New Strategies in Glioblastoma: Exploiting the New Biology

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Running Title: Exploiting the New Biology of Glioblastoma

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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 last two decades with median survival of only about 15 months despite aggressive neurosurgery, radiation and chemotherapy. Nevertheless, the last 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 yet to impact on the natural history of the disease or on patient long-term outcome. Nevertheless, it is hoped with time such approaches will lead to more effective treatments but issues such as the unique biology and anatomy of the central nervous system (CNS), impaired drug delivery, poor preclinical models with resultant non-predictive preclinical screening and poor clinical trial design potentially impede the rapid development of such new therapies. In this paper, we will review the excitement and challenges that face the development of effective new treatments that exploit this new biology.
Background

Despite years of research into its biology and countless clinical trials of new therapies, the prognosis for patients with GBM remains dismal (1). Newly diagnosed patients will often present with either new focal neurological 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 a MRI scan that reveals the tumor. Although a definitive diagnosis of GBM can never be made solely on the basis of an MRI scan, there are pathognomonic features of GBM that include T2 and FLARE signal abnormalities, gadolinium enhancement and sometimes mass effect all in a non-vascular distribution. The pathological correlates of these radiological findings include hypercellularity, heterogeneity and invasiveness of a glial cell type, nuclear atypia, endothelial proliferation and necrosis (2).

Although much has been learned recently regarding the biology of GBM over the last 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 least a biopsy for histological confirmation of the diagnosis. Furthermore, with improved intraoperative neurosurgical guidance technology, most patients can undergo a more extensive resection of their tumor with a reasonable margin of safety. Secondary to the diffuse invasiveness of the GBM, however, a truly complete
surgical resection can never be achieved since large portions of intervening and eloquent areas of normal brain would have to be removed along with the infiltrative tumors cells. Thus, fractionated radiation therapy has been the mainstay of GBM treatment for nearly thirty years (3). A series of clinical trials conducted over that time period has defined the optimal dose-fractionation schema and the target volume. Generally speaking patients are treated in fractions of 200 cGy/daily for 5 times per week for 30 fractions. Escalating the dose above this amount runs the risk of significant damage to normal brain tissue. The target volume has been refined over the years from whole brain radiation 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, the fact is that tumor recurrence is almost always within the initial radiation volume rather than at some distant site.

Despite optimal surgical resection of radiation therapy, most GBMs recur within 5-8 months after the completion of the radiation. A series of phase 3 trials conducted over the last few decades have suggested that the addition of some chemotherapy (most often an alkylating agent such as nitrosoureas) given either with or after the radiation therapy can cause a small, but statistically significant increase in survival. Although the choice of which drugs are optimal remained somewhat controversial, a randomized phase 3 trial of surgery/radiation 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 GBMs recur following standard treatment 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 GBM is, unfortunately limited with only the nitrosoureas demonstrating any kind of clinical benefit; and that is marginal (5). As will be discussed below, the antiangiogenic drug, bevacizumab, was recently approved for recurrent GBM 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 two thousand GBM patients 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 radiation became standard treatment of GBM over 4 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 is required.

**On the Horizon**

Any effective therapeutic strategy will have to consider at least four properties of GBMs that make them different than 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, GBMs reside in
eloquent and non-expendable normal tissue limiting treatment to those modalities that will not cause unacceptable long-term neuro-cognitive dysfunction. Third, GBMs reside within the central nervous system (CNS) often referred to as an immunological sanctuary secondary 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 secondary to the unique and relatively impermeable microvascular architecture within the brain known as the blood-brain barrier (BBB) (11).

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

**Signal transduction inhibition/targeted therapy**

*The promise.* Much has been learned over the last several years about the genomic makeup of GBM 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 GBMs 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, anti-sense 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 anti-apoptotic molecules that are often unregulated in p53 mutant cells (e.g., Bcl2) (15).

