Immunotherapy coming of age: What will it take to make it standard of care for glioblastoma?

Amy B. Heimberger and John H. Sampson

Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, Texas (A.B.H.); Division of Neurosurgery, Department of Surgery (J.H.S.), Preston Robert Tisch Brain Tumor Center (J.H.S.), Department of Pathology (J.H.S.), Duke University Medical Center, Durham, North Carolina

With the recent approval by the FDA of an immunotherapy for prostate cancer and another positive immunotherapy trial in melanoma, immunotherapy may finally be coming of age. So what will it take for it to become part of the standard treatment for glioblastoma? To put this question into perspective, we summarize critical background information in neuro-immunology, address immunotherapy clinical trial design, and discuss a number of extrinsic factors that will impact the development of immunotherapy in neuro-oncology.

Keywords: clinical trial, glioblastoma multiforme, immunotherapy, malignant gliomas.

Immune Privilege

The failure of immunotherapeutics to exert an effect against tumors within the brain has been attributed to “immunological privilege” secondary to the absence of a lymphatic drainage system within the brain, the presence of the blood–brain barrier (BBB), and a paucity of resident specialized antigen-presenting cells (APCs) within the CNS. However, all these premises have now been substantially discounted. For example, cerebrospinal fluid (CSF) has been shown to drain via the Virchow–Robbin spaces to the deep cervical lymphatics via perivascular sheaths and through the nasal submucosa. Antigens within the CNS enter the cervical lymph nodes by these routes and result in immune activation with a distinct hierarchy. This hierarchy is characterized by strong antibody responses and priming of cytotoxic T cell responses but an absence of delayed-type hypersensitivity (DTH) responses, with a skewing toward a nontumor Th2 phenotype. Although naive T cells are not found within the CNS, T cells and antibodies have access to antigens within the CNS, indicating that the BBB does not form an absolute barrier to immune responses. Activated T cells are permitted to patrol the CNS in an antigen-independent and apparently unrestricted manner and return to the systemic circulation. These cells exit through the cribriform plate and reach the nasal mucosa and, eventually, cervical lymph nodes. Evidence suggests that T cells that encounter their cognate antigen are retained within the CNS, but some studies suggest that they do not proliferate and instead undergo apoptosis. Other studies have shown the proliferation of antigen-specific T cells, specifically within tumors, and differentiation into cells with enhanced effector function. Microglia and macrophages have been shown to act as resident APCs within the CNS. Dendritic cells (DCs) are present in both the choroid plexus and meninges. CNS microglia with the phenotypic and functional characteristics of both macrophages and DCs express class II antigens and T cell costimulatory molecules that are capable of antigen presentation when not associated with tumors. Astrocytes, though capable of antigen presentation, are poor APCs and probably do not play a primary role in immune activation.

Based largely on the low globulin protein concentration within the CSF, it was generally believed that antibodies do not penetrate the BBB. However, antibodies do rapidly accumulate within the CSF and brain parenchyma after passive or active peripheral immunization in experimental animals and are distributed throughout the CNS according to kinetics similar to those in other peripheral organs, albeit at a ratio of...
apparent increase in CNS metastases, particularly the cause of this apparent increase in brain metastases in patients with HER2-positive breast cancer remain HER2 positive; therefore, it is not clear what amount of antibody that penetrates the CNS is sufficient to manifest an antitumor effect. Cumulatively, these data indicate that CNS metastases from breast cancer remain HER2 positive, with diminished responsiveness of T-helper cell activity. Many cancers, including gliomas, secrete factors such as prostaglandin E2, interleukin (IL)-10, vascular endothelial growth factor, and transforming growth factor (TGF)–β that are capable of suppressing cytotoxic responses of T cells against tumor targets, downregulating major histocompatibility complex (MHC) expression, suppressing T cell proliferation, and inhibiting the maturation of DCs. The absence or low expression of costimulatory molecules within the CNS also gives an immune escape advantage to cancer cells because costimulatory signals are essential for differentiation of functional tumor-specific CD8+ T-effector cells. Furthermore, the expression of costimulatory inhibitory molecules like B7-H1 that are expressed in malignant gliomas (especially with PTEN gene loss) can further inhibit immune responses.

