New Strategies in Glioblastoma: Exploiting the New Biology

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Abstract

Glioblastoma is one of the deadliest human cancers. There have been few significant therapeutic advances in the field over the past two decades, with median survival of only about 15 months despite aggressive neurosurgery, radiotherapy, and chemotherapy. Nevertheless, the past 5 years has seen an explosion in our understanding of the genetic and molecular underpinnings of these tumors, leading to renewed optimism about potential new therapeutic approaches. Several of the most promising new approaches include oncogenic signal transduction inhibition, angiogenesis inhibition, targeting canonical stem cell pathways in glioblastoma stem cells, and immunotherapy. As promising as many of these approaches appear, they have not had an impact yet on the natural history of the disease or on patient long-term outcomes. Nevertheless, it is hoped that with time such approaches will lead to more effective treatments, but issues such as the unique biology and anatomy of the central nervous system, impaired drug delivery, poor preclinical models with resultant nonpredictive preclinical screening, and poor clinical trial design potentially impede the rapid development of such new therapies. In this article, we review the excitement and challenges that face the development of effective new treatments that exploit this new biology. Clin Cancer Res; 21(9): 1984-8. ©2015 AACR.

Background

Despite years of research into its biology and countless clinical trials of new therapies, the prognosis for patients with glioblastoma remains dismal (1). Newly diagnosed patients will often present with either new focal neurologic abnormalities such as weakness or sensory abnormalities in one or more extremities, cranial nerve dysfunction, seizure, or visual disturbance, or they may present with more general symptoms such as headache or change in mental status or cognition. Following such a clinical presentation, almost all patients inevitably end up undergoing an MRI scan that reveals the tumor. Although a definitive diagnosis of glioblastoma can never be made solely on the basis of an MRI scan, glioblastoma has pathognomonic features that include T2 and FLARE signal abnormalities, gadolinium enhancement, and sometimes mass effect all in a nonvascular distribution. The pathologic correlates of these radiologic findings include hypercellularity, heterogeneity and invasiveness of a glial cell type, nuclear atypia, endothelial proliferation, and necrosis (2).

Although much has been learned regarding the biology of glioblastoma over the past decade, standard treatment for this disease remains largely generic and not biologically based. With the very rare exception of tumors intrinsic to the brain stem, patients with concerning lesions on a diagnostic MRI or CT scan undergo at some point an invasive diagnosis such as de
tomography or biopsy. The target volume has been re
defined over the years from a whole-brain radiotherapy to the area of brain involved by infiltrating tumor cells as defined by the FLARE signal abnormality on MRI.

Although the extent of tumor cell invasion likely goes far beyond the radiographic abnormalities, tumor recurrence is almost always within the initial radiotherapy volume rather than at some distant site. Despite optimal surgical resection of radiotherapy, most cases of glioblastoma recur within 5 to 8 months after the completion of the radiation. Results from a series of phase III trials conducted over the past few decades have suggested that the addition of some chemotherapy (most often an alkylating agent, such as nitrosour
eas) given either with or after the radiotherapy can cause a small, but statistically significant, increase in survival. Although the choice of which drugs are optimal remained somewhat contro
croversial, a randomized phase III trial of surgery/radiotherapy plus temozolomide or placebo in nearly 700 patients demonstrated a highly significant extension in median survival by approximately 2 months. Even with this combination, however, median survival was only approximately 14 months (4).

When cases of glioblastoma recur following standard treat
tment as defined above, patients may be treated with repeat surgical re-resection, re-irradiation, or systemic therapy. None of these therapies, however, has ever been demonstrated to...
significantly prolong survival. The number of active drugs for recurrent glioblastoma is, unfortunately, limited, with only the nitrosoureas demonstrating any clinical benefit, which is marginal (5). As discussed below, the antiangiogenic drug bevacizumab was recently approved for recurrent glioblastoma based on what can be dramatic radiographic and occasional clinical responses (6, 7). Nevertheless, the overall impact of bevacizumab on the natural history of the disease has recently been called into question when two large randomized trials involving nearly 2,000 patients with glioblastoma failed to show a significant increase in overall survival when bevacizumab was used as part of the initial treatment (8, 9). Given this paucity of active drugs in the recurrent setting, clinical trials are often offered to patients at the time of tumor progression.

