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

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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"[1] 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.[2,3] 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[4] via perivascular sheaths and through the nasal submucosa.[5–7] Antigens within the CNS enter the cervical lymph nodes by these routes[8] and result in immune activation with a distinct hierarchy.[9] 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.[6,8–10] Although naïve T cells are not found within the CNS, T cells and antibodies have access to antigens within the CNS,[11–13] 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[14] and return to the systemic circulation. These cells exit through the cribriform plate and reach the nasal mucosa and, eventually, cervical lymph nodes.[15] Evidence suggests that T cells that encounter their cognate antigen are retained within the CNS,[16] but some studies suggest that they do not proliferate and instead undergo apoptosis.[17,18] Other studies have shown the proliferation of antigen-specific T cells, specifically within tumors, and differentiation into cells with enhanced effector function.[16] Microglia and macrophages have been shown to act as resident APCs within the CNS.[19] Dendritic cells (DCs) are present in both the choroid plexus[20] and meninges.[21] CNS microglia,[22] with the phenotypic and functional characteristics of both macrophages and DCs,[23,24] express class II antigens and T cell costimulatory molecules[24–26] 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.[17]

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[18] and are distributed throughout the CNS according to kinetics similar to those in other peripheral organs, albeit at a ratio of

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~0.1%–5% of the titer of antibodies found in the serum.[27–30] 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,[31,32] 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).[29,30] Furthermore, PET imaging has recently shown directly that trastuzumab can penetrate brain metastases.[33] 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.*

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.[34]). Primed CD8+ cytotoxic T cells gain CNS access[14,35]; 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 microevironment.[36] 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 E_2, 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,[37–39] and inhibiting the maturation of DCs.[40] The absence or low expression of costimulatory molecules within the CNS[36] also gives an immune escape advantage to cancer cells because costimulatory signals are essential for differentiation of functional tumor-specific CD8+ T-effector cells.[41–44] 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.[45]

Many studies have demonstrated that Tregs are responsible for inhibition of tumor reactive effector T cells, and the elimination of Tregs by any of several different strategies successfully enhances antitumor immunity mostly in murine models.[46–50] Similarly, CD4+CD25+FoxP3+ Treg-mediated suppression has also been demonstrated in several human cancers with increased numbers of these cells.[51–54] Collectively, these data indicate that Tregs cannot only inhibit the initial systemic immune activation but also prevent the effector responses in the tumor microenvironment. Increasingly, other immune populations such as immunosuppressive myeloid cells[55] and M2 macrophages[56,57] within the tumor microenvironment are being shown to participate and mediate tumor immunosuppression.

A variety of investigators have also shown that cancer stem cells suppress immune response,[58–61] indicating that this is probably a generalized property of pluripotent stem cells. Distinct pathways and mechanisms that are used by the cancer stem cells, if they exist, will need to be clarified to specifically target cancer stem cell–mediated immunosuppression. It may be possible to vaccinate against cancer stem cells through the use of immunologic manipulations that enable the generated effector responses to overwhelm the immunosuppressive capacity of the tumor.[62–64] Nevertheless, the grim reality is that recurrence and persistence are hallmark features of gliomas, and in most circumstances, the intrinsic immune system of the patient, unaided, is unable to eradicate the cancer stem cells that are the progenitors that give rise to recurrence and progression. Thus, immunosuppression predominates in patients with high-grade gliomas and poses barriers for successful antitumor immunotherapy.

A rudimentary way to overcome this tumor-mediated immunosuppression 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.

Table 1. Clinical efficacy data from representative immunotherapy clinical trials

Agent delivered/site	Phase	Sponsor or centers involved	Results
PEP-3-KLH + GM-CSF (ACTIVATE)/ systemic[120]	II	Duke University Medical Center/ The University of Texas MD Anderson Cancer Center/ Celldex	Median survival time = 2.4 years; newly diagnosed; $n = 23$
PEP-3-KLH + GM-CSF with TMZ (ACT II)/ systemic[121]	II	Duke University Medical Center/ The University of Texas MD Anderson Cancer Center/ Celldex/Pfizer	Median survival time = 1.9 years; newly diagnosed; $n = 21$
DCs + PEP-3-KLH/systemic[109]	I/II	Duke University Medical Center	Median survival time = 1.8 years; newly diagnosed; $n = 14$
Personalized peptide vaccines (4)/systemic[122]	I	Nigata University	Median survival time = 1.7 years; recurrent GBM; $n = 17$
DCs + CMV systemic[123]	II	Duke University Medical Center	Median survival time not reached yet but will exceed 1.6 years; newly diagnosed GBM; $n = 13$
DCs + autologous tumor lysates[124]	II	University of Leuven and Wurzburg	Median survival time from relapse = 0.8 years; recurrent GBM; $n = 56$
DCs + acid-eluted tumor peptides/ systemic[125]	I/II	Cedars Sinai Medical Center	Median survival time = 1.3 years; newly diagnosed GBM; $n = 7$
DCs + tumor homogenate/systemic[108]	II	Cedars Sinai Medical Center	Median survival time = 1.8 years for immune responders vs 1.2 for nonimmune responders; newly diagnosed GBM; $n = 11$; median survival time = 1.6 years for immune responders vs 1.1 for nonimmune responders; recurrent GBM; $n = 21$
DCs + acid-eluted tumor peptides/ systemic[126]	I	UCLA	Median survival time = 2.0 years; newly diagnosed and recurrent GBM patients; $n = 12$
Autologous tumor cells with TGF-β2 antisense/systemic[127]	I	Advanced Biotherapies/NovaRx	Median survival time = 1.4 years; progressing GBM patients at enrollment; $n = 6$
Poly-ICLC/systemic[128]	II	North American Brain Tumor Coalition	Median survival time = 1.4 years; newly diagnosed GBM patients; $n = 30$
Autologous whole tumor with GM-CSF and adoptive transfer of CD3-activated lymphocytes/systemic[129]	II	Tvax Biomedical	Median survival time = 1.0 years; recurrent malignant glioma; $n = 19$