Patients with cancer, and especially those with malignant gliomas, have a variety of heterogeneous, redundant mechanisms that contribute to their overall state of immune suppression, and these mechanisms serve as a barrier to effective immunotherapy. Generalized manifestations of immune impairment in these patients include low peripheral lymphocyte counts, reduced DTH reactions to recall antigens, impaired mitogen-induced blastogenic responses by peripheral blood mononuclear cells (PBMCs), and increased numbers of regulatory T cells (Tregs; reviewed in Dey et al. ). Primed CD8+ cytotoxic T cells gain CNS access; however, the lack of tumor eradication indicates that the T cells are functionally impaired. This has been confirmed with ex vivo studies demonstrating a lack of effector/activated T cells in the glioma microenvironment. More specifically, adaptive immune responses are noticeably deficient, with diminished responsiveness of peripheral T cells associated with impaired early transmembrane signaling through the T cell receptor/CD3 complex. In addition, reduced immunoglobulin synthesis by B cells in vitro from the peripheral blood of patients with intracranial tumors appears to be related to diminished T-helper cell activity. Many cancers, including gliomas, secrete factors such as prostaglandin 4

Immunosuppression

Patients with cancer, and especially those with malignant gliomas, have a variety of heterogeneous, redundant mechanisms that contribute to their overall state of immune suppression, and these mechanisms serve as a barrier to effective immunotherapy. Generalized manifestations of immune impairment in these patients include low peripheral lymphocyte counts, reduced DTH reactions to recall antigens, impaired mitogen-induced blastogenic responses by peripheral blood mononuclear cells (PBMCs), and increased numbers of regulatory T cells (Tregs; reviewed in Dey et al. ). Primed CD8+ cytotoxic T cells gain CNS access; however, the lack of tumor eradication indicates that the T cells are functionally impaired. This has been confirmed with ex vivo studies demonstrating a lack of effector/activated T cells in the glioma microenvironment. More specifically, adaptive immune responses are noticeably deficient, with diminished responsiveness of peripheral T cells associated with impaired early transmembrane signaling through the T cell receptor/CD3 complex. In addition, reduced immunoglobulin synthesis by B cells in vitro from the peripheral blood of patients with intracranial tumors appears to be related to diminished T-helper cell activity. Many cancers, including gliomas, secrete factors such as prostaglandin

~0.1%–5% of the titer of antibodies found in the serum. Despite this, it remains unclear as to what levels of antibody are sufficient to mediate effector functions in the brain, including antitumor effects, and how binding kinetics and antigen distribution affect these parameters. Soon after the approval of trastuzumab for HER2-positive breast cancer, concern arose over an apparent increase in CNS metastases, particularly in the context of excellent systemic control. This was taken as evidence that antibodies like trastuzumab cannot cross the BBB at levels sufficient to have a therapeutic effect, even in the context of metastatic intraparenchymal tumor or leptomeningeal disease. However, the cause of this apparent increase in brain metastases in patients with HER2-positive breast cancer is probably multifactorial. Subsequent studies have shown that trastuzumab does enter the CSF, but at significantly lower concentrations (~420:1), even in the context of whole-brain radiation therapy, which is thought to disrupt the BBB (76:1), or leptomeningeal disease (<49:1). Furthermore, PET imaging has recently shown directly that trastuzumab can penetrate brain metastases. However, what has also been shown is that CNS metastases from breast cancer remain HER2 positive; therefore, it is not clear what amount of antibody that penetrates the CNS is sufficient to manifest an antitumor effect. Cumulatively, these data indicate that tumors in the CNS should not be considered “off-limits” to immunotherapy and that the therapeutic nihilism surrounding the application of immunotherapy to primary and metastatic tumors of the brain needs to be eliminated.