In summation, since radiotherapy became standard treatment of glioblastoma over four decades ago, our improvements in therapy have only extended the median survival of these patients from approximately 9 months to 15 months. Clearly, new treatments and a new approach are required.

On the Horizon

Any effective therapeutic strategy will have to consider at least four properties of glioblastoma that make it different from other systemic tumors. First, these tumors do not metastasize but rather diffusely invade into normal brain parenchyma, ultimately resulting in treatment failure and patient death. Second, glioblastomas reside in eloquent and nonexpansible normal tissue, limiting treatment to those modalities that will not cause unacceptable long-term neurocognitive dysfunction. Third, glioblastomas reside within the central nervous system (CNS), often referred to as an immunologic sanctuary due to impaired systemic immune responsiveness, thereby challenging the applicability of lessons learned from, and recent success of, systemic immunotherapy (10). Finally, one needs to contend with altered and impaired systemic delivery of many drugs and biologics to the CNS due to the unique and relatively impermeable microvascular architecture within the brain known as the blood–brain barrier (BBB; ref. 11).

There are a number of new strategic areas of therapeutic development that look potentially promising for the treatment of glioblastoma over the next several years—four of them are highlighted below.

Signal Transduction Inhibition/Targeted Therapy

The promise

Much has been learned over the past several years about the genomic makeup of glioblastoma through large efforts like The Cancer Genome Atlas (TCGA) and The Glioma Molecular Diagnostic Initiative (GMDI) at the NCI. First, it is apparent that although individual cases of glioblastoma may have a diverse array of specific genetic and epigenetic changes, most result in the deregulation of the PI3K, the p53, and the RB pathways (12). Thus, efforts are ongoing to target different nodes along these particular pathways with small-molecular inhibitors, antisense molecules, or monoclonal antibodies. For example, there have been a number of efforts to target the PI3K pathway using inhibitors of the PI3K complex itself, AKT, and the mTOR complex (13). Similarly, a number of clinical trials are currently looking at inhibitors of the cyclin-dependent kinases to target the deregulation of the RB pathway (14). Finally, the inactivation of p53 is being addressed through evaluation of inhibitors of MDM2, small molecules that stabilize endogenous p53 and inhibitors of apoptotic molecules that are often unregulated in p53-mutant cells (e.g., Bcl2; ref. 15).

Along with global targeting of the deregulated signaling pathway, ongoing efforts are in place to try and target individual driver-mutated proteins on a tumor-by-tumor basis—the so-called "actionable mutations." The most commonly mutated or deregulated in glioblastoma is the epidermal growth factor receptor (EGFR), which is amplified in about 60% of glioblastomas, of which about 40% have a deletion of the extracellular domain of the protein (EGFRv3) resulting in a low-level constitutively active receptor (16). Other less commonly deregulated genes that have been targeted in clinical trials include the platelet-derived growth factor receptor (PDGF), cMet, PI3K, and the FGFR (17–19). Other mutations that are not infrequently seen in certain subtypes of glioblastoma that await development of clinical targeting strategies include the neurofibromatosis-1 gene (NF1), chromatin H3.3, and ATRX (the latter two found most commonly in pediatric high-grade gliomas; ref. 20). One mutation that appears particularly promising for therapeutic intervention is a gain-of-function mutation in the IDH1 and IDH2 genes that results in an "oncometabolite" and a downstream genomic hypermethylated genotype (GCCIMP; ref. 21). The IDH1 gene is mutated in nearly 70% to 80% of low-grade gliomas and in about 7% of cases of glioblastoma (22). Although there is growing evidence suggesting that mutated IDH1 contributes to the pathogenesis of the disease, it is not clear whether it remains a driver of the glioblastoma phenotype. Nevertheless, early data with a new inhibitor of the mutated IDH1 gene product in multiply relapsed and refractory acute leukemia look potentially promising, lending hope to its potential in treatment of glioblastoma.

