CENTRAL NERVOUS SYSTEM TUMORS

Bridging Science and Clinical Practice: How to Use Molecular Markers When Caring for a Patient with Brain Cancer

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Technical advances in genomic and proteomic profiling and bioinformatics have resulted in the identification of a large number of possible prognostic, predictive, and diagnostic molecular markers in glial tumors. Increasingly, clinical trials are incorporating tissue analyses to prospectively and retrospectively study the value of these and yet-to-be defined markers. Once validated, markers form the basis for increasingly stringent classification schemes and the development of personalized, targeted therapies. Descriptions of molecular marker findings, many not validated as clinically relevant, are filling pathology reports, and patients arrive to clinic with the latest journal article requesting marker assessment. Although some practitioners may choose to incorporate these findings into clinical decision making, empirical data supporting these decisions is limited to a few specific circumstances. This article reviews three markers—codeletion of 1p/19q, methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter, and the presence of IDH1/2 mutation—for which there exists high or moderate levels of evidence of current clinical utility for guiding diagnostic, prognostic, and treatment decisions.
The studies validated 1p/19q codeletion as a favorable prognostic marker for OS. In RTOG 9402 patients with 1p/19q codeletion had median OS of at least 7 years compared with 2.8 years for those without the codeletion (p < 0.001). Although neither study had a chemotherapy-only arm or a delayed radiation arm, on the assumption that radiation at recurrence after upfront PCV would be effective, these results led some practitioners to delay radiation until progression in patients with codeletions to avoid late cognitive effects. The poor survivals of patients with nondeleted tumors led some to use the radiation and temozolomide (TMZ) regimen used for treating GB.

Now with a median follow-up of 11.3 years, outcomes have been further clarified. In RTOG 9402, the OS of patients with 1p/19q codeletions who received neoadjuvant PCV was 14.7 years compared with 7.3 years (p = 0.03) for those who received only radiation. The addition of PCV to radiation had no impact on the OS of patients without 1p/19q codeletions (2.6 vs. 2.7 years; p = 0.39). Similar but not quite statistically significant data were found for adjuvant PCV (p = 0.059) in EORTC 26951.

These studies suggest that neoadjuvant or adjuvant PCV is highly effective for 1p/19q codeleted tumors. Yet not all neuro-oncologists are adopting this strategy, with some opting to replace PCV with TMZ or continuing to defer radiation until progression. The reasoning behind these choices is varied and includes the increased toxicity of the PCV regimen, the proven efficacy of TMZ in GB, and studies that, although not formally designed to compare PCV with TMZ, suggest equal efficacy (studies also exist suggesting superiority of PCV).

In both studies, the divergence of survival curves for patients with 1p/19q codeletion did not occur until after 5 years suggesting that not all patients with codeletion benefit equally from PCV plus radiation. It also suggests that treatment at recurrence in some patients can be effective and that delay of radiation may not be detrimental.

Survival curves for patients without codeletion also diverged in favor of PCV and radiation. In RTOG 9402, 18% of patients without codeletion were alive at 10 years although median OS was 2.6 years, suggesting a benefit of PCV and radiation in a subgroup not defined by 1p/19q codeletion.

How do these studies direct treatment decisions for patients with anaplastic tumors without 1p/19q codeletions? For this group, neither RTOG 9402 nor EORTC 26951 demonstrated a survival benefit from PCV, and only EORTC 26951 showed a significant increase in progression-free survival (PFS) with the addition of PCV (median, 1.2 vs. 0.7 years; p = 0.026). These poor outcomes are similar to those seen in GB. Therefore, some practitioners treat GB in these patients with radiation and TMZ even though there are no clear data supporting this as effective. The Chemoradiation and Adjuvant Temozolomide in Nondeleted Anaplastic Tumors (CATNON) study may provide direction. This study has four arms: (1) radiation, (2) radiation and concurrent TMZ, (3) radiation followed by TMZ, and (4) radiation with concurrent and adjuvant TMZ. Eligibility is predicated on the absence of the 1p/19q codeletion and not histology, and the study is ongoing.

**Recommendations**

Patients with newly diagnosed 1p/19q codeleted anaplastic tumors should not receive treatment with radiation only. There are no prospective studies comparing responses to PCV and TMZ in this population, and available studies are not definitive. Thus, evidenced-based practice directs the use of PCV and radiation; TMZ can be considered if PCV is not tolerated. The decision to delay radiation is fraught with difficulty as data are scant; however, as noted above, for some patients treatment at recurrence appears to be effective. This author would consider delay of radiation in a young adult with 1p/19q codeletion and with previously defined good prognostic factors such as gross total resection and high performance status.

Although oncologists consider PCV toxicities relatively mild compared with those produced by other multiagent regimens, for some systemic cancers, the trade-off can be a durable remission. Patients with glioma rarely have durable remissions, and one must weigh even moderate toxicity against impact on quality of life and expected improvement in OS. Some practitioners (who have been prescribing PCV consistently even after TMZ became available) do not stringently recommend dietary restrictions to patients. The avoidance of foods high in tyramine because of weak inhibition of monoamine oxidase by procarbazine results in patients receiving an extensive list of foods to eliminate, many of which are staples of many diets. A fascinating review of food restriction and procarbazine written more than 30 years ago persuasively asserts that these food interactions are of minor clinical significance. Because food restriction can adversely affect quality of life and lead to unwanted weight loss, if PCV is to be used more commonly, perhaps after 30 years it is time to readdress this issue.

**KEY POINTS**

- Codeletion of 1p/19q is a predictive marker in anaplastic oligodendroglioma and can be used for treatment decisions.
- MGMT promoter methylation status is a predictive marker in newly diagnosed glioblastoma (GB); however, at this time, it likely should be used only for treatment decisions in elderly patients.
- IDH1/2 mutation is a strong prognostic marker in all grades of gliomas but does not currently have predictive value.
- Triple-mutant glioma (intact 1p/19q, unmethylated MGMT promoter, and wild-type IDH1/2) identifies a distinct subclass of glioma with poor outcome; this status can guide treatment decisions, especially at recurrence.
- The use of other markers for treatment decisions outside of clinical trials is not empirically based.
The presence of 1p/19q codeletion can support a pathologic diagnosis of oligodendroglioma, especially for cases in which histology does not show many classic oligodendroglial features (e.g., perinuclear halos, rounded nuclei). However, up to 20% of GB can have oligodendroglial features and of these, 5% to 25% will have 1p/19 codeletion. Thus, the presence of 1p/19q codeletion is not by itself sufficient to diagnose oligodendroglioma.

Tumors that can mimic oligodendroglioma include dysplastic neuroepithelial tumors (DNET), neurocytomas, clear cell ependymoma and small-cell variants of anaplastic astrocytoma and GB. These tumors have varying clinical outcomes and treatment approaches. Because they do not exhibit 1p/19q codeletion, the detection of the deletion is a useful ancillary diagnostic tool.

**MGMT PROMOTER METHYLATION STATUS**

**Use in Clinical Decision Making**

**Prognostic.** Positive prognostic factor for improved outcome in anaplastic gliomas and GB regardless of therapy type (radiation, chemotherapy, or both).