Numerous immune population, including monocytes, have been shown to play a role in immune suppression. Generalized immune suppression is by attempting to resect as much of the tumor as is feasibly possible—an approach that has been used in several recent clinical trials (Table 1). This also provides the opportunity to minimize the patient’s dependence on immunosuppressive steroids, a possible confounding factor during active immunotherapeutic approaches. However, not all patients have disease that is amenable to surgery, and thus alternative approaches that can target key molecular hubs that mediate multiple mechanisms of immunosuppression need to be identified and targeted.
The signal transducer and activator of transcription 3 (STAT3) has been shown to be a potent regulator of anti-inflammatory responses by suppressing innate and adaptive immunity. The STAT3 pathway becomes constitutively active in diverse tumor-infiltrating immune cells, markedly impairing their effector responses. STAT3 also increases the functional activity of the immunosuppressive Tregs. Furthermore, we have recently demonstrated that the cancer stem cells are dependent on the STAT3 pathway in mediating immunosuppression that can be reversed with p-STAT3 inhibitors, indicating that this pathway is a molecular hub of tumor-mediated immunosuppression.

Targeting these molecular hubs is a paradigm shift from previous approaches that attempted to overwhelm the tumor with effector responses by now focusing therapeutic attention on controlling tumor-mediated immune suppression in a comprehensive, global fashion and perhaps allowing intrinsic recognition of tumor-associated antigen (TAA) and tumor-specific antigen (TSA). Interestingly, adult malignant gliomas appear to express more TAA and TSA than gliomas within pediatric patients, likely related to better immunologic reactivity (ie, less immune suppression) in the pediatric patient relative to the adult and makes the use of agents that can profoundly control tumor-mediated immune suppression therapeutically compelling. Thus, if we are going to be successful in treating patients with glioblastoma multiforme (GBM) with immunotherapy, we will also have to carefully consider and combat the matrix of immunosuppressive mechanisms operating in these patients.

**Clinical Trial Design**

With immunologically based therapeutics, a maximum tolerated dose cannot be identified in most cases. This is especially true with biologic agents in which there is a limitation to the amount of “drug” that can be feasibly generated. Thus, in many cases, what can be obtained during a phase I clinical trial of an immunotherapeutic is a maximum feasible dose. Furthermore, for noncytotoxic agents and most immunotherapeutics, efficacy and toxicity are often not clearly dose related. Added
to this is the practical matter that the chances of dose escalation are very high with standard 3 + 3 designs, even when the true rate of toxicity is quite high. Shown below is the probability of escalating from one dose to another as a function of the true unknown toxicity rate in a standard 3 + 3 trial:

<table>
<thead>
<tr>
<th>True toxicity rate (%)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of dose escalation</td>
<td>0.97</td>
<td>0.91</td>
<td>0.71</td>
<td>0.49</td>
<td>0.31</td>
<td>0.17</td>
</tr>
</tbody>
</table>

As can be seen from this chart, there is a >90% probability of further dose escalation even when the dose-limiting toxicity rate is as high as 10% in a 3 + 3 trial design. These toxicity rates have seldom been seen within the context of cancer immunotherapeutics. Thus, in the context of clinical trials of immunotherapeutics, there are little data to support a traditional phase I dose-escalation approach based on toxicity assessment alone.

An alternative goal of “dose-escalation” immunotherapeutic trials has been to determine the most effective dose. These studies usually list immune response as a primary endpoint because most investigators realize that the variability inherent in survival endpoints prohibits obtaining the answer to this question with a reasonable number of patients. Although a laudable goal, this is also often ill-conceived because of the variability in immune responses seen among patients as well as the small magnitude of the responses, which makes these assessments prohibitive in early-phase trials as well. For example, consider a clinical trial in which even a fairly dramatic doubling of immune response is sought between groups given different doses of a vaccine. Suppose the expected mean immune response in Group 1 is 1.0 U and that in Group 2 is 2.0 U. What sample size is required to demonstrate this difference? Consider the 4 following scenarios that assume a 2-tailed test conducted at the 0.05 level of significance with 80% power:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean response (U)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scenario #1</td>
<td>Scenario #2</td>
</tr>
<tr>
<td>One</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Two</td>
<td>2.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Sample size</td>
<td>6</td>
<td>24</td>
</tr>
</tbody>
</table>