Finally, there is hope that some genes/proteins that are not actually mutated, but are central nodes for the aberrant signaling pathways within the gene regulatory network (GRN) of the glioblastoma, may be useful targets. For instance, gene expression analysis has been able to clearly define at least four major subtypes of glioblastoma (11). These subtypes appear to have unique GRNs or at least unique GRN modules that might contain critical nodes ripe for targeting (23). For example, it was demonstrated that C/EBPβ and STAT3 are central nodes in the mesenchymal subtype of glioblastoma and that genetic approaches to inhibit these nodes result in dramatic antitumor effects preclinically (24). Thus, there are significant efforts ongoing to define such nodes in specific molecular subtypes of glioblastoma and to develop therapeutics targeted toward them.

The challenge

A diverse group of therapeutic agents that supposedly target various signal transduction inhibitors, such as EGFR, PDGF, SRC, and RAS, to name but a few, have uniformly proven to be inactive in glioblastoma (25). Why that happens remains unclear and may merely reflect a problem with the drug itself, such as inadequate drug delivery across the BBB. More ominously, however, these negative clinical trials may reflect that these targets may not be drivers of glioblastoma biology. Glioblastoma can quickly develop alternate signaling pathways overriding inhibition of the targeted signaling molecule, or the dramatic intratumoral heterogeneity found within gliomas may allow for rapid selection for nonsensitive clones (26). Further efforts are needed to build studies that will better define whether the experimental

Exploiting the New Biology of Glioblastoma
therapeutic is actually getting to the target and inhibiting it and, if so, the mechanisms of resistance. Ultimately, it is likely that should signal transduction inhibition bring about major therapeutic responses and clinical benefit, combinations of inhibitors will likely be necessary.

**Antiangiogenesis**

The promise

From the characteristic blush seen on cerebral angiography to the endothelial proliferation prototypic and diagnostic of glioblastoma, the vascularity and angiogenic nature of glioblastoma have been well known for decades. Indeed, a large number of preclinical trials have demonstrated the potential therapeutic benefit of inhibiting glioblastoma-associated angiogenesis (27). Early pessimism in the approach due to the clinical failure of several small-molecule inhibitors (i.e., angiotatin, endostatin, and thalidomide) gave way to unbridled optimism with the dramatic radiographic (and sometimes clinical) responses seen in recurrent glioblastoma with bevacizumab. Unfortunately, radiographic responses to bevacizumab tend to be short lived, and the overall impact on survival appears to be small at best (6–9). This has led to and will continue to produce a series of clinical trials evaluating the activities of combinations of anti-VEGF therapies with non–VEGF-targeted antiangiogenic agents (i.e., integrin inhibitors such as cilengitide), other signal transduction inhibitors, and immunotherapeutic approaches (28).

The challenge

Although bevacizumab has clearly been a step forward in the treatment of the recurrent glioblastoma, its use has raised a number of questions regarding how the drug is really working and the approach of antantiangiogenesis in glioblastoma itself (29). Despite the impressive radiographic response, it has been difficult to demonstrate a significant survival advantage with bevacizumab, raising the question of how much of the radiographic effects from bevacizumab are truly antiangiogenic versus merely a vascular stabilizing effect. Furthermore, as mentioned above, bevacizumab use is associated with a highly invasive pattern of recurrence consistent with preclinical data demonstrating that VEGFR inhibition induces an epithelial-to-mesenchymal–like transition (EMT) and tumor stem cell state (30). Indeed, glioblastomas that are recurrent after bevacizumab treatment appear to have a pan-resistant phenotype to almost all drugs tested to date (31). In addition, it is curious that other small-molecule inhibitors of VEGFR have shown little activity in this disease, as have monoclonal antibodies and small-molecule inhibitors of other angiogenic targets such as PDGFR and aVb3 integrin (32, 33). Thus, ongoing attempts to add second antiantiangiogenic agents to bevacizumab may not be promising because the patterns of recurrence do not appear to be primarily angiogenic. Rather, attempts to target the invasive phenotype and/or the biologic basis for the EMT/stem cell–like transition following VEGFR inhibition may be much more promising. The targeting of cMET, a primary mediator of glioma invasion and one induced following VEGFR inhibition, is one such example of this promising approach (34).