**Predictive.** Yes, of benefit to alkylating chemotherapy in GB including in older patients.

**Diagnostic.** Can aid in distinguishing tumor progression from pseudoprogression.

**Background**

The EORTC/National Cancer Institute of Canada (NCIC) trial that demonstrated the benefit of TMZ in newly diagnosed GB also confirmed the relationship between clinical outcome and tumor levels of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT). MGMT levels were indirectly determined by analysis of MGMT promoter methylation, an epigenetic change that silences gene transcription. MGMT promoter methylation was an independent favorable prognostic factor (p < 0.001) and predictive of response to TMZ and radiation. Patients with methylated MGMT promoters who received TMZ in addition to radiation had median OS of 21.7 months compared with 15.3 months for those receiving radiation alone (p = 0.007). Patients with unmethylated MGMT showed a small but not quite significant (p = 0.06) survival benefit from TMZ compared with radiation. Temozolomide concurrent with radiation and 6 months of adjuvant TMZ became the standard of care, but not just for patients with methylated MGMT. In the absence of a therapeutic alternative to offer patients with nonmethylated tumors and considering the small benefit that some of these patients received, outside of a clinical trial TMZ is not withheld from patients on the basis of MGMT methylation status.

Some practitioners use MGMT status as the basis for continuing adjuvant TMZ beyond the 6-month regimen used in the EORTC/NCIC trial, assuming this would translate into better outcomes. On the basis of data showing that prolonged exposure to TMZ depletes cellular MGMT, others asked whether TMZ could be used to overcome TMZ resistance in...
nonmethylated tumors. The RTOG 0525 study of newly diagnosed GB addressed these scenarios. In this study, after completion of radiation with concurrent TMZ, patients were randomly assigned to undergo 12 cycles of standard 5-days-on, 23-days-off TMZ dosing or lower-dose TMZ on a 21-days-on, 7-days-off schedule. The final results confirmed that MGMT status was a positive prognostic factor for improved outcome. However, comparison of the two dosing arms showed no significant difference in OS or median PFS. Patients in the 21/28 arm also had more grade 3 or 4 episodes of lymphopenia and fatigue. Thus, neither longer exposure to TMZ in patients with MGMT methylation nor the use of (theoretically) MGMT-depleting dose-dense TMZ in nonmethylated tumors affected outcome. These phase III data do not support a change in the standard 5/28 dosing regimen or the use of TMZ beyond six adjuvant cycles.

Recently a predictive role for MGMT promoter methylation was demonstrated in two trials studying older patients (≥ 60 years) with GB and anaplastic glioma. The NOA-08 study randomly assigned patients to receive radiation or TMZ, and showed no difference in median OS between the treatments. MGMT promoter methylation was associated with prolonged OS (11.9 months for methylated compared with 8.2 months for nonmethylated; p = 0.014) for the group as a whole, and longer PFS if the patient received TMZ compared with radiation (8.4 vs. 4.6 months). In contrast, patients without MGMT promoter methylation had longer PFS when receiving radiation (4.6 vs. 3.3 months). The NORDIC trial randomly assigned older patients to undergo a standard 6 weeks of radiation, hypofractionated radiation delivered over 2 weeks, or TMZ alone. Temozolomide and hypofractionated radiation each provided better OS than did standard radiation. MGMT promoter methylation was associated with significantly better OS in patients who received TMZ (9.7 vs. 6.8 months; p = 0.03) but not in those who received radiation (8.2 vs. 7.0 months; p = 0.88). Thus, in older patients with high-grade glioma, MGMT promoter methylation is predictive of response to TMZ. Because older patients without MGMT methylation do not seem to derive a benefit from TMZ, it is reasonable to consider radiation alone with hypofractionated dosing decreasing overall time needed for treatment.

Recommendations
Because the phase III EORTC/NCIC study included patients 70 years of age or younger, knowledge of MGMT status in this age group is not needed currently to move forward to initial standard postsurgical care for a patient with GB. Outside of a clinical trial or medical contraindication, patients with newly diagnosed GB should be offered TMZ. The predictive value of MGMT status in older patients should be taken into consideration when making treatment recommendations—in particular, the choice of short-course hypofractionated radiation for patients with nonmethylated MGMT and TMZ without radiation for those with methylated MGMT.

MGMT Promoter Methylation Status as a Diagnostic Marker
MGMT promoter methylation status can assist in distinguishing tumor progression from pseudoprogression that occurs in 20% to 30% of patients receiving radiation and TMZ. Several reports have shown that patients with GB who have MGMT promoter methylation have an increased rate of developing pseudoprogression compared with those without methylation. In the appropriate clinical context and in association with other data such as advanced MRI, MGMT methylation status can be helpful to support a diagnosis of pseudoprogression.

IDH1/2 Mutation
Use in Clinical Decision Making
Prognostic. A positive prognostic marker in low-grade, anaplastic glioma, and although rarely found, in GB.

Predictive. Not defined at this time.

Diagnostic. Useful to distinguish glioma from other entities.

Background
Mutations of IDH1 and, less commonly, IDH2 are found in more than 50% to 80% of low-grade and anaplastic gliomas and secondary GB (those that progress from lower-grade astrocytomas) but only 5% to 10% of primary (de novo) GB. A positive prognostic value of IDH1 mutation is supported by many studies. In one, patients with low-grade gliomas and IDH1 mutation had 4.7-year median PFS and 42% five-year OS compared with 1.4 years PFS and 14% five-year OS for those with wild-type IDH. A study of patients with anaplastic astrocytoma and GB found that IDH1 mutation was the strongest independent prognostic factor for good outcome. In general, for patients with GB and IDH1 mutation, studies report median OS ranging from 24 to 36 months compared with 9 to 15 months for wild-type IDH1 GB.

Mutation of IDH1 is strongly associated with younger age (< 50 years), TP53 mutation, 1p19q codeletion and MGMT promoter methylation; 90% to 100% of 1p/19q codeleted gliomas have IDH1 or -2 mutations. In contrast tumors with wild-type IDH1 often do not have 1p/19q codeletion or TP53 mutation. A study of triple-negative (wild-type IDH and TP53, 1p/19q intact) low-grade glioma demonstrated that these tumors more commonly involved the insula, were large in size, had a more infiltrative MRI pattern, and occurred in older patients compared with tumors with IDH1 and TP53 mutation and 1p/19q codeletions. Patients with triple-negative tumors had a 54% 5-year OS compared with 91% for those with IDH1 and TP53 mutations and methylated MGMT.

Recommendations
Although the presence of IDH1/2 mutation provides prognostic information, this has not yet translated into a change in practice.
in clinical practice, and a predictive role of the mutation has not yet been defined. Triple-negative tumors likely constitute a unique entity with poor clinical outcomes, and this can be taken into consideration but should not direct treatment decisions.

