Immunotherapy for Primary Brain Tumors: No Longer a Matter of Privilege

Peter E. Fecci1, Amy B. Heimberger2, and John H. Sampson1

Abstract
Immunotherapy for cancer continues to gain both momentum and legitimacy as a rational mode of therapy and a vital treatment component in the emerging era of personalized medicine. Gliomas, and their most malignant form, glioblastoma, remain as a particularly devastating solid tumor for which standard treatment options proffer only modest efficacy and target specificity. Immunotherapy would seem a well-suited choice to address such deficiencies given both the modest inherent immunogenicity of gliomas and the strong desire for treatment specificity within the confines of the toxicity-averse normal brain. This review highlights the caveats and challenges to immunotherapy for primary brain tumors, as well as reviewing modalities that are currently used or are undergoing active investigation. Tumor immunosuppressive countermeasures, peculiarities of central nervous system immune access, and opportunities for rational treatment design are discussed.

See all articles in this CCR Focus section, "Discoveries, Challenges, and Progress in Primary Brain Tumors."
Clin Cancer Res; 20(22); 5620–9. ©2014 AACR.

Disclosure of Potential Conflicts of Interest
J.H. Sampson is a consultant/advisory board member for CellDex Therapeutics and reports receiving a commercial research grant and licensing fees from CellDex Therapeutics for intellectual property related to the EGFRvIII peptide vaccine (CDX-110). A.B. Heimberger is a consultant/advisory board member for Bristol-Myers Squibb; holds patents on WP1066 and the immune modulatory miRNA portfolios; and reports receiving a research grant from Merck and licensing fees from CellDex Therapeutics for intellectual property related to the EGFRvIII peptide vaccine (CDX-110). No potential conflicts of interest were disclosed by the other author.

Editor’s Disclosures
The following editor(s) reported relevant financial relationships: S.E. Bates reports receiving a commercial research grant from Celgene via CRADA with NCI.

CME Staff Planners’ Disclosures
The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives
Upon completion of this activity, the participant should be able to identify the various modes of immunotherapy employed for primary brain tumors and to understand historical and contemporary challenges in brain tumor therapeutic testing and efficacy.

Acknowledgment of Financial or Other Support
This activity does not receive commercial support.

Introduction
In 2010, the FDA approved two immunotherapies, sipuleucel-T (PROVENGE; Dendreon Corp.; ref. 1) and ipilimumab (Yervoy; Bristol-Meyers Squibb) for the treatment of metastatic hormone-refractory prostate cancer and metastatic melanoma, respectively, ushering in a new era for cancer immunotherapy. The state of such approaches for primary brain tumors (most frequently glioblastoma) remains, by comparison, in its adolescence, sustaining the “growing pains” specific to the immunologic peculiarities of glioblastoma and the central nervous system (CNS). This review highlights the current context, clinical applications, and challenges to successful immunotherapy for primary brain tumors, focusing on glioblastoma.
Context: The (Fading) Question of Immune Privilege

In light of historical notions about CNS immune privilege, relying on a collection of seemingly "brain-banished" immune cells to deliver a strategic antitumor "smart bomb" would appear ill-advised. Such notions draw their origins from the studies of Medawar in the 1940s, in which allo- geneic skin grafts transplanted onto the brains of experimental animals escaped rejection (2). Subsequent CNS studies highlighted vague nascent antigen presentation, low HLA-expression, blood–brain barrier (BBB)–imposed restrictions for immune access, and absent lymphatic participation, all conjuring the singular perception of the brain as an immunologic void.

As early as the 1980s, revised views of the CNS as more "immunologically distinct" were increasingly advanced (3). Nascent CNS mechanisms for antigen uptake/transport, T-cell priming, and immune access are increasingly apparent and remain areas of interest for study. It is now accepted that intracerebral antigens move through cerebrospinal fluid in the subarachnoid space, along the olfactory nerve, and across the cribriform plate to the nasal mucosa, where they subsequently drain into cervical lymph nodes (CLN; refs. 4, 5). The CLN may be a requisite initiator to adaptive CNS immune responses, possessing unclear interplay with several brain-resident glial cells that have the capacity to mediate their own mode of HLA-restricted antigen presentation (6).

