well as genomic complexity of the disease during therapeutic planning. In addition, one must consider the tumor left behind, the regions not biopsied or studied in terms of molecular profile, and drug distribution and pharmacodynamic effects.

Therapeutic agents that are approved for non-oncology indications but which have known CNS activity and evidence for potential activity in cancer may also be useful in treating CNS malignancies. This strategy of drug repositioning capitalizes on known safety information and BBB penetration for target compounds and is being explored by various groups in multiple cancer types, including the recently launched Coordinated Undermining of Survival Paths (CUSP9) trial by the International Initiative for Accelerated Improvement of Glioblastoma Care. A prime example of this drug repositioning approach is disulfiram, a drug used to manage chronic alcoholism. Disulfiram has recently been found by several groups to have preclinical activity in glioblastoma and is currently being evaluated in clinical trials (NCT01907165, NCT01777919).

Various approaches for increasing drug delivery are also being explored. One approach is to optimize local delivery of a drug behind the BBB, such as was done with Carmustine-loaded polymer implants. Other local delivery strategies,
including convection-enhanced delivery, have also been pursued in an effort to more widely distribute therapeutic agents in the target tissue. Strategies to improve drug delivery through chemical or mechanical disruption of the BBB have also experienced renewed interest, such as use of microbubble-enhanced focused ultrasound to induce local BBB disruption. Recent advances in nanoparticle systems have provided new opportunities for drug-loaded nanoparticle delivery to intracranial tumors through various modes of delivery, including convection-enhanced delivery, systemic administration, and intranasal delivery. While these advances hold promise for improving drug delivery and efficacy, the challenge of selecting effective, molecularly informed therapeutic options remains.

### Selected Examples in Glioblastoma Molecular Profiling

As a conceptual example to illustrate these principles, we applied whole-genome and -exome sequencing to 13 archival recurrent glioblastoma samples and mapped the genomic alterations identified to potential CNS-active therapeutics. Several of the identified alterations overlapped those found in primary GBM, applied evidence scale as we used in the primary GBM samples revealed several potentially targetable alterations.

Potentially actionable alterations and the candidate therapeutic agents for selected recurrent glioblastoma samples are displayed in Table 2. In sample 2, the potentially actionable level 2 alterations include *EGFR* amplification, *EGFRvIII* expression, and *CDKN2A* deletion. As mentioned above, *EGFR* is frequently altered in GBM but has been challenging to effectively target, with several of the EGFR inhibitors showing reduced activity against variant III and/or poor CNS activity. Afatinib, a dual EGFR/ERBB2 irreversible inhibitor, has shown preclinical activity against EGFRvIII. While the activity of afatinib in glioblastoma remains to be demonstrated, activity against brain metastases has been reported. Next-generation EGFR inhibitors with improved BBB penetration, such as dacomitinib, are currently in clinical trials in GBM (NCT01112527). Various repositioned therapies with potential activity against activated EGFR can also be considered. Propranolol is a nonselective beta-adrenergic receptor antagonist approved for hypertension, angina pectoris, migraine prophylaxis, and, recently, infantile hemangioma. Propranolol is known to cross the BBB and has demonstrated anticancer activities in various tumor types.

For sample 11, a *TSC2* deletion and *BRAF* V600E mutation were detected, suggesting dual activation of PI3K/mTOR and mitogen-activated protein kinase (MAPK) signaling pathways. Loss of heterozygosity of *TSC1* and *TSC2*, negative regulators of mTOR complex 1 (C1) activity, occur at low frequency in GBM. Recently, loss of *TSC1* was shown to result in mTORC1