Few (if any) immunotherapy studies demonstrate a doubling in mean immune response, and fewer still have such consistent responses that variability is reduced to the levels shown in the above table. As a result, large cohorts of patients will be required at each dose to obtain meaningful data that are sufficiently powered. As such, we do not recommend that the immunotherapeutic clinical trials be devised to detect differences in immune response with different doses of an immunotherapeutic.

Most of the phase II immunotherapeutic clinical trials conducted to date typically enroll small numbers of patients with unique eligibility criteria (Table 1) that preclude robust analysis of confounding prognostic variables because of insufficient statistical power and the lack of robust databases such as those that are maintained by the FDA for other diseases. Although comparisons with such databases using a historical cohort matched to enrollment criteria and prognostic variables may have some value, in the last 10 years, in part owing to the introduction of new agents and our more aggressive care of this patient population, the outcome for patients with GBM has rapidly shifted. For example, in the definitive clinical trial supporting the efficacy of temozolomide (TMZ) in patients newly diagnosed with GBM, the median survival time was initially reported to be 15 months. However, more contemporaneous clinical trials evaluating the efficacy of TMZ have demonstrated a median survival interval of 18.2 months. Although one could argue that these differences may be attributed to subtle differences in the timing of administration of the TMZ (concomitant with and after radiation therapy vs strictly after radiation therapy), these differences probably reflect changes in the treatment regimens of these patients, especially upon tumor recurrence. The survival benefit of more intensive medical intervention is further supported by an analysis of the median survival time of GBM patients who, based only on the criterion of being enrolled in a clinical trial regardless of the treatment agents, had a median survival time of 19.6 to 21 months. Thus, because the historical cohort consists of retrospective data, its use as a comparative population may not reflect current treatment responses and could convey a false sense of response unless the databases are large, homogeneous, and tightly regulated. We therefore recommend that given the recent shift in the survival time of GBM patients, randomization to a control standard-of-care arm (or an equivalent) is essential during phase II testing.

It is apparent that immunotherapy has been more successful in other tumors than GBM. For example, 2 immunotherapeutic agents, interferon (IFN)-α and IL-2, have long been approved by the FDA for stages II and III melanoma patients who had relatively low malignancy burdens based on the response rates of 10%–33% and prolongations in survival. More recently, immunotherapy has been shown to be efficacious even in advanced prostate cancer and unresectable stage III and IV melanoma, indicating that significant tumor burden can be overcome using immunotherapy approaches. What remains unclear is whether the degree or types of operational mechanisms of immune suppression are fundamentally different between malignant gliomas and other types of malignancy. As we move forward in the design of immunotherapeutic clinical trials, we recommend stratifying our patients based on the amount of residual disease and on the operational mechanisms of immunosuppression that are occurring. For example, it would seem appropriate to stratify patients according to the Treg fraction for clinical
trials testing anti-Treg approaches.\(^8^9\) This would serve to identify those patients likely to benefit from those agents rather than patients who are relying on other mechanisms of immune suppression. Furthermore, Parsa et al.\(^{45}\) have suggested that patients who have lost tumor suppressor PTEN function and thus have upregulated B7-H1 may not be suitable candidates for active immunotherapeutic approaches at all. In the future, factors that may predict immunotherapeutic resistance—such as B7-H1 expression, Treg level, PTEN deletion, STAT3 phosphorylation, and CD133 expression—could be used as part of an immunosuppressive genetic signature\(^9^0–9^2\) to stratify patients enrolling in immunotherapeutic clinical trials. Thus, we recommend that in future immunotherapeutic clinical trials, patients be stratified based on residual disease and that the evaluation of markers that may reflect immunotherapeutic resistance (such as B7-H1, Tregs, PTEN, p-STAT3, and CD133 expression) be included in the context of a secondary endpoint or as stratification variables in the trial design.