Regardless, T cells (and other immune effectors) must be granted access to the CNS to mediate these primed responses. Restrictions for such access are imposed by the BBB, which is designed to restrict the promiscuous transport of proteins and other molecules from the circulation to the parenchyma, and which also limits immune cell transit. The BBB likely does not represent the unpassable seal to immune cell trafficking initially purported, however (7). This is particularly true in instances of its disruption, often the case in the setting of glioblastoma (8, 9). Even when it remains undamaged, circulating immune cells are capable of penetrating an intact BBB to perform routine immune surveillance functions (10, 11).

Although the molecular events underlying immune trafficking to the CNS are still emerging (12), several studies have reported on the chemokines and adhesion molecules that may be critical (13), some proposing a 'CNS homing' phenotype that may be influenced by T-cell expression of the α4β1 integrin (14). Ultimately, the identity and phenotype of immune cells penetrating CNS tumors, the means by which they are not infrequently foiled, and the possibilities for enhancing their homing capacities and antitumor functionality represent important areas of investigation.

Clinical Applications: Immunotherapeutic Approaches to Glioblastoma

Employed immunotherapeutic modalities for glioblastoma now encompass a wide variety of approaches (Table 1; Fig. 1), the major categories of which are discussed below.

Surface-directed passive immunotherapies (antibodies and targeted toxins)

Antibody and targeted toxin therapies remain some of the oldest investigated immunotherapies for brain tumors (reviewed in ref. 15). The ultimate goal is specific binding of a molecule or receptor on the tumor surface, with the deployed agent serving in one of a number of defined capacities: as biologic response modifiers (i.e., EGFR blockade; ref. 16) or as delivery vehicles for tumoricidal toxins (i.e., diphtheria, Pseudomonas; refs. 17, 18) or radionucleotides (131I; ref. 19).

Many clinical trials have been conducted over the years, most of these being phase I/II studies. Classically, surface targets have included EGFR, tenascin, transferrin receptor, and the IL13 and IL4 receptors. The nonpermissiveness for large protein passage across the BBB often limits treatment delivery to intrathecal routes or directly into resection cavities, but some recent phase II successes are reported employing systemic antibody delivery to pediatric patients with diffuse intrapontine glioma (where delivery into a resection cavity is precluded; ref. 16).

This treatment mode is further limited by the passivity of the instigated immunity, with the duration of immune response tethered to the half-life of the agent delivered. Persistent treatment effects can develop, but likely depend on the recruitment of subsequent T-cell immunity. Some contemporary antibody therapies then aim to solicit and direct T cells not otherwise specific for tumor by employing bispecificity for a tumor target and the T-cell receptor [bispecific T-cell engagers (BiTEs)]. These agents remain in preclinical testing (20).

Adoptive lymphocyte transfer

Multiple strategies have looked to precipitate T-cell activation, with the most "simple" being direct enlistment of T cells via adoptive lymphocyte transfer. Here, autologous T cells are harvested, trained/expanded/activated ex vivo against tumor, and transferred back to patients either alone or in conjunction with other so-handled immune cells, such as dendritic cells. In its earlier renditions, adoptive lymphocyte transfer included the transfer of a variety of immune populations, not just T cells. These have included peripheral blood mononuclear cells (21), lymphokine/mitogen-activated killer cells (LAK; ref. 22), tumor-infiltrating lymphocytes (TIL; ref. 23), and cytotoxic T lymphocytes (CTL; refs. 24, 25), administered either systemically (preclinical data support tumor trafficking; ref. 26) or into the tumor cavity. Targets have varied, and newer renditions have combined adoptive lymphocyte transfer with active vaccination (27) and/or prior myelosuppressive regimens (ref. 28; NCT00693095) in efforts to promote survival and functional expansion of the transferred cells in vivo (active trials: NCT0114427, NCT01801852).

Beyond ensuring cell survival, an additional "rate-limiting step" for adoptive lymphocyte transfer therapy has been the generation of large numbers of functional tumor-specific T cells ex vivo. One solution has been the genetic modification of T cells to express a chimeric antigen receptor (CAR), which specifically binds to tumor antigens in an MHC-unrestricted
Table 1. Immunotherapeutic approaches to glioblastoma