![Fig. 2](image_url). Genomic alterations in recurrent glioblastoma tumors. (A) The spectrum of alterations identified within the cohort of recurrent GBM samples was mapped against the subset of frequently altered genes previously identified in primary GBM. Red indicates copy number gain, green is copy number loss, and an asterisk (*) indicates nonsynonymous mutation, where missense mutations are colored blue, nonsense mutations colored purple, and frameshift mutations colored orange. Tan indicates a structural variant. Chromatin remodeling gene alterations are colored gray and include missense, nonsense and splice site mutations. The most dominant molecular subtype for each sample, based on the gene expression classifications of Verhaak et al., is shown. C, classical; M, mesenchymal; N, neural; P, proneural; ind, indeterminant. The bar chart indicates frequency of genomic alteration for each gene within the TCGA primary GBM dataset (white bars) and the recurrent GBM samples presented here (black bars).
hyperactivation and to stimulate malignant gliomagenesis in the presence of oncogenic signals. Lack of tuberous sclerosis complex (TSC1/TSC2) expression has been associated with mTORC inhibitor sensitivity, suggesting potential efficacy of an mTOR pathway inhibitor such as everolimus or temsirolimus in this context. Both everolimus and temsirolimus have literature evidence supporting their brain distribution, though studies of these agents have thus far shown minimal clinical activity in GBM. The BRAF V600E mutation is a low-frequency, hot spot mutation, occurring in 2% of primary adult GBM. Several targeted agents have gained FDA approval for BRAF V600E-mutant melanoma, including vemurafenib, dabrafenib, and trametinib. Though not yet tested in adult GBM, mutant BRAF inhibitors have shown efficacy in a V600E-positive pediatric brainstem ganglioglioma, and most recently, a clinical case of V600E-positive pediatric relapsed GBM, suggesting potential efficacy for these inhibitors in BRAF V600E-mutant brain tumors. Selection of a compound with activity against BRAF V600E with anticipated CNS activity can be challenging. Literature evidence indicates that both vemurafenib and dabrafenib are substrates for active efflux by P-glycoprotein and breast cancer resistance protein, thus limiting their distribution to the brain. According to the FDA approval documents, trametinib crosses the BBB, achieving a concentration of ≈20% of that in plasma following multiple doses. However, trametinib did not significantly inhibit extracellular signal-regulated kinase phosphorylation in the mouse brain, raising questions as to whether the concentration achieved is sufficient for antitumor activity. Mebendazole,
a microtubule-targeting, anti-helminthic agent with demonstrated efficacy against parasitic infections of the CNS, has recently been shown to have direct inhibitory activity against several oncogenic kinases, including BRAF V600E. Systemic mebendazole treatment significantly increased survival in 2 orthotopic mouse models of glioma, suggesting potential for repositioning this therapy into GBM. A phase I study of mebendazole in newly diagnosed high-grade glioma is currently under way (NCT01729260). Thus, combination of an mTOR inhibitor with a BRAF/MEK pathway inhibitor, with additional considerations for CNS activity, may constitute a potential therapeutic strategy for cases with dual MAPK/PI3K activation.

Together, these examples highlight the likely requirement for combination therapy in this molecularly complex and heterogeneous tumor type, and the need to consider BBB penetration characteristics of potential therapeutic agents during treatment selection.

Concluding Remarks and a Prospective Trial Design

In a recent phase I trial, a patient with MET-amplified, recurrent GBM showed a rapid and durable clinical response following treatment with the MET inhibitor crizotinib. Though this proof-of-principle case provides encouraging evidence for efficacy of a selected targeted agent based on molecular profiling in GBM, the challenge remains to successfully apply this approach within the larger context of GBM. The use of unselected single agents in large patient clinical trials for GBM has proven futile for all of the reasons described above. Given the clinical, phenotypic, and genomic heterogeneity that we know exists, a more rational selection of treatment for our patients is needed. Indeed, the treating physician is increasingly receiving whole-exome or other genomic molecular profiles obtained from a patient’s tumor, along with treatment suggestions based upon those profiles. In many cases, multiple agents are suggested within these reports. The challenge remains as to how to use this information in a rational way, particularly outside of the context of a prospective clinical trial.

The strategy we propose, and have now started (NCT02060890), is to obtain multiple biopsies of patients at the time of surgery, within both the enhancing as well as the more infiltrative, non-enhancing regions of disease. Subsequently, we perform extensive genome-wide profiling and select drugs that we anticipate may modulate actionable targets within the remaining, diffuse regions of the lesion. Drug selection is individualized, and multiple agents (up to 4) are allowed. “Rules” for drug selection are implemented using the specialized drug pharmacopeia designed for this trial, taking into account the potential number of agents (dose, sequence, knowledge of overlapping toxicity, CNS pharmacokinetics) and the safety of using combination therapy. The drugs chosen are carefully considered with knowledge about the patient’s past treatment history and concomitant therapies, with the assistance of a multispecialty molecular tumor board that drafts a report to the treating physician. Additional tumor samples are also collected for future in vitro and in vivo xenograft testing, as well as blood samples obtained over time to assess for circulating tumor DNA that may help with noninvasive biomarker development in the future.

Whenever possible, paired samples from each patient at the time of relapse will be taken in order to validate the strategy and to assess potential mechanisms of resistance. We are currently testing the feasibility and safety of this strategy in a limited recurrent glioblastoma patient sample, in patients who are otherwise felt to be surgical candidates. If safe and feasible, additional clinical studies testing efficacy using multiple pre-specified targeted agents would be possible, either alone or with repositioned drugs from the US Pharmacopeia.

While we are just beginning the process of assessment of the feasibility of this strategy, with the goal of prospective efficacy trials, we recognize that much more research is needed. For instance, far more precise noninvasive assessment of biologic and metabolic changes within tumor, particularly within the nonenhancing infiltrating tumor regions, with early time points is clearly needed. Additional research is also needed to optimize drug-to-tumor delivery strategies to ensure biologically adequate distribution and pharmacodynamic changes within these regions. Small, informative, tissue-based clinical trials that take into account the individual molecular features of patients and provide early “go” or “no go” decisions are needed and should be prioritized over unselected, large, population-based strategies. Early signals of efficacy or proof of concept need to be quickly validated, with the goal of final proof of efficacy in controlled studies.

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