**Immune response monitoring**

To date, no T cell–based immune response measure has been universally validated in cancer immunotherapy (Table 2).\(^9^4–1^0^6\) This is partly because of the lack of standardization or even definitive agreement on prioritization of these assays. It is also likely that evaluating a single immune cell population will be insufficient because antitumor immune responses are probably an orchestrated effort among a variety of immune cell populations that are not captured in popular rudimentary assays. Attempts to resolve this issue have included ascertaining polyvalent immune responses using multiparameter flow analysis. These assays, while accounting for more global immune responses, may also still reflect the functional status or overall immune responsive nature of a subset of patients and only in a specific compartment at a specific time.

The best data available for defining an immune surrogate with clinical response come from infectious disease studies\(^1^0^1–1^0^3\) in which an increasing proportion of polyfunctional T cells, T cells that simultaneously secrete IFN-\(\gamma\), tumor necrosis factor-\(\alpha\), and IL-2 along with coordinated expression of CD107\(\alpha\) as a marker for cytotoxicity, prospectively predict long-term nonprogressors in patients with human immunodeficiency and cytomegalovirus (CMV) infection.\(^1^0^1,1^0^4,1^0^5\) Increased numbers of polyfunctional antitumor T cells have also predicted improved antitumor efficacy, albeit only in animal models to date.\(^1^0^6,1^0^7\)

In addition to polyfunctional T cell responses, there may be some hints in support of other immune surrogates in cancer. For example, Wheeler et al.\(^1^0^8\) showed a correlation between IFN-\(\gamma\) production and survival. In the phase III study that demonstrated the efficacy of sipuleucel-T (PA2024) (Provenge; Dendreon), the stimulation index of fresh T cells in response to antigen was \(\sim 8\)-fold higher in the treatment group than in the controls.\(^8^6\) Of note, we conducted a similar analysis on the peptide-based vaccine targeting epidermal growth factor receptor vIII and used this same measure of immune response and were widely criticized during peer review.\(^1^0^9\) In addition to the lack of consistency and validation in T cell–based immunologic monitoring, immune monitoring has generally neglected other immune effectors such as monocytes, natural killer cells, and antibody-dependent cellular cytotoxicity, which may in fact be the mechanism of in vivo activity that should be more comprehensively evaluated in the context of these trials. In the interim, we should focus on validated surrogate immune markers from other fields and aggressively attempt to validate them in this field.

**Toxicity and adverse events**

The lack of significant toxicity, such as autoimmunity–associated, with immunotherapeutic approaches for GBM may indicate that insufficient immune responses are being generated for tumor eradication. In the case of melanoma immunotherapeutics, autoimmune responses have been shown to correlate with treatment response for both IL-2 and IFN-\(\alpha\).\(^1^1^0,1^1^1\) Although autoimmunity is not a perfect correlate of success in immunotherapy for melanoma, such autoimmune responses do indicate that robust immune responses against the target cell type can be obtained. A recent case of a patient with a brain metastasis from melanoma treated with cytotoxic T lymphocyte antigen–4 antibodies demonstrated that when strong immune responses against brain antigens are unleashed, the immune response will probably have side effects.\(^1^1^2\) Similar adverse events were seen in the immunization trials against amyloid-\(\beta\) in the treatment of Alzheimer’s disease in which 15% of patients developed severe encephalitis induced by T cells. Examination of the patients’ brains appeared to indicate that the inflammatory response was able to clear typical Alzheimer’s neuropathology.\(^1^1^3\) Thus, if adverse events are an indication of strong effective immune stimulation, its absence from clinical immunotherapy trials could be of significance. However, enthusiasm for generating significant effector responses needs to be counterbalanced with the consideration that an expanding mass of inflammation within the relatively closed compartment of the CNS could result in herniation or fatal autoimmunity against CNS antigens.\(^1^1^4\) These adverse autoimmune events could also be reflective of the lack of tumor specificity. Thus, we recommend targeting TSA in the context of approaches that generate robust immune effector responses. However, this specificity can limit the durable response by the development of antigen negative tumor clones.\(^1^1^5\)