<table>
<thead>
<tr>
<th>Class</th>
<th>Subtype</th>
<th>Target type</th>
<th>Comments</th>
<th>Active trials (examples)</th>
<th>References (examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Biologic modifier</td>
<td>Surface molecules/receptors (i.e., HER2, EGFR, tenasin). Can also be used to target other cells as with anti-CD25 and TR1.</td>
<td>Direct activity (i.e., receptor blockade or Fc-mediated cytotoxicity). Local administration more common, unless targeting suppressive immune cells.</td>
<td>NCT01475006, NCT00600054</td>
<td>(16)</td>
</tr>
<tr>
<td>Antibody/ligand</td>
<td>Toxin delivery</td>
<td>Surface molecules/receptors (commonly IL13R, IL4, and transferrin receptor)</td>
<td>Targets toxins to tumor. Common toxins have been altered with diphtheria and Pseudomonas toxins. Tumor linked to IL13 or IL4.</td>
<td>NCT00880061</td>
<td>(17, 18)</td>
</tr>
<tr>
<td>Antibody/ligand</td>
<td>Toxin delivery</td>
<td>Surface molecules/receptors (commonly EGFR and tenasin)</td>
<td>Targets radionucleotide to tumor, commonly 131I.</td>
<td>NCT00003478, NCT0002753 (both completed)</td>
<td>(19)</td>
</tr>
<tr>
<td>Adaptive lymphocyte transfer</td>
<td>Lymphocyte, immune effector</td>
<td>Whole tumor antigen, TAA(s), CMV</td>
<td>Difficult production, survival/persistence in vivo can be enhanced with concomitant vaccination or prior myeloconditioning.</td>
<td>NCT0114427, NCT01801852</td>
<td>(21–28)</td>
</tr>
<tr>
<td>Adoptive lymphocyte transfer</td>
<td>CARs</td>
<td>Whole tumor antigen, TAA(s), CMV</td>
<td>Chimeric antigen receptor links otherwise nonspecific T cells to tumor surface antigens. Still requires autologous lymphocyte harvests.</td>
<td>NCT01454596, NCT01109095, NCT00730613, NCT01082926</td>
<td>(29–31)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Tumor cell ± GM-CSF</td>
<td>Whole tumor antigens</td>
<td>Difficult production. Newer forms employ GM-CSF secreting bystander lines (i.e., K-562).</td>
<td>NCT00694330</td>
<td>(37, 38)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>DC</td>
<td>Whole tumor antigens, TAA(s), CMV</td>
<td>Multiple methods for production, loading, maturing and delivering, all relatively laborious and none standardized. Relies on nascent T cells for effect unless combined with adoptive lymphocyte transfer.</td>
<td>NCT01808820, NCT00045968</td>
<td>(39–50)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Antigenic/peptide(s)</td>
<td>TAA(s) (to date commonly EGFRVIII, IL13Ra2, survivin, EphA2, and WT1)</td>
<td>Rely on nascent DC and T cells to effect function. Scalable, &quot;off-the-shelf production.&quot; HLA-restricted use.</td>
<td>NCT02149225, NCT01920191, NCT02078648, NCT01480479</td>
<td>(41, 54, 55)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>HSPPC</td>
<td>Unidentified tumor peptides</td>
<td>HSPs shuttle peptides to MHC I, enhancing presentation. Antigens remain unidentified.</td>
<td>NCT02122822, NCT01814813</td>
<td>(58)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>DNA/viral</td>
<td>TAA(s), cytokine delivery, CMV</td>
<td>Virus used to coat DNA for gene delivery into and expression by APC.</td>
<td>Preclinical to date</td>
<td>(59–62)</td>
</tr>
<tr>
<td>Oncolytic virus</td>
<td>HSV, adenovirus, polio</td>
<td>Direct tumor cell lysis/immune recruitment</td>
<td>Avails of predilection for tumor. Some effects immune-mediated.</td>
<td>NCT02031965, NCT00931931, NCT02197169, NCT01491893</td>
<td>(63–65)</td>
</tr>
<tr>
<td>Immune checkpoint blockade</td>
<td>Anti–CTLA-4</td>
<td>Nonspecific T-cell activation, TR1 inhibition</td>
<td>Perpetuates T-cell activation. FDA approved for metastatic melanoma.</td>
<td>NCT02017717</td>
<td>(67–73)</td>
</tr>
<tr>
<td>Immune checkpoint blockade</td>
<td>Anti–PD-1/PD-L1</td>
<td>Nonspecific T-cell activation, TR1 inhibition</td>
<td>Perpetuates T-cell activation. May be better tolerated than anti–CTLA-4.</td>
<td>NCT02017717</td>
<td>(67, 72, 74–78)</td>
</tr>
</tbody>
</table>