**IDH1/2 Mutation as a Diagnostic Marker**

The absence of IDH1/2 mutation in other entities makes it a very useful diagnostic marker for distinguishing glioma—in particular, low-grade glioma—from other low-grade tumors such as pilocytic astrocytoma and from nontumor entities such as gliosis, ischemia, or radiation-induced damage, and between primary and secondary GB. \(^2\) Because 60% to 80% of pilocytic astrocytomas show BRAF duplication or express a BRAF fusion protein not found in other gliomas, the combination of IDH mutation analysis and BRAF testing is highly useful in cases with indeterminate histologic features. This can be particularly valuable when there is limited tissue availability.

**CONCLUSION**

Many factors determine the clinical value of a molecular marker, including the reproducibility of the laboratory methods used for marker measurement and the stringency of the data that determined the markers’ clinical value. The testing method for some markers, such as IDH1/2 mutation, is relatively straightforward and reproducible. In contrast, multiple techniques are used for measuring MGMT promoter methylation. A recent National Comprehensive Cancer Network Task Force report on the clinical utility of molecular markers used a level-of-evidence system. \(^2\) The highest level of evidence (1A) was given to a biomarker that had been evaluated in at least one adequately powered and specifically designed prospective controlled trial. For gliomas, only 1p/19q codeletion achieved level 1A and some may argue this because both RTOG 9402 and EORTC 26951 were amended after initiation to allow the study of the relationship of 1p/19q status with outcomes. Furthermore, 1p/19q codeletion was not absolutely predictive of response to PCV and radiation, and there was a subpopulation without codeletion that seemed to benefit from these treatments. In the absence of effective alternatives or additional markers to define these subpopulations, are neuro-oncologists prepared to withhold chemotherapy from patients without 1p/19q codeletion? This is similar to the scenario that has occurred in GB in the absence of specific therapies for tumors without MGMT promoter methylation.

Although there are clinical scenarios in which markers can support diagnostic and treatment decisions, it is not readily apparent that we are ready to make decisions solely on the basis of these markers. For example, are the data so strong that we are compelled to offer a 21-year-old with a 1p/19q codeleted AO upfront radiation and PCV? Are we so sure that we cannot delay radiation? Until unequivocal or further supporting robust data are available, neuro-oncologists and their patients will decide how to apply this new knowledge, weighing the goals of increasing survival while maintaining quality of life.

**Disclosures of Potential Conflicts of Interest**

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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How to Use Molecular Markers When Caring for a Patient with Brain Cancer: 1P/19Q as a Predictive and Prognostic Marker in the Neuro-oncology Clinic

M. J. van den Bent, MD

OVERVIEW

Although the central role of 1p/19q codeletion in oligodendroglioma was established almost two decades ago, apart from clear prognostic significance the implications for clinical care have been less clear. This has changed with the long-term follow-up analysis of the EORTC and RTOG trials on procarbazine, lomustine, and vincristine (PCV) chemotherapy in anaplastic oligodendroglioma. These have shown that 1p/19q loss in these tumors is predictive of overall survival benefit of the addition of PCV chemotherapy to radiotherapy.

In 1998 a National Institutes of Health study group gave the following definition of markers: “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Markers can have indeed a variety of clinical significances: they can be useful when diagnosing a disease, when assessing prognosis in an individual patient, when measuring response to treatment or predicting outcome to treatment.

DIFFERENT TYPES OF MARKERS

The role of markers can be manifold, depending on the information they carry. Markers can be considered diagnostic, when they give information about the presence or absence of a disease. Examples are serum CA 15.3 in breast cancer and prostate-specific antigen in prostrate cancer. Markers can be considered prognostic, if they provide prognostic information irrespective of the treatment given. Although this will in general not lead to an altered treatment, they can still support decisions on intensity of treatment (e.g., the decision not to treat in the face of poor prognostic markers) or they can be used to stratify patients in randomized trials to ensure a well-balanced patient population across treatment arms. An example here is serum lactate dehydrogenase (LDH) in non-Hodgkin lymphoma (NHL), which is part of the prognostic index for NHL.

Markers can also be predictive for outcome to a specific treatment, which implies that if present the treatment is more or less likely to be successful. Here, a distinction can be made between qualitative and quantitative predictivity. These terms refer to the question of whether the marker simply predicts more treatment activity (assuming the marker is associated with a favorable treatment response) or whether there will be no treatment effect at all or even an adverse effect in the absence of the marker (qualitative marker). HER2/neu presence is an example of such a qualitative marker in breast cancer; no effect is to be expected from herceptin in the absence of HER2/neu expression. The current knowledge suggests that 1p/19 codeletion is both a disease marker, a prognostic marker, and a predictive marker.

BIOMARKER DEVELOPMENT

Biomarker development is as cumbersome as the conduct of randomized phase III trials, and as a rule of thumb similar numbers of patients are required. All too often data are presented from small series in which a trend toward a better outcome in the presence of a certain biologic marker is found, which result is as a rule decorated with a nice p value. Even in the presence of these nice p values, positive and negative predictive values can be disappointing, and in the case of predictive markers, tests for interaction should also be statistically significant. Then, confirmatory tests in independent datasets are required. It is important to realize that biomarkers investigated in uncontrolled series can only suggest prognostic information. From such projects one cannot determine whether a marker is correlated to the outcome of a specific treatment. That requires biomarker assessment in a well-controlled trial.

THE 1P/19Q TRANSLOCATION

1p/19 codeletion was the first genomic biomarker that heralded promise for widespread use in neuro-oncology. In 1994, Reifenberger and colleagues described the combined loss of 1p/19q as typical for oligodendroglial tumors. In 1997 research on low-grade mixed oligoastrocytoma showed that...
these tumors had either 1p/19q codeletion or TP53 mutations, suggesting these lesions could be used to distinguish between the tumors from oligodendrogial and from astrocytic lineage. Subsequently, in another seminal paper it was demonstrated that these 1p/19 codeleted tumors represented the PCV-chemotherapy sensitive oligodendrogliomas. Despite that, non-1p/19q codeleted tumors still had a 25% response rate to PCV. Further studies showed that 1p/19q codeleted tumors represent a specific subset of tumors, which are more often frontally located, with a more indolent clinical behavior, with more often a classical oligodendrogial morphologic appearance, and also responsive to temozolomide chemotherapy and radiotherapy. Several papers focused on radiologic features, although with somewhat different conclusions, but ring enhancement is rare for a 1p/19q codeleted oligodendroglioma. It was then shown that this 1p/19q codeletion is actually a t(1;19)(q10;p10) translocation after which 1p and 19q are lost.

Despite the quantitative predictive effect of 1p/19q loss for response of recurrent oligodendrogial tumors to PCV chemotherapy, the 2006 reports on the large adjuvant PCV randomized studies failed to show a predictive value of 1p/19q status, although these trials established a clear prognostic role of 1p/19q status in anaplastic oligodendrogliomas. The long-term follow-up data from both studies, however, show that in the 1p/19q codeleted anaplastic oligodendrogial tumors, the addition of PCV to radiation therapy significantly improves overall survival with a hazard ratio (HR) reduction of 0.55-0.60 (EORTC study: HR 0.56; 95% CI, 0.31-1.03; RTOG study: HR 0.59; 95% CI, 0.37-0.95). It is noteworthy that each single trial would not have allowed the conclusion that 1p/19q deletion is predictive for outcome to adjuvant PCV chemotherapy, but the combined data showing exactly the same long-term effect is the convincing observation.