**Extrinsic Factors**

We are in the midst of a transformation in the pharmaceutical industry, and it is one that may not be favorable for the development of novel therapeutics for primary brain tumors. Although the approval of TMZ...
<table>
<thead>
<tr>
<th>Immune assay</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DTH</strong></td>
<td>Easy to perform</td>
<td>Cutoff for positive response not standardized—subjective</td>
</tr>
<tr>
<td>Injection of an antigen intradermally and measurement of erythema and/or in duration after 48–72 hours</td>
<td>May correlate with T cell proliferative responses¹³⁰</td>
<td>Amount of antigen not standardized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May not be antigen specific¹³¹,¹³²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be a surrogate marker for better performance status/less immune suppression</td>
</tr>
<tr>
<td><strong>Peptide MHC tetramers</strong></td>
<td>May correlate with cytotoxicity assays¹³³</td>
<td>Antigen must be known</td>
</tr>
<tr>
<td>Soluble, fluorescently labeled, MHC-peptide complex that binds to antigen-specific T cells</td>
<td>May correlate with T cell avidity for antigens¹³⁴</td>
<td>Tetramer positive cells may not kill target¹³⁵</td>
</tr>
<tr>
<td></td>
<td>Can be used to select antigen-specific T cell for further analysis¹³⁶</td>
<td>Only MHC I tetramers are available routinely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May bind both naïve and memory T cells¹³⁷</td>
</tr>
<tr>
<td><strong>Lymphoproliferative assay</strong></td>
<td>Can be performed directly on peripheral blood samples</td>
<td>In vitro culture conditions can alter results</td>
</tr>
<tr>
<td>Purified T cells or PBMCs are stimulated with antigen in the presence of irradiated autologous APCs. After 72–100 hours, proliferation is measured and compared with the proliferative index of cells without antigen</td>
<td></td>
<td>May reflect the overall immune suppression state of patient</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High proliferation by a few cells or low levels of proliferation by a few cells would give a similar stimulation index</td>
</tr>
<tr>
<td><strong>ELISA/multiplex flow cytometric assay</strong></td>
<td>Easy to perform</td>
<td>Definition of positive results differs</td>
</tr>
<tr>
<td>PBMCs are incubated with an antigen. Then the supernatant from the culture is harvested, and specific cytokines are detected</td>
<td></td>
<td>Is not based on individual cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Measures the ability of the cells to secrete cytokines and not necessarily the in vivo characteristics</td>
</tr>
<tr>
<td><strong>ELISPOT</strong></td>
<td>Reliably detects the number of antigen-specific T cells¹³⁸</td>
<td>Cell viability is lost; so unable to perform subsequent functional assays</td>
</tr>
<tr>
<td>A microtiter plate is coated with a cytokine-specific antibody and then incubated with the T cells. Each spot represents a single cell secreting the cytokine of interest. Precursor frequency is determined by the total number of cells placed into the wells</td>
<td>Can be rapidly read with computerized plate readers, making it suitable for a large-scale study</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intracellular cytokine detection by multiparameter flow cytometry</strong></td>
<td>Can evaluate multiple immune populations simultaneously</td>
<td>Cell viability is lost; so unable to perform subsequent functional assays</td>
</tr>
<tr>
<td>In vitro T cell stimulation followed by prevention of cytokine secretion, surface staining, fixation, permeabilization, and staining with antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cytotoxicity assays</strong></td>
<td>Thought to be relevant marker for in vivo antitumor activity</td>
<td>Requires in vitro stimulation that may alter the activity from the in vivo state</td>
</tr>
<tr>
<td>Mix T cells or PBMCs with labeled antigen, expressing and measuring release of the target</td>
<td></td>
<td>Because autologous tissue is difficult to obtain, other targets are used that may not reflect tumor biology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Does not measure perforin, granzymes, or Fas-Fas-ligand cytotoxic killing—only direct cytotoxicity</td>
</tr>
</tbody>
</table>
demonstrated that drugs used for GBM can be profitable, this enthusiasm may not be sustained. According to Munos,116 pharmaceutical companies have on average developed only one new drug or biologic agent per year over the last 60 years. As a result, new drugs must be priced tremendously high and serve a large patient population to meet investors’ expectations, which means few, if any, drugs or biologics developed specifically for GBM will appear attractive. Moreover, large pharmaceutical companies, which have the capital to perform the required large and expensive phase III trials, are shifting their focus from early discovery and development to marketing. This is partially in response to demands from investors for nearer-term returns, but it leaves a significant void in early development that cannot be met by the current funding levels available to investigators from the National Institutes of Health. Even the cost of providing data for an initial investigational new drug application can mean an insurmountable financial burden (usually in the range of $1–2 million or more) for the investigator. This frequently does not even include manufacturing of the agent. Added to this is the cost for the initial clinical trials. Even for simple, off-the-shelf immunotherapeutics such as a peptide vaccine, the cost can be as high as $20 000 per patient/year in addition to the standard of care; thus, for a small and probably insufficiently powered clinical trial enrolling 25 patients, the cost to the investigator could be >$500 000.117 The price tag further escalates if the trial extends to other institutions because an extensive data and regulatory infrastructure needs to be put in place.