Abbreviations: APC, antigen-presenting cell; HSPPC, heat shock protein-peptide complexes; HSV, herpes simplex virus; TAA, tumor-associated antigen.
fashion (29, 30). CARs are fusion genes comprised of a single-chain variable fragment (scFv) antibody or other extracellular domain recognizing the tumor-associated antigen (TAA) of interest, linked to intracellular signaling modules that mediate T-cell activation upon ligation of the CAR's extracellular domain. Upon gene transfer of the CAR into T cells (using viral vectors or electroporation; ref. 31), the transduced T cell acquires specificity for the targeted TAA, while retaining its endogenous TCR. As a result of this construct, use is limited to cell surface targets, such as IL13R, EGFRvIII, and HER2 (phase I/II trials are ongoing or recently completed: NCT01454596, NCT01109095, NCT00730613, NCT01082926).

Vaccines

Much of the immunotherapeutic work in glioblastoma to date has been vaccine based. Tumor vaccines encompass a broad range of approaches, including cell-based, antigenic, DNA, and viral-derived strategies. Most are intended as therapeutic modalities, initiated after tumor detection. The most prominent exceptions are cervical and hepatocellular carcinomas, in which the identification of human papillomavirus and hepatitis B etiologies, respectively (32, 33), confers the ability to vaccinate prophylactically against a cancer. Most cancers do not have an identified microbial precipitant, and the ability to vaccinate against a viral target is not similarly afforded. In the case of glioblastoma, the detection of tumor-borne cytomegalovirus (CMV) antigens has sparked debate about whether CMV might be etiologic or simply re-expressed/reactivated in an immunosuppressive local environment (34, 35).

Early tumor vaccines comprised "killed or inactivated" tumor cells, eventually genetically engineered to elaborate a variety of immune-stimulating cytokines, most famously,
granulocyte-macrophage colony-stimulating factor (GM-CSF; ref. 36). Versions of GM-CSF secreting tumor cell vaccines have been used for glioblastoma (37, 38), often revealing technical difficulties (38). Current generations are accompanied by an allogeneic tumor cell line (K-562) secreting GM-CSF. These vaccines have completed phase I testing, and results await publication (NCT00694330).

More commonly, vaccine-based therapies for glioblastoma have used dendritic cells (DC; refs. 39–50), and most of these treatments have demonstrated some level of efficacy in phase I/II studies. Definitive phase III evidence for efficacy remains lacking, however, and production is labor intensive and expensive, with nearly all generating DCs from peripheral blood monocytes with the aid of GM-CSF and IL4. DCs have been loaded/pulsed with synthetic versions of glioma-associated antigens/peptides (41, 51, 52); whole tumor cell lysates (40, 43, 45–48); or electroporated/pulsed/transfected with tumor cell or even tumor stem cell RNA (49, 50). After loading, DCs are often matured with a cocktail (often some combination of TNFα, IL1β, IL6, PGE2), or more recently with poly(I:C), a dsRNA mimic, before being delivered, typically intradermally. Presently, there are at least 11 open DC vaccine trials for adult and/or pediatric glioma in the United States (NCT0108820, NCT01792505, NCT2010606, NCT01902771, NCT01655283, NCT01204684, NCT01957956, NCT02049489, NCT00626483, NCT00049568, and NCT01522820), as well as an additional trial for medulloblastoma/PNET (NCT01326104).

In contrast to cell-based vaccines, "antigenic" vaccines involve the delivery of a protein or peptide antigen itself, often in conjunction with an immune-stimulating adjuvant. This is, in effect, an attempt at in vivo pulsing of nascent DCs. Advantages include scalable, "off-the-shelf" production but HLA restrictions and reliance upon potentially dysfunctional nascent immune cells impose limitations. Currently identified glioma-associated antigens (GAA) include IL13Ra2, HER2, gp100, TRP2, EphA2, survivin, WT1, SOX2, SOX11, MAGE-A1, MAGE-A3, APM2, SART1, and CMV proteins. In addition, EGFRvIII and the IDH1 WT1, SOX2, SOX11, MAGE-A1, MAGE-A3, AIM2, SART1, and CMV proteins have been loaded/pulsed with synthetic versions of glioma-associated antigens/peptides (41, 51, 52); whole tumor cell lysates (40, 43, 45–48); or electroporated/pulsed/transfected with tumor cell or even tumor stem cell RNA (49, 50). After loading, DCs are often matured with a cocktail (often some combination of TNFα, IL1β, IL6, PGE2), or more recently with poly(I:C), a dsRNA mimic, before being delivered, typically intradermally. Presently, there are at least 11 open DC vaccine trials for adult and/or pediatric glioma in the United States (NCT0108820, NCT01792505, NCT2010606, NCT01902771, NCT01655283, NCT01204684, NCT01957956, NCT02049489, NCT00626483, NCT00049568, and NCT01522820), as well as an additional trial for medulloblastoma/PNET (NCT01326104).