Is 1p/19q all there is, when it comes to predicting benefit from PCV chemotherapy? Probably not. Both trials suggest that other molecular features may allow assessment of benefit to PCV. In particular, gene expression arrays and genome-wide methylation studies have yielded interesting results. Other single gene candidate markers are IDH and MGMT promoter methylation. Which technique offers the best predictive power remains to be established. It is also clear that the increased responsiveness of p/19q codeleted tumors is not limited to PCV, but includes temozolomide and radiation therapy. Still, absence of 1p/19q loss does not imply a patient will not benefit from chemotherapy. It is at this point in time, however, uncertain if a patient with a grade 3 tumor will benefit in a clinically significant way from the addition of adjuvant chemotherapy to radiation therapy in the absence of the 1p/19q codeletion.

ASSESSMENT OF 1P/19Q CODELETION

With these combined data, the prognostic and predictive roles of 1p/19q have been clearly established. Assessing 1p/19q status may also help in case of diagnostic uncertainty with oligodendrogial mimics (e.g., central neurocytoma). Assessing 1p/19q status should now be considered in all grade 2 and grade 3 tumors, especially in the presence of oligodendrogial features. A variety of techniques exist to assess 1p/19q status, and efforts have been made to correlate these to each other (with in general good but certainly not complete correlations). Both loss of heterozygosity and FISH are used frequently, for 1p/19q FISH an extensive guideline exist. It is important to realize that the 1p36.6 probe frequently used to assess loss of 1p can also be deleted in glioblastoma that show the loss of only the tip of 1p. If LOH is used for assessment of 1p/19q status, care should be taken that several markers on the 1p arm are absent. Indeed, both the entire arm of 1p and 19q are lost. Grade 3 tumors not infrequently show loss of 19q without additional 1 loss. Laboratories conducting 1p/19q analysis should participate to quality programs that include ring tests.

NOVEL DISCOVERIES

Next-generation sequencing (Illumina HiSeq platform) has identified in 1p/19q codeleted oligodendrogial tumors novel and probably inactivating mutations in the CIC and FUBP genes. In a total of 34 1p/19q codeleted tumors 18 (53%) mutations were identified in the CIC gene located on 19q, and 5 (15%) in the FUBP gene located on 1p. No CIC mutations were identified in tumors without 19p loss. Subsequent studies confirmed the high mutation rate of CIC in 1p/19q codeleted tumors but only rarely in tumors without the deletion. Taken together, CIC mutations appear to occur in 50% to 70% of 1p/19 coded tumors and FUBP mutations in 15%. CIC mutations occur almost invariably with IDH mutations, and occur almost exclusively in 1p/19q codeleted grade 2 and 3 oligodendrogial tumors. They are exceedingly rare in other brain tumors. Cic is a downstream component of receptor kinase pathways (RTK-RAS-RAF-MAPK). Cic blocks transcription through binding to regulatory regions, and is negatively regulated by RTK signaling, which blocks the function of cic through MAPK mediated phosphorylation. This results in the degradation of cic. FUBP mutations may result in MYC activation. Although fascinating new developments, the clinical significance of these mutations is currently unknown.
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PRACTICAL APPROACH OF 1P/19Q TESTING IN 2013 NEURO-ONCOLOGY

In general, the assessment of markers should lead to alterations in the management of patients. The clinical value of a marker that is prognostic only is limited, as it will not change management of the patient.

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References

CENTRAL NERVOUS SYSTEM TUMORS

Controversies in Antiangiogenesis Therapy: Point/Counterpoint

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Antiangiogenic Therapy for Glioblastoma: The Challenge of Translating Response Rate into Efficacy

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OVERVIEW

Glioblastoma are one of the mostly vascularized tumors and are histologically characterized by abundant endothelial cell proliferation. Vascular endothelial growth factor (VEGF) is responsible for a degree of vascular proliferation and vessel permeability leading to symptomatic cerebral edema. Initial excitement generated from the impressive radiographic response rates has waned due to concerns of limited long-term efficacy and the promotion of a treatment-resistant phenotype. Reasons for the discrepancy between high radiographic response rates and lack of survival benefit have led to a focus on identifying potential mechanisms of resistance to antiangiogenic therapy. However, equally important is the need to focus on identification of basic mechanisms of action of this class of drugs, determining the optimal biologic dose for each agent and identify the effect of antiangiogenic therapy on oxygen and drug delivery to tumor to optimize drug combinations. Finally, alternatives to overall survival (OS) need to be pursued using the application of validated parameters to reliably assess neurologic function and quality of life.

A pathophysiological hallmark of glioblastoma is the expression of VEGF and other pro-angiogenic cytokines, which, in turn, stimulate endothelial cell proliferation, migration, and survival. This leads to the formation of a highly abnormal tumor vasculature characterized by hyperpermeable vessels, increased vessel diameter, and abnormally thickened basement membranes. This abnormal vascular network not only promotes tumor growth but may also impair the efficacy of cytotoxic chemotherapy and radiation by enhancing tumor hypoxia and compromising intratumoral delivery of chemotherapy.

The utilization of angiogenesis inhibitors for the treatment of glioblastoma has brought hope that blocking this central component of gliomas will inhibit tumor growth and prolong patient survival. Early, uncontrolled studies have been encouraging. Both the pan-VEGF receptor (VEGFR)-2 tyrosine kinase inhibitor cediranib and the anti-VEGF-A antibody bevacizumab demonstrated impressive radiographic response rates and prolongation of progression-free survival (PFS) in single arm phase II clinical trials. With a strong tail wind, these agents entered into phase III clinical trials for recurrent (cediranib) and newly diagnosed (bevacizumab) glioblastoma. Although these agents may improve symptoms and allow for reduction in steroid use, to date, they have not demonstrated an improvement in OS compared with standard of care therapy. These data require reflection on potential mechanisms by which antiangiogenic therapy could augment benefit associated with current approaches, and identify mechanisms that may limit the ability of these agents to improve patient survival.

In this review and at our presentation at the 2013 American Society of Clinical Oncology Annual Meeting, we will explore the potential biologic mechanisms of action and reasons why antiangiogenic therapy should be effective in the treatment of glioblastoma. Point by point, we will describe potential flaws in current approaches to inhibit angiogenesis and possible mechanisms of resistance limiting efficacy. Finally, we will describe areas that require new insights as well as approaches that may improve understanding and better exploit these therapies to meaningfully improve patient outcome.