The increasing complexity of conducting clinical trials in the United States is further confounded by the increasing legal issue of indemnification. Multi-institutional clinical trials are advantageous because they rapidly enroll patients and may reduce institutional bias; however, negotiating participating institution indemnification can result in unexpected delays and further escalate clinical trial costs. Increasingly, as a result of this and other financial pressures, pharmaceutical companies are conducting clinical trials outside the United States.118 In fact, in just under 10 years (1997–2007), the percentage of clinical trials registered with the FDA to be conducted in the United States dropped from 86% to 57%, whereas sites in places such as India and China rose from 5% to 29%.119 As a result, very creative strategies will be needed to translate any immunotherapeutic for GBM beyond the dubious single-institution early-phase trial and to demonstrate sufficient efficacy to attract the interest of companies with sufficient resources to bring these agents to market. These extrinsic factors pose a real threat to the emergence of a standard-of-care immunotherapy for GBM and will need to be addressed creatively.

Conclusions and Recommendations

Although the benefits of immunotherapy are becoming evident in other fields, its use in neuro-oncology remains limited. To advance these promising approaches toward a standard of care will require that lessons learned from basic science investigations in neuro-immunology and immunotherapy in other fields be applied creatively and cost-effectively. A number of conclusions and recommendations can be derived from our review.

1. Immune responses exert therapeutic effects within the CNS; therefore, immunotherapy is a viable approach to CNS malignancies.

2. Although tumors within the CNS are not completely protected from an immune attack, sufficiently potent immune responses need to be generated to overcome profound immunosuppression, or the immunosuppression has to be minimized by tumor resection or with agents that target tumor-mediated immunosuppression globally or at key molecular hubs. Successful approaches will probably incorporate both.

3. Clinical trial design should be carefully considered. Traditional paradigms may not be informative. An immune response endpoint may not yield meaningful results. Given the recent shifting in GBM patient survival time, randomization should be strongly considered even during early testing. Furthermore, the selection criteria during early phases of clinical trial testing should be the same as in the final registration clinical trial. Immunotherapeutic prognostic markers need to be identified and accounted for as secondary endpoints.

4. Immunologic surrogates that predict the efficacy of immunotherapeutic approaches in cancer are not currently available but are desperately needed. Clues as to which responses are important may come from existing studies or from infectious disease investigations and may not be as expected. Effective antitumor immune responses may require coordinated actions among several components of the immune system, all of which may need to be monitored. Immune monitoring results will become useful only when they are standardized and prospectively validated.