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To date, peptide vaccine trials in glioma have targeted WT1 (54, 55) and EGFRvIII (41), with ongoing trials targeting collections of GAA, including IL13Ra2, survivin, EphA2, and WT1 (NCT02149225, NCT01920191, and NCT02078648). A study targeting the same antigens in pediatric glioma continues to show tremendous promise and awaits publication (NCT01130077). One of the few phase III immunotherapy trials for glioma is an active study (NCT01480479) targeting EGFRvIII. "CDX110-04" is an international multicenter double-blind clinical trial of rindopepimut (EGFRvIII peptide vaccine; CellDex), in which approximately 700 patients with newly diagnosed, resected, EGFRvIII-positive glioblastoma, upon completion of standard chemoradiotherapy, are randomized to receive either rindopepimut/GM-CSF or control (keyhole limpet hemocyanin), in combination with standard adjuvant temozolomide.

A unique tumor cell-derived approach administers essentially multiple nonidentified peptides in the form of heat shock protein–peptide complexes (HSPPC). HSPPCs are stress-induced proteins that chaperone intracellular peptides from the proteasome to the endoplasmic reticulum, mediating transfer to MHC I. One such HSPPC using the tumor-isolated HSP glycoprotein-96 (gp-96; HSPPC-96; Vtespen, formerly Oncophage) has served as a vaccination platform in phase III trials for metastatic melanoma and renal cell carcinoma with no survival benefit observed (56, 57). A phase I study published for glioma in 2012 demonstrated safety as well as antigen-specific peripheral immune responses in 11 of 12 treated patients (58). Two further early-phase clinical trials are ongoing (NCT02122822, NCT01814813, and NCT00293423).

There are a variety of viral-based anticancer approaches being explored today for glioblastoma, ranging from immune-targeting antigen-delivery systems (59–61) to tumor-targeting suicide gene delivery vectors (62) to directly oncolytic viruses (63–65). The latter two strategies classically use viruses with specific tissue predilections, with the neural preferences for herpes simplex and polioviruses creating roles in glioma (reviewed in ref. 66). Viruses have also served as the antigenic target of interest, and as discussed above, studies have uncovered the selective re-expression of latent CMV proteins within glioma cells (34, 35), proffering a potent immunologic target. Multiple clinical trials targeting CMV are currently open (NCT00626483, NCT01109095, and NCT00693095).

**Immune checkpoint blockade**

The physiologic provisions for routine immunologic shutdown are termed "immune checkpoints" and are furnished by molecules on activated T cells, signaling via which precipitates their inactivation (CTLA-4) or even apoptosis (PD-1). Conversely, blockade or antagonism of these same molecules and their intracellular signaling pathways can potentiate T-cell responses, and even render them insensitive to tumor-mediated inhibition (67).

CTLA-4 blockade increases the availability of CD28 costimulation, thereby amplifying/perpetuating T-cell activation and either directly or indirectly inhibiting T_{reg} activity, as T_{reg}s similarly express CTLA-4 at high levels (68). Resultant T-cell activation is global and antigen nonspecific, creating a response that is potent, but not inherently "directed." Promising phase III results led to FDA approval of anti–CTLA-4 (ipilimumab) for patients with metastatic melanoma in 2010 (69). Although preclinical studies have proven extremely promising (70–72), multicenter clinical trials in glioblastoma are only now being initiated (NCT02017717). Clinical experience with CNS disease to date has been solely in patients harboring small intracranial melanoma metastases (73), experience which proved safe, yielding no instances of CNS autoimmunity.
Programmed death-1 (PD-1, CD279) is a member of the CD28 family expressed on activated T cells, B cells, dendritic cells, and macrophages (67, 74). PD-1 engages two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), both members of the B7 family. PD-L1 is expressed on a variety of immune and nonhematopoietic cells, whereas PD-L2 is restricted to myeloid cells. The PD-1 pathway functions to downmodulate inflammatory responses under physiologic conditions and may be exploited by cancers en route to immunologic escape. PD-1 is also highly expressed on T<sub>reg</sub>, and signaling enhances their suppressive function upon ligand engagement. The molecule is detected on a large proportion of TILs, and PD-1 ligands (especially PD-L1) are upregulated on the surface of numerous tumor types, including glioblastoma (67), a phenomenon linked to inferior clinical outcomes in a variety of cancers (75–77).