KLH, keyhole limpet hemocyanin; poly-ICLC, polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose; GM-CSF, granulocyte-macrophage colony–stimulating factor.

The signal transducer and activator of transcription 3 (STAT3) has been shown to be a potent regulator of anti-inflammatory responses by suppressing innate[65–68] and adaptive immunity.[69] The STAT3 pathway becomes constitutively active in diverse tumor-infiltrating immune cells,[69] markedly impairing their effector responses.[69,70] STAT3 also increases the functional activity of the immunosuppressive Tregs.[71–73] 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,[60] 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,[74] likely related to better immunologic reactivity (ie, less immune suppression) in the pediatric patient relative to the adult[75] 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[76] and toxicity[77] 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:

True toxicity rate (%)	5	10	20	30	40	50
Probability of dose escalation	0.97	0.91	0.71	0.49	0.31	0.17

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.[77]

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:

Group	Mean response (U)	Standard deviation			
		Scenario #1	Scenario #2	Scenario #3	Scenario #4
One	1.0	0.1	0.5	1.0	2.0
Two	2.0	0.2	1.0	2.0	4.0
Sample size		6	24	82	318

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.[78] However, more contemporaneous clinical trials evaluating the efficacy of TMZ have demonstrated a median survival interval of 18.2 months.[79] 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[80] to 21 months.[81] 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.[82–85] More recently, immunotherapy has been shown to be efficacious even in advanced prostate cancer[86] and unresectable stage III and IV melanoma,[87] 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[53,54,88] for clinical

trials testing anti-Treg approaches.[89] 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[90–93] 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).[94–100] 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[101–103] in which an increasing proportion of polyfunctional T cells, T cells that simultaneously secrete IFN-γ, tumor necrosis factor-2α, and IL-2 along with coordinated expression of CD107a as a marker for cytotoxicity, prospectively predict long-term nonprogressors in patients with human immunodeficiency and cytomegalovirus (CMV) infection.[101,104,105] Increased numbers of polyfunctional antitumor T cells have also predicted improved antitumor efficacy, albeit only in animal models to date.[106,107]

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.[108] showed a correlation between IFN-γ 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 ~8-fold higher in the treatment group than in the controls.[86] 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.[109] 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-α.[110,111] 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.[112] Similar adverse events were seen in the immunization trials against amyloid-β 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.[113] 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.[114] 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.[115]

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 2. Immune assays commonly used in immunotherapeutic clinical trials

Immune assay	Advantages	Disadvantages
DTH		
Injection of an antigen intradermally and measurement of erythema and/or in duration after 48–72 hours	Easy to perform	Cutoff for positive response not standardized—subjective
	May correlate with T cell proliferative responses[130]	Amount of antigen not standardized
		May not be antigen specific[131,132]
		May be a surrogate marker for better performance status/less immune suppression
Peptide MHC tetramers		
Soluble, fluorescently labeled, MHC-peptide complex that binds to antigen-specific T cells	May correlate with cytotoxicity assays[133]	Antigen must be known
	May correlate with T cell avidity for antigens[134]	Tetramer positive cells may not kill target[135]
	Can be used to select antigen-specific T cell for further analysis[136]	Only MHC I tetramers are available routinely
		May bind both naïve and memory T cells[137]
Lymphoproliferative assay		
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	Can be performed directly on peripheral blood samples	In vitro culture conditions can alter results
		May reflect the overall immune suppression state of patient
		High proliferation by a few cells or low levels of proliferation by a few cells would give a similar stimulation index
ELISA/multiplex flow cytometric assay		
PBMCs are incubated with an antigen. Then the supernatant from the culture is harvested, and specific cytokines are detected	Easy to perform	Definition of positive results differs
		Is not based on individual cells
		Measures the ability of the cells to secrete cytokines and not necessarily the in vivo characteristics
ELISPOT		
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	Reliably detects the number of antigen-specific T cells[138]	
	Can be rapidly read with computerized plate readers, making it suitable for a large-scale study	
Intracellular cytokine detection by multiparameter flow cytometry		
In vitro T cell stimulation followed by prevention of cytokine secretion, surface staining, fixation, permeabilization, and staining with antibody	Can evaluate multiple immune populations simultaneously	Cell viability is lost; so unable to perform subsequent functional assays
Cytotoxicity assays		
Mix T cells or PBMCs with labeled antigen, expressing and measuring release of the target	Thought to be relevant marker for in vivo antitumor activity	Requires in vitro stimulation that may alter the activity from the in vivo state
		Because autologous tissue is difficult to obtain, other targets are used that may not reflect tumor biology
		Does not measure perforin, granzymes, or Fas-Fas-ligand cytotoxic killing—only direct cytotoxicity

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 neuroimmunology 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.

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