Along with global targeting of deregulated signaling pathway, there are ongoing efforts 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 GBM is the epidermal growth factor receptor (EGFR) which is amplified in about 60% of GBMs, 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 fibroblastic growth factor receptor (FGFR) (17-19). Other mutations that are not infrequently seen in certain subtypes of GBMs 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 (20). One mutation that appears particularly promising for therapeutic intervention is a gain of function mutation in the IDH1 and IDH2 genes that result in an “oncometabolite” and a downstream genomic hypermethylated genotype (“GCIMP) (21). The IDH1 gene is mutated in nearly 70-80% of low-grade gliomas and in about 7% of GBMs (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 GBM phenotype. Nevertheless, early data
with a new inhibitor of the mutated IDH1 gene product in multiply relapse and refractory acute leukemia looks potentially promising lending hope to its potential in GBM.

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 regularity network (GRN) of the GBM may be useful targets. For instance, gene expression analysis has been able to clearly define at least 4 major subtypes of GBM (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/EBPbeta and STAT3 are central nodes in the mesenchymal subtype of GBM and that genetic approaches to inhibit these nodes results in dramatic anti-tumor effects preclinically (24). Thus, there are significant efforts ongoing to define such nodes in specific molecular subtypes of GBM and 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 GBM (25). Why that is 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 GBM biology, GBMs can quickly find alternate signaling pathways overriding inhibition of the targeted signaling molecule, and/or the dramatic intratumoral heterogeneity found within gliomas allows for rapid selection for non-sensitive clones (26). Future efforts will need to build studies that will better define if the experimental 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.

**Anti-angiogenesis**

*The promise.* From the characteristic blush see on cerebral angiography to the endothelial proliferation prototypic and diagnostic of GBM, the vascularity and angiogenic nature of GBMs have been well known for decades. Indeed, a large number of preclinical trials have demonstrated the potential therapeutic benefit of inhibiting GBM-associated angiogenesis (27). Early pessimism in the approach secondary to the clinical failure of several small molecule inhibitors (angiostatin, endostatin, thalidomide) gave way to unbridled optimisms with the dramatic radiographic (and some times clinical) responses seen in recurrent GBM 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 produce a series of clinical trials evaluating the activities of combinations of anti-VEGF therapies with non-VEGF targeted anti-angiogenic 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 GBM, its use has raised a number of questions regarding how the drug is really working and the approach of anti-angiogenesis in GBM itself (29). Despite the impressive radiographic response it has been difficult to
demonstrate a significant survival advantage with the use of bevacizumab raising the question of how much of the radiographic effects from bevacizumab are truly anti-angiogenic 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 (EMT)-like and tumor stem cell state (30). Indeed, GBMs recurrent after bevacizumab treatment appear to have a pan-resistant phenotype to almost all drugs tested to date (31). Additionally, 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 αvβ3 integrin (32, 33). Thus, it seems that ongoing attempts to add second anti-angiogenic agents to bevacizumab may not hold the most amount of promise since the patterns of recurrence do not appear to be primarily angiogenic. Rather, attempts to target the invasive phenotype and/or the biological 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 (GSCs)**

*The promise.* A growing body of literature suggests that all GBM cells within a given tumor are not identical either in their genomic and/or epigenetic makeup and thus not in their biological behavior including their tumorigenic capability. Indeed, it appears that the GBM 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 (GSCs) that possess the capability of reconstituting the entire GBM 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 a GBM 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 suggests that these GSCs are particularly refractory to standard genotoxic insults such as conventional radiation and chemotherapy (36). Nevertheless, it is possible that GSC have their own specific vulnerabilities related to their dependence on several canonical stem cell signaling pathways such as Notch, Wnt and Hedgehog 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 potential possibility of therapeutically targeting such pathways with acceptable side effects. Nevertheless, early clinical studies with gamma secretase inhibitors (notch inhibitors) have demonstrated significant gastrointestinal toxicity thought to be an on target effect against colonic stem cells (37). Newer generation of Notch inhibitors are 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 begun in patients with recurrent GBM based on preclinical data showing synergistic cytotoxicity of such combinations (38). If targeting canonical
stem cell pathways will ultimately prove to be therapeutically useful for GBM, patient selection will be of central importance since it appears that there is great variability in which pathways are active in one GSC population 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 GBM for more than two decades. Early efforts focused on cytokine administration such as IL-2 and interferons resulting in CNS toxicities but no reproducible anti-tumor effects. More recently the field has been dominated by efforts to induce anti-tumor 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 data that proponents point to as evidence of both anti-tumor activity and positive therapeutic benefit are prolonged progression-free survival and/or overall survival of the relatively small cohort of patients treated in the phase 1 and phase 2 trials compared to 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. It is somewhat concerning 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 anti-tumor effect was the report demonstrating a significantly decreased number of EGFRv3 expressing GBM cells in tumors that have recurred following long term vaccination with an anti-VEGFRv3 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 s predominant clonal population within a given tumor, one would see radiographic evidence of an anti-tumor response. In aggregate, however, at least this much has been learned and is encouraging. First, the often-stated proclamation that the CNS is an immunological sanctuary has been shown to not be true and the both efferent and afferent arms of the immune system can be active against select antigens within the CNS. Furthermore, GBM 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 3 trials are ongoing or will soon be initiated so that once and for all we will have objective evidence of whether any of these vaccine approaches are truly impacting on the natural history of the disease.

In addition to tumor vaccines, there is excitement in the potential of immune checkpoint modifiers, anti-CTLA4 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 check point inhibitors will generate significant anti-GBM responses. Thus, the optimism for their success rest largely on circumstantial evidence such as the demonstrated that GBMs do tend to express the PD1-L and melanoma brain metastases have responded to such therapies (44). Nevertheless, issues related to the degree of BBB breakdown between GBMs and systemic metastases, the innate immunogenicity between these tumor types and the presence of a GBM-induced immunosuppressive microenvironment demonstrate the danger of extrapolating therapeutic success in one tumor type to another (45).

Finally the development of immunotherapies utilizing chimeric antigen receptors (CARs), one of the newest, most sophisticated and potentially promising approaches to immunotherapy, is just beginning to be explored in GBM. One such approach is using a CAR directed to the antigenic portion of the EGFRv3 fusion epitope whereas another is attempting to develop CARs against CMV antigens which some claim are expressed in most GBMs (46). Issues related to access of the genetically modified, CAR expressing immune effector cells to GBM cells located behind an intact BBB and in an immunosuppressive microenvironment, immunoediting and the relative paucity of true GBM-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 GBM 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 biological 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 often tendency to call an immunological intervention promising based on an in vitro surrogate assay of immunological responsiveness and/or improved patient outcome (e.g., prolonged PFS or survival) when compared to historical controls. Such studies inevitably suffer from patient selection bias making such comparisons unreliable at best. Experience from the anti-CTL4 and anti-PD1 immunotherapeutic studies in melanoma and other systemic cancers demonstrated that when a truly active and effective anti-tumor immunological response is generated, tumor shrinkage occurs. There is no reason to believe that it should not be seen in GBM (albeit with the possibility of an initial worsening of the MRI scan secondary to immune cells infiltration). Thus, early phase trials of new immunotherapeutic approaches should in the future be more rigorous in insisting in radiographic tumor response as a true indication of effective immunologic anti-tumor activity and such strategic approaches should be moved into well designed, placebo controlled randomized phase 3 trials as soon as feasible.

**Conclusion**

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 utilizing such approaches have generally been disappointing to date but we are early on in the development of such therapeutics. A better scientific understanding of basic biological principles of drug delivery to the CNS, immune surveillance in the CNS, more predictive imaging technologies of brain...
tumors, and novel clinical trial designs will all be necessary if we hope to realize the potential promise of this new biology.

**References**


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