Clinical trials with anti–PD-1 (MDX-1106) and anti–PD-L1 (MDX-1105) monoclonal antibodies have been conducted in patients with various solid tumors with promising response rates (78). In some contrast to the results with anti–CTLA-4, anti–PD-1 mAbs appear to be better tolerated, although potentially lethal pneumonitis has been observed (67). Clinical trials of anti–PD-1 in glioblastoma are set to begin (NCT02017717) and will use a combination arm with ipilimumab, given an expectation for synergy (72).

Challenges: Designing, Effecting, and Monitoring Our Success

There is no step along the advance from planning to implementing to assessing immunotherapeutic deployment that does not pose a defined set of challenges to be acknowledged and met. Beginning with trial design, the relative infrequency of glioblastoma limits the obtainable power for single institution studies, which have dominated the landscape as phase I and II studies to date. Large phase III studies become similarly challenging to construct, and a search of clinicaltrials.gov reveals just three active phase III trials that can be classified as immune-based therapies (NCT00045968, NCT01759810, NCT01480479), all of which have required willing industry sponsors (Northwest Biotherapeutics, NeuroVita Clinic, CellDex). In addition, some trials target newly diagnosed patients, whereas others enlist patients with recurrence, the latter of whom have almost invariably undergone a variety of previous regimens, many with potential immunologic consequences. Even “newly diagnosed” patients will have typically seen dexamethasone, an established lymphocyte modulator and immunosuppressant. Therefore, trial design must standardize across such influences, as well as strive for multi-institutional recruitment.

Once implemented, immunotherapies face a unique set of contextual difficulties posed specifically by glioblastoma and the severity of its immunologic influence. Glioblastomas are now increasingly recognized as among the most immunosuppressive of solid tumors. Cellular immunity is particularly damaged, with T-cell deficits proving both profound and widespread (79). A thorough review of glioma’s capacities for soliciting immune compromise is beyond the scope of this account, although exists recently in the literature (3). A brief introduction is offered here, however.

Therapies aimed at stimulating T-cell immunity depend on some abundance of T cells, yet T-cell lymphopenia is one of the oldest documented immune shortfalls for patients with glioblastoma, harkening back to the studies of Brooks and colleagues in the 1970s (80). Often, lymphopenia has been attributed to the effects of treatment with chemotherapy (temozolomide) and dexamethasone, and while these undoubtedly contribute, increasing evidence is that they merely exacerbate a lymphopenia (particularly CD4) that is already present in a substantial number of treatment-naive patients (81). Investigations into the source of such lymphopenia are currently under way and yielding interesting results about compartmental T-cell redistributions.

Those T cells that do remain in the circulation are hampered by anergy (82, 83), IL2 system dysfunction (84), TH2-biased responsiveness (85), decreased NKG2D expression (86), and inhibition by disproportionate representations of suppressive regulatory T cells (T<sub>reg</sub>; ref. 81), all products of uniquely potent glioblastoma systemic influences and extrinsic mechanisms for immune escape. T cells that do manage activation and tumor trafficking find themselves faced with equally impressive local and intrinsic means of tumor evasion, including more T<sub>reg</sub> (87), indoleamine 2,3-dioxygenase expression (88), downregulated MHC and B7 family proteins (89, 90), increased PD-L1 (91), PTEN loss (92), STAT3 expression/activation (93), TGFB and IL10 production (94), MICA/B secretion (95, 96), and HLA-E expression (97), all of which serve to sidestep or directly undermine those immune cells present (Fig. 2). Our own sampling of TILs in glioma specimens yields phenotypes rich in CD95, PD-1, PD-L1, CTLA-4, LAG3, and Tim3, strongly indicating immune exhaustion, defined by poor effector function, sustained expression of inhibitory receptors, and an altered transcriptional state (98). We can therefore no longer be satisfied with simply “delivering” immune cells to target, but must better know the fate of those cells and arrive at standardized biomarker and radiographic surrogates/goals for realized immunity across studies. The question is no longer solely one of privilege.