MECHANISMS OF ACTION

There are a number of hypothesized mechanisms of action of antiangiogenic agents in the treatment of solid tumors. These mechanisms are not mutually exclusive and may be operative simultaneously or at different time points during the course of antiangiogenic treatment. The first hypothesis is the classical view that antiangiogenic agents have a direct effect on tumor endothelium resulting in endothelial cell apoptosis, ultimately leading to a cytostatic effect on new blood vessel growth. Consequently, there are effects on vascular function, including vasoconstriction, decreased...
permeability, and decreased perfusion resulting in decreased delivery of oxygen and nutrients to the tumor. The resulting “tumor starvation” inhibits tumor growth. Moreover, when used in combination therapy, VEGF pathway inhibitors may sensitize glioma-associated endothelial cells to cytotoxic therapy and counteract a surge in VEGF expression and endothelial precursor cell recruitment induced by genotoxic stress from chemotherapy and radiation.\(^4\)\(^-\)\(^6\) A second hypothesis is that antiangiogenic agents achieve antitumor effects by vascular normalization.\(^7\) Preclinical and clinical studies in a number of solid tumors, including glioblastoma, have demonstrated that there is a transient period of “vascular normalization” following administration of anti-VEGF therapies characterized by reduced vessel diameter and permeability, improved vessel perfusion, reduction in tumor interstitial pressure, and improved tumor oxygenation.\(^8\)\(^-\)\(^10\) In aggregate, these changes enhance delivery and efficacy of cytotoxic chemotherapy during the “normalization window.” Vascular normalization might also improve the efficacy of ionizing radiation.\(^9\) In mouse models of glioblastoma, VEGFR2 inhibition produced a transient period of reduced tumor hypoxia (i.e., a vascular normalization window) during which radiation had a synergistic effect with anti-VEGFR2 therapy. During this normalization window, pericytes are recruited to blood vessels by activation of Ang-1/Tie2 signaling.\(^9\) These vessels are more efficient at oxygen delivery, thus augmenting the effects of radiation. However, the effect of anti-VEGF agents on chemotherapy delivery to tumors is incompletely understood. In a small PET study in patients with non-small cell lung cancer, there was reduced perfusion and net influx rate of radiolabeled docetaxel within 5 hours after the administration of bevacizumab.\(^10\) However, other studies demonstrate that perfusion increases in a subset of glioblastoma patients within 24 hours after treatment with anti-VEGF therapy and those patients with improved perfusion have longer OS.\(^11\)\(^,\)\(^12\) A third hypothesis is that antiangiogenic agents may have activity against glioblastoma stem-like cells (GSCs). GSCs seem to be at least partially responsible for resistance to genotoxic treatments through activation of DNA damage checkpoint response and an increase in DNA repair capacity. In preclinical studies, these GSCs exhibit upregulation of VEGF expression, form highly angiogenic tumors in mice, and reside in aberrant perivascular stem cell niches supported by endothelial cells.\(^13\) In these models it has been demonstrated that antiangiogenic agents disrupt the perivascular-stem cell niche and contribute to the death of GSCs.\(^13\) Treatment of GSCs with bevacizumab or metronomic chemotherapy also suppresses their tumorigenicity in animal models.\(^13\)\(^,\)\(^14\) A fourth hypothesis is that antiangiogenic agents disrupt pro-angiogenic signaling from bone marrow–derived myeloid cells thereby suppressing tumor vascularization.\(^15\) Angiogenesis is dependent on diverse cell populations in the tumor microenvironment, including inflammatory cells of myeloid lineage like mast cells, dendritic cells, eosinophils, neutrophils, and macrophages—all sources of VEGF and other proangiogenic factors. VEGF and the closely related placental growth factor (PIGF) are potent myeloid cell chemokines that attract VEGF receptor 1 (VEGFR1)+ monocytes to tumors. VEGFR1+ cells appear to be important in sustaining glioblastoma angiogenesis.\(^15\) Preclinical experiments demonstrate that elimination of VEGFR1 signaling in bone marrow–derived myeloid cells decreases tumor angiogenesis and growth. Thus, the antitumor effects of antiangiogenic agents may be at least partially mediated by disruption of VEGFR1 signaling and reduction of tumor-infiltrating VEGFR1+ monocytes. Resistance to antiangiogenic agents may be conferred by eventual infiltration of additional bone marrow–derived macrophages that promote aggressive mesenchymal features and increased stem cell marker expression.\(^16\)

**KEY POINTS**

- Antiangiogenic therapy rapidly reduces vascular permeability and cerebral edema, which corresponds with high radiographic response rates, symptom improvement, and reduced corticosteroid requirement.
- Despite multiple potential mechanisms by which antiangiogenic therapy may augment the efficacy of chemotherapy and radiation, benefits to patients may not translate into improved survival.
- Effect on drug delivery, vascular endothelial growth factor-independent angiogenesis, transformation to a mesenchymal phenotype, and a heterogeneous microenvironment may contribute to a lack of therapeutic efficacy.
- Future efforts should address specific knowledge gaps regarding mechanism of action, identification of patient subsets more likely to benefit, informative biomarkers, the value of alternative outcome measures, and the development of therapies to improve efficacy.

**VASOGENIC CEREBRAL EDEMA**

VEGF, originally termed vascular permeability factor, increases the permeability of tumor vessels leading to vasogenic brain edema. Primary and metastatic brain tumors are often associated with vasogenic edema, which contributes to neurological morbidity. Vasogenic brain edema is a primary reason why a large proportion of patients with brain tumor require treatment, often long term, with corticosteroids. Clinical and radiographic studies of patients with glioblastoma treated with anti-VEGF agents demonstrate both anti-edema and steroid-sparing effects.\(^8\)\(^,\)\(^17\) In addition, preclinical studies of cediranib, a pan-VEGF receptor tyrosine kinase inhibitor, demonstrated potent antiedema effects in three different orthotopic murine glioma models.\(^18\) In these studies, cediranib significantly alleviated edema through rapid normalization of tumor vasculature. Of note, survival time was prolonged (p < 0.05) with no change in tumor growth indicating that a potent antiedema effect may prolong survival in some orthotopic glioma models.\(^18\) Thus, a salutary benefit of vascular normalization in patients with brain tumors is
reduction in cerebral edema and sparing of steroid-related complications.

**PSEUDOPROGRESSION**

It is not uncommon to observe increased contrast-enhancement and surrounding T2/FLAIR hyperintensity within the radiation treatment field on MRI scans in patients with glioblastoma obtained within a few months of chemoradiation completion. While these radiographic findings raise the possibility of tumor progression, these changes may also reflect the cytotoxic effect of chemoradiation on the tumor and the tumor microenvironment, typically referred to as tumor “pseudoprogression.” The incidence of tumor pseudoprogression ranges from 28% to 66% in all patients with glioblastoma undergoing chemoradiation and typically occurs within 3 months after completion of concurrent radiation and temozolomide. The radiographic findings typically consist of an increased area of contrast enhancement and enlargement of noncontrast T2/FLAIR hyperintense signal surrounding the enhancement. Approximately one-third of patients are symptomatic from tumor pseudoprogression and may require treatment with corticosteroids. Bevacizumab appears to be efficacious in the treatment of radiation-related brain necrosis but has not been adequately studied or established as an effective therapy for symptomatic tumor pseudoprogression. There is some evidence to suggest that concurrent treatment of newly diagnosed patients with glioblastoma with chemoradiation and an inhibitor of VEGF may reduce the incidence of pseudoprogression. Reduction in the incidence of pseudoprogression may be a secondary benefit of the vascular normalizing effects of anti-VEGF treatment, which may in turn spare patients from neurological symptoms and steroid usage. This benefit may be of particular value among patients with large, unresectable tumors in order to permit better tolerance of chemoradiation.

**RESISTANCE TO ANTIANGIOGENIC THERAPY**

The radiographic response rate of antiangiogenic therapy in glioblastoma is very high, but the duration of response on average lasts approximately 4 months. “Vascular response,” or “pseudoresponse,” referring to a reduction in contrast enhancement in the absence of tumor growth inhibition, can occur rapidly, even within 24 hours of antiangiogenic therapy administration. The rapid responses and short duration of benefit from these therapies have prompted the suggestion that there is little intrinsic antitumor activity of these agents and that the main benefit may be due to indirect effects attributable to reduced cerebral edema. In light of recent preliminary data failing to show an improvement in OS for newly diagnosed patients treated with bevacizumab together with the standard of care, all glioblastoma may be intrinsically resistant to these agents. This is not to say that antiangiogenic therapy might not be a useful tool for the treatment of glioblastoma, but at this point, evidence for the ability of these agents to augment survival is lacking. Here we provide a counterpoint to the discussion above and describe several potential mechanisms of resistance that may limit the efficacy of antiangiogenic therapy in glioblastoma.

**THE BALANCE BETWEEN NORMALIZATION AND EXCESSIVE VESSEL PRUNING**

As described above, antiangiogenic therapy-mediated vascular normalization may normalize an otherwise chaotic tumor vasculature to improve oxygenation, drug delivery, and radiation therapy efficacy. As eloquently described by Jain and colleagues this process requires a balance of pro and antiangiogenic factors. However, an overabundance of antiangiogenic factors, as may occur with excess antiangiogenic therapy, may tip the balance toward a devascularized and hypoxic environment. Several animal models and human biopsy studies have identified increases in tumor hypoxia following prolonged antiangiogenic therapy administration. As opposed to the beneficial effects of oxygenation, tumor hypoxia is a well-known mediator of resistance to chemotherapy and radiation and may contribute to resistance mechanisms described below.

**ANTIIANGIOGENIC THERAPY MAY LIMIT DRUG DELIVERY**

There appears to be a complex interaction between the potential benefit provided by vascular normalization and drug delivery. Although some reports have demonstrated an improvement in radiation sensitivity following vascular normalization, these benefits may be limited to the period immediately following antiangiogenic drug dosing. Time from initiation of drug administration as well as dose of antiangiogenic may limit the potential benefit provided by the vascular normalization window. For example, low-dose antiangiogenic therapy resulted in improved temozolomide delivery in an orthotopic glioma xenograft model, but high-dose antiangiogenic therapy decreased delivery compared to untreated controls. Translation of high- and low-dose antiangiogenic therapy to the human GBM experience has not been explored and poses a challenge when designing clinical trials to evaluate combination therapies. Furthermore, recent data suggest that antiangiogenic therapy decreases delivery of small molecules such as erlotinib to glioma xenograft tumors (personal communication, J Sarkaria). Implications of these data to the neuro-oncology community are enormous. Utilizing maximum tolerated dose as opposed to the optimal biologic dose of antiangiogenic therapy is the approach currently used for patients. It is unclear if this is a “high” or “low” dose and thus the effect of dose on drug delivery to patients remains unknown. There are numerous completed and ongoing clinical trials combining antiangiogenic therapy with chemotherapy and molecularly targeted agents. These studies have failed to demonstrate improved outcomes compared
to the antiangiogenic agent alone. At this time, it is not known if this is due to a lack of synergy between the combinations or if there is antagonism resulting from a reduction in drug delivery following antiangiogenic dosing. Future combination approaches should incorporate techniques to ensure that intratumoral drug delivery is not impeded by concurrent use of antiangiogenic therapy.

**TUMOR VESSELS INSENSITIVE TO ANTIANGIOGENIC THERAPY**

Although small vessel pruning is one of the most prominent and oft cited benefits of antiangiogenic therapy, only certain blood vessels within the tumor microenvironment are likely to be affected. Recent studies suggest that only mother vessels and glomeruloid microvascular proliferations contain VEGFR2-expressing endothelial cells and are susceptible to anti-VEGF therapies. Conversely, feeding arteries and draining veins are less sensitive to anti-VEGF strategies due to lower expression of VEGF receptors. The histologic prominence of glomeruloid vascular proliferation and the rapid reduction in contrast enhancement on MRI suggest that VEGF plays a dominant role in permeable blood vessels in glioblastoma. Although the vast majority of patients treated with antiangiogenic therapies have some reduction in contrast enhancement, it is not clear to what degree these imaging changes correlate with a reduction in tumor vascularity. Anti-VEGF therapy may not eliminate blood flow to tumor via larger non-VEGF-dependent vascular structures.

**VEGF-INDEPENDENT ANGIOGENESIS**

Many groups have demonstrated that inhibition of VEGF-A in glioma tumor models promotes both tumor and stromal-mediated VEGF-A-independent angiogenesis. A plethora of pro-angiogenic factors have been shown to increase including VEGF-C, VEGF-D, PIGF, PDGF, basic fibroblast growth factor, and others. It is unclear how many human glioblastomas switch to VEGF-A-independent angiogenesis. Interestingly, only a fraction of tumors progressing during treatment with an antiangiogenic agent develop new contrast enhancement and frequently this contrast enhancement is atypical. MRI images display punctate and scattered enhancement as opposed to the typical ring-enhancement of glioblastoma. Analysis of tumor cells biopsied from enhancing areas of human glioblastoma progressing on bevacizumab revealed a distinct set of upregulated genes, an increase in tumor cell proliferation but no changes in tumor vascularity compared to their pretreated samples suggesting that bevacizumab may not reduce tumor vasculature in all tumors. Importantly, tumor cells requiring oxygen and nutrients may grow and migrate along (co-opt) normal blood vessels within the brain. This juxtaposition of tumor cells along the normal vasculature may obviate the tumor’s need for neoangiogenesis for survival and may provide a route for tumor infiltration to distant areas of the brain protected by the blood brain barrier, as described in more detail below.

**MYELOID CELL-MEDIATED RESISTANCE**

Although initially associated with reduced recruitment of monocytes and macrophages, antiangiogenic therapy eventually promotes the recruitment of multiple myeloid cell types. These cells are associated with diverse aspects of an evasive tumor phenotype. It is known that M2-skewed tumor associated macrophages contribute to angiogenesis, assist with immune evasion, and promote tumor invasion. It is important to note that macrophages account for only a fraction of bone marrow–derived cells recruited to resistant tumors. Elimination of macrophages in a glioma stem cell xenograft model using a colony stimulating factor 1 receptor inhibitor did not improve the efficacy of antiangiogenic therapy alone and promoted resistance consistent with described mechanisms of resistance common to many antiangiogenic therapies (unpublished data, J. de Groot). In fact, attraction of macrophages to tumor may eliminate regions of hypoxia and improve sensitivity to therapy highlighting the challenges of inhibiting just one myeloid cell type in the complex tumor microenvironment. Equally or more important, neutrophils, Tie-2 expressing monocytes, and myeloid-derived suppressor cells all contribute to a highly resistant tumor through complex microenvironment interactions between tumor, endothelial cells and the microenvironment. Several studies have indicated the importance of targeting neutrophils in antiangiogenic therapy resistance. In animal models, both the JAK/STAT inhibitor AZD1480 and an inhibitor of Bv83 in combination with antiangiogenic therapy reduced infiltration of neutrophils and led to significant prolongation of animal survival and reduced tumor size.

**AUTOPHAGY-MEDIATED RESISTANCE**

In addition to direct effects on tumor vasculature, antiangiogenic therapy has been shown to minimally induce glioma cell apoptosis in animal models. These indirect effects are small, but glioblastoma may utilize autophagy to prevent cell death. Glioblastoma are inherently hypoxic, which may be further exacerbated by antiangiogenic therapy. Glioma cells may utilize autophagy to confer a survival advantage when exposed to this hypoxic microenvironment. Additionally, resistance to chemotherapy may in part be mediated by autophagy leading to a more resistant phenotype consistent with the absence of efficacy of standard therapies. Attempts to overcome pro-survival autophagic pathway activation will require a more detailed understanding of target protein for inhibition as well as the overall consequences of autophagy inhibition.

**MESENCHYMAL AND INVASIVE TRANSFORMATION**

Finally, there is the concern that antiangiogenic therapy promotes tumor invasion. In animal models, invasion of glioma cells during antiangiogenic therapy is thought to occur through vessel co-option, which has been demonstrated in human and animal histologic studies. Attempts to
overcome this invasive phenotype in preclinical models have been variably successful.\textsuperscript{34,38} However, the effect of this phenotypic shift in human disease remains controversial.\textsuperscript{39} At the heart of this debate is the fact that glioblastomas are highly invasive in the absence of antiangiogenic therapy and all glioblastomas ultimately progress on standard and experimental therapy. At a minimum, inhibition of vascular permeability with anti-VEGF therapies leads to a decoupling between contrast enhancement visualized on MRI and its expected (nonenhancing) tumor growth/infiltration. This phenomenon occurs in a third or fewer cases and results in clinical progression in the absence of worsening enhancing tumor burden.

For at least a decade, the proto-oncogene MET has been implicated in resistance to antiangiogenic therapy.\textsuperscript{40} Some groups have identified activation of this pathway in glioblastoma,\textsuperscript{41} whereas others have not,\textsuperscript{16,42} possibly reflecting the complexity of the disease, differences in animal models, and heterogeneous tumor cell responses to therapy. Additionally, the spectrum of molecular alterations associated with resistance to antiangiogenic therapy includes the induction of mesenchymal genes,\textsuperscript{16} which are known to promote invasion in glioblastoma, a proneural to mesenchymal phenotype, epithelial-mesenchymal transition, and metastasis in other solid tumors. An increase in stem cells\textsuperscript{16} and extracellular matrix proteins\textsuperscript{28,34} may also reflect selection for highly resistant cells, which may contribute to therapeutic resistance. Resistance of antiangiogenic therapy-treated tumors remains a formidable problem and will require new approaches to elucidate and overcome these complex and likely interwoven contributing mechanisms.

**CHALLENGES FOR THE FUTURE**

With the exception of temozolomide, evaluation of a wide array of therapeutics for patients with glioblastoma over the past few decades has demonstrated, at best, short-term benefit for a small minority of patients. In contrast, most patients with glioblastoma derive some benefit from bevacizumab, and to varying degrees, other VEGF/VEGFR-targeting therapeutics. The durability of clinical benefit for most patients is limited. These results suggest that mechanisms of resistance represent a major challenge for antiangiogenic therapies. Furthermore, many fundamental questions regarding the application of these agents for patients with glioblastoma remain unanswered. Answers to these questions may hold the key to overcoming current limitations and future challenges.

Proliferative blood vessel formation is a characteristic feature of glioblastoma, and several pathophysiologic mechanisms, including vascular co-option, angiogenesis, vasculogenesis, vascular mimicry, and glioblastoma-endothelial cell transdifferentiation, contribute to this process. Paradoxically, glioblastoma vasculature is dysfunctional due to ultrastructural, functional, and biochemical abnormalities leading to poor blood flow and increased vessel permeability, which in turn contribute to elevated interstitial pressure and tissue hypoxia/acidosis. Furthermore, glioblastoma tumors are intrinsically infiltrative and capable of adapting to hypoxic/acidotic microenvironments. In these contexts, it is unclear how truly dependent glioblastoma tumors are on angiogenesis and, beyond that, how dependent they are on VEGF. From this perspective, a survival benefit associated with VEGF inhibitor therapy would be unexpected and many would predict intrinsic resistance to be the norm for glioblastoma tumors. Nonetheless, cumulative clinical data suggests that patients with glioblastoma derive benefit from VEGF/VEGFR inhibitors. A major clinical challenge is therefore how to effectively prove and measure these clinical benefits (net clinical benefit).

Several fundamental questions regarding antiangiogenic agents for glioblastoma remain unanswered. A basic unknown is their underlying mechanism of action and, in particular, whether they elicit bona fide antitumor activity. The antipermeability capability of VEGF/VEGFR inhibitors, with their potential to decrease tumor-associated edema and mass effect, is undisputed. The critical question however remains, what is happening to the underlying tumor cells? Are they being starved and shifted toward apoptosis and diminished proliferation as indicated by some preclinical studies?\textsuperscript{24} Are glioblastoma stem cells preferentially targeted? Or does blocking VEGF diminish immunosuppression that may in turn enhance antitumor immune responses?\textsuperscript{44}

**DETERMINING THE OPTIMAL BIOLOGIC DOSE**

As discussed previously, one proposal is that judicious dosing of antiangiogenic agents may achieve a “sweet spot” to effectively normalize aberrant tumor vasculature. Specifically, optimally dosed antiangiogenic agents may prune abnormal tumor vasculature in a limited manner while avoiding vessel obliteration associated with more aggressive dosing. The former process can enhance intratumoral blood flow and improve efficacy of coadministered therapeutics,\textsuperscript{7} while the latter process may detrimentally effect delivery and increase hypoxia/acidosis in the tumor microenvironment. Advanced MRI studies among recurrent patients with glioblastoma following cediranib administration support the vascular normalization mechanism.\textsuperscript{8} Nonetheless, if the normalization mechanism is truly relevant for glioblastoma, a critical question then becomes dosing schedules. Few studies have included alternative dosing schedules of antiangiogenic agents, but none has formally tested less intensive compared with more intensive dosing. A recent report demonstrates that bevacizumab decreases radiolabeled docetaxel delivery among patients with lung cancer.\textsuperscript{10} Whether antiangiogenics can limit intratumoral delivery of concurrently administered therapeutics among patients with glioblastoma remains to be studied. Before initiating expensive and time-consuming clinical trials, efforts are needed to determine the optimal biologic dose that improves drug delivery and oxygenation. Further mechanistic-based studies of antiangiogenic agents and their effect on delivery are clearly needed for patients with malignant glioma.
IDENTIFICATION OF PATIENTS THAT BENEFIT

Another unanswered question is whether informative biomarkers can predict outcome for antiangiogenic therapy. This deficiency remains a shortcoming of antiangiogenic agents across oncology, although recent studies suggest that circulating levels of the short isoforms of VEGF-A may correlate with outcome. Validated biomarkers could potentially serve two key roles. First, they may identify patients more likely to benefit, for whom antiangiogenic agents should be prioritized, or conversely, those patients who are less likely to respond, for whom alternative agents should be considered. Second, biomarkers offer the potential for early determination of progressive tumor among initially responding patients. The latter role offers particular value given current limitations associated with routinely used MRI response assessment methods as discussed below. A wide array of potential biomarkers have demonstrated encouraging early data among patients with glioblastoma, but none have been validated, including advanced MRI applications, PET strategies, measurement of plasma markers or circulating endothelial cells, and the measurement of hypoxia markers from archival tumor samples. One intuitive and easily assessed biomarker appears to be early radiographic response.

Along these lines, another important question is whether antiangiogenic agents may effect subsets of patients with glioblastoma differently. Glioblastoma tumors are currently defined by a spectrum of genetic abnormalities and gene microarray expression studies identify three to four distinct subclasses. Linked expression of mesenchymal and angiogenic genes has been associated with poor prognosis. Future studies may therefore consider patient stratification or enrichment based on genetic characterization.

ESTABLISHING THE CLINICAL BENEFIT OF ANTIANGIOGENIC THERAPY

Benefit from antiangiogenic therapy varies widely across cancers. Among responsive tumors, antiangiogenics consistently achieve greater PFS increments and less prominent, or in some cases, lack of OS benefit. A similar pattern has emerged for glioblastoma. Specifically, among patients with recurrent glioblastoma, the BRAIN study as well as several additional trials, reported PFS-6 rates of approximately 40%, compared to 10% to 15% achieved among historic cohorts. In contrast, median OS on the BRAIN study was eight to 10 months compared to six and seven months reported in historic data. Preliminary results of the AVAglio study, a placebo-controlled, randomized phase III evaluation of bevacizumab for patients with newly diagnosed glioblastoma confirmed similar findings, although mature follow-up is lacking and the effect of cross-over by control patients to receive bevacizumab at progression is unclear. Specifically, median investigator-reported PFS for the bevacizumab and placebo patients were 10.6 and 6.2 months, respectively (p < 0.0001; hazard ratio = 0.64), while OS at 1-year was 72% compared with 66%, respectively (p = 0.052).

These data raise a critical unanswered question regarding the value of various metrics of outcome. Specifically, is there a meaningful value of prolonging PFS independent of OS? Although prolonging OS remains the gold standard, PFS is more controversial, particularly if not clearly validated to predict OS. For most cancers, it is not unreasonable to question whether therapies that prolong PFS are of substantive merit if OS is not prolonged. However, the effect of underlying tumor progression is not the same for all cancers. For example, while progressive enlargement of a metastatic lung nodule or a new hepatic lesion may bode for poor overall outcome, in general these findings usually harbor minimal acute effect for patients with solid tumor. In contrast, progression of inherently infiltrative and destructive central nervous system tumors like glioblastoma typically translates into worsened/new neurologic deficits and chronic corticosteroid dependence, both of which erode quality of life.

Thus, a critical consideration in assessing the value of prolonging PFS, is the effective application of validated parameters to reliably assess neurologic function and quality of life. In a single-center, retrospective series, patients with recurrent glioblastoma who received bevacizumab had a nearly twofold higher independent living score, compared to patients treated without bevacizumab. Similarly, preliminary AVAglio study results demonstrate that bevacizumab recipients reported higher scores on five predetermined quality of life parameters and maintained a Karnofsky Performance Status of 70 or higher compared to placebo controls. Another important correlate of overall well-being among neuro-oncology patients is dependence on chronic corticosteroid dosing to reduce symptoms from tumor-associated cerebral edema and mass effect. The sequelae of chronic corticosteroid dependence on quality of life are substantial including incapacitating proximal myopathy, osteoporosis and pathologic fracture, marked truncal weight gain, hypertension, and hyperglycemia. Of note, both the BRAIN and AVAglio studies reported reduced corticosteroid dosing.

A wide array of metrics to assess quality of life are available for future glioblastoma studies including measures of overall performance, standardized neurocognitive evaluations and validated self-reporting quality of life and symptom inventories adapted for neuro-oncology patients. In addition, an objective scale of neurologic function for neuro-oncology patients is being developed and will integrate into the RANO criteria (personal communication, D. Reardon).

The utility of assessing neurologic function and other readouts reflecting quality of life, is further heightened by the co-nundrum frequently encountered by clinicians attempting to radiologically assess response among patients with malignant glioma undergoing antiangiogenic therapy. Traditional MRI response assessment based on enhancing tumor frequently provides an unreliable surrogate of underlying tumor activity in this setting and was one of the important bases for the delineation of the RANO criteria. Nonetheless, a significant challenge for our neuroradiology colleagues is further refinement of advanced imaging techniques to
more reliably assess underlying tumor activity following antiangiogenic therapy.

NEW SALVAGE THERAPIES NEEDED IN THE ERA OF ANTIANGIOGENIC THERAPY

Finally, given that all patients with malignant glioma ultimately progress following bevacizumab, a major unanswered question is defining effective therapies for patients with bevacizumab-resistant disease. Outcome for such patients is dismal with no effective therapy currently defined. As discussed previously, better understanding of factors underlying acquired resistance to VEGF/VEGFR therapies may provide critical insight to design effective therapies for these patients. In the meantime, one approach frequently used in daily practice is bevacizumab continuation beyond initial progression, usually in combination with a new or different chemotherapeutic. A recently reported phase III study validated the benefit of this approach among patients with colorectal cancer.\(^5\) A recent retrospective analysis among patients with recurrent glioblastoma demonstrated that bevacizumab continuation beyond initial progression modestly improved PFS and OS over nonbevacizumab therapies.\(^5\) A prospective study to evaluate bevacizumab continuation beyond initial progression is being planned for patients with glioblastoma. Importantly, innovative therapeutics focusing on novel malignant glioma targets remain critically needed.

CONCLUSION

Targeting angiogenesis has been a prominent focus of clinical research for glioblastoma over the past five years. Although some answers have been obtained, the cumulative knowledge gained from myriad conducted clinical trials and associated efforts have failed to resolve many fundamental questions. New data suggesting that antiangiogenic therapy may not prolong overall patient survival when added to the standard of care, raises multiple questions regarding the true benefit of these drugs for our patients. Future studies should prioritize addressing specific knowledge gaps regarding mechanism of action, identification of patient subsets more likely to derive durable benefit, informative biomarkers, the value of alternative outcome endpoints, and effective therapy to delay or overcome resistance.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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