**Glioma Initiating/Stem Cells**

The promise

A growing body of literature suggests that all glioblastoma cells within a given tumor are not identical in either their genomic and/or epigenetic makeup and thus not in their biologic behavior, including their tumorigenic capability. Indeed, it appears that the glioblastoma cells that make up a tumor constitute a hierarchical structure spanning the spectrum from terminally differentiated cells to rapidly amplifying cells to cells with neural stem cell–like properties (GSC) that possess the capability of reconstituting the entire glioblastoma and all of its cell types (35). If true, then it would stand to reason that these cells must ultimately be destroyed if any long-term control, let alone cure, of glioblastoma is to be achieved. Thus, efforts have begun to identify small-molecular inhibitors and/or cytotoxic agents active against glioma stem cells as well as immunotherapeutic strategies targeted at antigenic epitopes found selectively on this stem cell population.

The challenge

Unfortunately, recent data suggest that these GSCs are particularly refractory to standard genotoxic insults such as conventional radiotherapy and chemotherapy (36). Nevertheless, it is possible that GSCs have their own specific vulnerabilities related to their dependence on several canonical stem cell signaling pathways, such as the Notch, Wnt, and Hedgehog (Hh) pathway. Although normal tissue-specific stem cells may likewise depend on one or more of these pathways, most normal differentiated cells do not, thereby opening the possibility of therapeutically targeting such pathways with acceptable side effects. Nevertheless, early clinical studies with γ-secretase inhibitors (notch inhibitors) have demonstrated significant gastrointestinal toxicity thought to be an on-target effect against colonic stem cells (37). A newer generation of Notch inhibitors is now being developed, as are HH inhibitors that will be tested alone and in combination with other targeted therapies. For example, trials of Hh inhibitors combined with PI3K inhibitors have been started in patients with recurrent glioblastoma based on preclinical data showing synergistic cytotoxicity of such combinations (38). Whether targeting canonical stem cell pathways will ultimately prove to be therapeutically useful for glioblastoma, patient selection will be of central importance because there appears to be great variability in which pathways are active in any one GSC population as opposed to another.

**Immunotherapy**

The promise

As much for the relative ineffectiveness of older treatments, as for the scientific rationale, there has been an interest in immunotherapeutic approaches for glioblastoma for more than two decades. Early efforts focused on cytokine administration, such as IL2 and interferons, resulting in CNS toxicities but no reproducible antitumor effects. More recently, the field has been dominated by efforts to induce antitumor-specific immunity using a variety of vaccine-type approaches (39). Such efforts have ranged from the very simple, such as vaccinating with lysates of the patient’s own tumor, to approaches as sophisticated as in vitro MHC loading of tumor-derived peptides onto isolated autologous dendritic cells (40). The field has been largely dominated by a series of isolated efforts that have not particularly built on prior experience or data, making interpretation of the promise of these strategic approaches difficult. Indeed, most of the proponents of immunotherapy for brain tumors point to as evidence for the antitumor activity and positive therapeutic benefit of such treatments the prolonged progression-free survival and/or overall...
survival of the relatively small cohort of patients treated in the phase I and II trials compared with historical controls. Needless to say, however, these conclusions are significantly confounded by selection bias of patients more likely to have better prognostic factors than patients in historical control groups. Another concern is that to date few, if any, vaccine approaches have consistently and reproducibly demonstrated significant radiographic reductions in tumor size from a vaccine intervention. Maybe the most promising observation suggestive of a true vaccine-mediated antitumor effect was the report of a significantly decreased number of EGFRV3-expressing glioblastoma cells in tumors that have recurred following long-term vaccination with an anti-VEGFR3 target vaccine (CDX-110), consistent with the phenomenon known as immunoediting (41). Nevertheless, one would think that if the vaccine was active enough to increase overall survival and destroy a predominant clonal population within a given tumor, one would see radiographic evidence of an antitumor response. In aggregate, however, at least this much has been learned and is encouraging. First, the often-stated proclamation that the CNS is an immunologic sanctuary has been shown to be untrue, and both the efferent and afferent arms of the immune system can be active against select antigens within the CNS. Furthermore, glioblastoma vaccines have generated evidence of immunogenicity and have been well tolerated to date with no evidence of induction of a CNS-associated autoimmune reaction (42). Most promising in the vaccine field is the fact that finally a number of randomized phase III trials are ongoing or will soon be initiated, so that we will have objective evidence of whether any of these vaccine approaches are truly affecting the natural history of the disease.

In addition to tumor vaccines, there is excitement in the potential of immune checkpoint modulators (anti–CTLA-4 and anti–PD-1 antibodies) based on the rather dramatic success they have generated in melanoma and, to a lesser extent, renal cell carcinoma and non–small cell lung cancer (43). Cautious optimism must be exercised, however, for in fact there is a paucity of preclinical data in adequate model systems that immune checkpoint inhibitors will generate significant anti-glioblastoma responses. Thus, the optimism for the success of these agents rests largely on circumstantial evidence, such as the demonstration that glioblastomas tend to express PD-L1 and brain metastases in melanoma have responded to such therapies (44). Nevertheless, issues related to the degree of BBB breakdown between glioblastoma and systemic metastases, the innate immunogenicity between these tumor types, and the presence of a glioblastoma-induced immunosuppressive microenvironment demonstrate the danger of extrapolating therapeutic success in one tumor type relative to another (45).

Finally, the development of chimeric antigen receptors (CAR), one of the newest, most sophisticated and potentially promising approaches to immunotherapy, is just beginning to be explored in glioblastoma. One such approach is using a CAR directed to the antigenic portion of the EGFRV3 fusion epitope, while another is attempting to develop CARs against CMV antigens that some claim are expressed in most glioblastomas (46). Issues related to access of the genetically modified, CAR-expressing immune effector cells to glioblastoma cells located behind an intact BBB and in an immune suppressive microenvironment, immunoediting, and the relative paucity of true glioblastoma-specific tumor antigens identified to date will be challenges to the ultimate success and overall utility of this approach.

The challenge

The field of immunotherapy for glioblastoma has been driven as much by faith in the promise of the strategy as by hard preclinical data. The field is in desperate need of a committed effort to better understand the biologic basis for the distinct interaction of the systemic immune systems with the CNS as well as unique aspects of the CNS immune response, including cells like microglia and reactive astrocytes. On the clinical side, the field needs to move past the all-too-common tendency to call an immunologic intervention promising based on an in vitro surrogate assay of immunologic responsiveness and/or improved patient outcome (e.g., prolonged progression-free or overall survival) when compared with historical controls. Such studies inevitably suffer from patient selection bias, making such comparisons unreliable at best. Experience from the anti–CTLA-4 and anti–PD-1 immunotherapeutic studies in melanoma and other systemic cancers have shown that when a truly active and effective antitumor immunologic response is generated, tumor shrinkage occurs. There is no reason to believe that it should not be seen in glioblastoma (albeit with the possibility of an initial worsening of visible on MRI due to immune cell infiltration). Thus, early-phase trials of new immunotherapeutic approaches should, in the future, be more rigorous in insisting on radiographic tumor response as a true indication of effective immunologic antitumor activity, and such strategic approaches should be moved into well-designed, placebo-controlled randomized phase III trials as soon as feasible.

Conclusions

The explosion in our understanding of the genetic and molecular biology of gliomas has led to a number of new strategic therapeutic approaches never previously envisioned. Initial trials using such approaches have generally been disappointing, but we are early on in the development of such therapeutics. A better scientific understanding of basic biologic principles of drug delivery to the CNS, immune surveillance in the CNS, more predictive imaging technologies for brain tumors, and novel clinical trial designs will all be necessary if we hope to realize the promise of this new biology.

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No potential conflicts of interest were disclosed.


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