Conclusions and Future Directions

Over the last three decades, tumor immunotherapy has forged forward with substantial strides, constituting a now legitimate and expanding mode of cancer therapy. Successful deployment against glioblastoma, however, requires increasing attention to the “immunologic idiosyncrasies” of gliomas and their microenvironment. We must acknowledge, understand, and counter the limitations imposed by relying on often impaired host cellular immunity to mediate our therapies in an immunologically “distinct” compartment. Such striving...
for immunologic potency, however, must be balanced by vigilance for autoimmune toxicities, particularly when choosing whole antigen approaches, as the brain is decidedly less tolerant of collateral inflammation than the prostate or skin. Conversely, these concerns must be weighed against fears for tumor immune escape when just a single or small number of antigens are targeted (99).

Immunotherapy is now poised to be a more ubiquitous component to the ever-emerging collage that will be personalized medicine. It will be the responsibility of immunotherapists, then, to determine its optimal place in the broadening context of complementary (or even cocanceling) therapies and tumor genetic backgrounds. Glioblastoma, as with cancer more generally, is now recognized as a constellation of genetically distinct diseases. The Cancer Genome Atlas project’s division of glioblastoma into proneural, neural, classical, and mesenchymal classifications highlights tumor phylogenies whose genetic makeup, patient characteristics, prognoses, and responses to traditional therapies all vary definitively (100). The immunophenotypes and efficacy of various immune-based therapies amidst the tumor classes remain almost entirely uncharacterized, however. Such characterization will be an important step to developing personalized treatment combinations predicated on pathologic diagnosis and the genomic technologies highlighted in the review by Gajjar and colleagues in this CCR Focus section (101) and therefore represents a vital future direction for glioblastoma immunotherapy.

Likewise, the revealing of glioblastoma subclasses may hold some relevance for understanding the differences between responders and nonresponders in immunotherapy trials, as well as between patients possessing normal versus defective cellular immunity (often strongly dichotomous). Practically speaking, this means that immunotherapy trials should begin to incorporate glioblastoma subclass and baseline immunophenotype into patient selection and grouping. Pretreatment factors such as lymphocyte count, steroid exposure, Treg fraction, and T-cell phenotype and responsiveness (as well as a variety of not yet determined immune markers) are likely to be just as important as (and possibly related to) proneural versus mesenchymal subtype in determining treatment responses and should constitute, at the very least, subgroup analyses in trials. Despite the challenges this will

Figure 2. Glioblastoma (GBM) immuno-evasive and -suppressive mechanisms. Glioblastoma uses a variety of mechanisms, both cell intrinsic and extrinsic, meant to sidestep or even directly counter host immune responses. Glioblastoma is pictured here as red cells amongst orange normal brain (glial cells). Inset on the left represents magnification of tumor, normal glia, and a CD8 T cell with typical exhausted phenotype. Glioblastoma cell-intrinsic mechanisms (visible on inset) include IDO expression [leading to recruitment of tumor-associated Tregs (black dotted arrow); ref. 88], downregulated MHC and B7 family proteins (89, 90), increased PD-L1 (91), PTEN loss (which can precipitate PD-L1 expression; ref. 92), STAT3 expression/activation (pleotropic immunosuppression; ref. 93), TGFβ and IL10 production (causing counterproductive TH2 shifts and elaborating Tregs; ref. 94), MICA/B secretion (inhibiting both T- and NK-cell activity; refs. 95, 96), and HLA-E expression (inhibiting NK cells; ref. 97). Cell-extrinsic mechanisms comprise effects on surrounding and systemic immune cells, and include lymphopenia and depressed cellular immunity. Patient T cells are hampered by anergy (82, 83), IL2 system dysfunction (84), TH2-biased responsiveness (85), decreased NKG2D expression (86), and inhibition by disproportionate representations of Tregs (81).
pose, it is the contextual understanding afforded that will permit us to move from simply proof of concept to a realizable goal of therapeutic efficacy.

Authors’ Contributions
Conception and design: P.E. Fecci, J.H. Sampson
Development of methodology: P.E. Fecci, J.H. Sampson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.B. Heimberger, J.H. Sampson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.H. Sampson
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References


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