5. New and creative development and marketing paradigms will definitely be needed if we are to achieve translation of immunotherapeutics for brain tumors into widely used therapeutics.

Acknowledgments

We thank Jim Herndon II, PhD, for statistical assistance. We thank Audria Patrick and David Wildrick, PhD, for editorial assistance.

Conflicts of interest statement. The authors and their respective institutions of The University of Texas MD Anderson Cancer Center and Duke University have received consulting fees, stock options, and licensing fees from Celldex Therapeutics and Pfizer.
**Funding**

Funding support came from the following grants: NIH –(5R01-CA133272-02, 3R01-CA133272-02S1, SP50-CA108786-03, SP50-CA127001-03, 1R25-NS065731-01, SP50-NS020023-27, 5R21-CA132891-02, 3R21CA132891-02S1, R01-CA097222, R01-CA097611, P50CA108786, R01-CA1208113, R43 AI 77225-01), Golfer Against Cancer, National Brain Tumor Foundation Translational Research Grant, Neurosurgery Research and Education Foundation, Shawn Hansen/American Brain Tumor Association, Adam Sliger Research Fund, Goodwin Fund for Clinical Trials Grant, National Brain Tumor Society, Dr Marnie Rose Foundation, Anthony Bullock III Foundation, PBTFUS, The Brain Tumor Society, Congress of Neurological Surgeons, Southeastern Brain Tumor Foundation, Accelerate Brain Cancer Cure/American Brain Tumor Association, AANS/CNS, and the Chandran Research Award.

**References**

23. Lowe J, MacLennan KA, Powe DG, Pound JD, Palmer JB. Microglial cells in human brain have phenotypic characteristics related to possible function as dendritic antigen presenting cells. J Pathol. 1989;159:143–149.


114. Wikstrand CJ, Bigner DD. Hyperimmunization of non-human primates
113. Orgogozo JM, Gilman S, Dartigues JF, et al. Subacute meningoence-
116. Munos B. Lessons from 60 years of pharmaceutical innovation.
118. Nundy S, Gulhati CM. A new colonialism?—conducting clinical trials in
123. Mitchell DA, Archer G, Bigner D, et al. RNA-loaded dendritic cells tar-
lysat-pulsed dendritic cells elicits antigen-specific, cytoxic T-cells in
poly-ICLC with radiation for adult patients with newly diagnosed supra-
tentorial glioblastoma: a North American Brain Tumor Consortium
patients with recurrent malignant glioma: preliminary results of using
autologous whole-tumor vaccine plus granulocyte-macrophage
colony-stimulating factor and adoptive transfer of anti-CD3-activated
130. Disis ML, Schiffman K, Gooley TA, McNeel DG, Rinn K, Knutson KL.
 Delayed-type hypersensitivity response is a predictor of peripheral
131. McNeel DG, Schiffman K, Disis ML. Immunization with recombinant
human granulocyte-macrophage colony-stimulating factor as a
vaccine adjuvant elicits both a cellular and humoral response to recom-
binant human granulocyte-macrophage colony-stimulating factor.
peptide-pulsed mature, monocyte-derived dendritic cells expands
descriptive cytotoxic T cells and induces regression of some metastases in
134. Yee C, Savage PA, Lee PP, Davis MM, Greenberg PD. Isolation of high
frequency antigen-specific cytotoxic T lymphocytes from peripheral blood.
Direct isolation, phenotyping and cloning of low-frequency antigen-
specific cytotoxic T lymphocytes from peripheral blood. Curr Biol.
1998;8:413–416.
Melan-A/MART-1-specific CD8 (+) T cells in a large proportion of
human histocompatibility leukocyte antigen (HLA)-A2 individuals.
136. Schmittel A, Keilholz U, Scheibenbogen C. Evaluation of the interferon-
gamma ELISPOT-assay for quantification of peptide specific T
lymphocytes from peripheral blood. J Immunol Methods. 1997;210: