

## Immunotherapy for Brain Cancer: Recent Progress and Future Promise

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### Abstract

Immunotherapy is emerging as the newest pillar of cancer treatment, with the potential to assume a place alongside surgical debulking, radiotherapy, and chemotherapy. Early experiences with antitumor vaccines demonstrated the feasibility and potential efficacy of this approach, and newer agents, such as immune checkpoint blocking antibodies and modern vaccine platforms, have ushered in a new era. These efforts are headlined by work in melanoma, prostate cancer, and renal cell carcinoma; however, substantial progress has been achieved in a variety of other cancers, including high-grade gliomas. A recurrent theme of this work is that immunotherapy is not a one-size-fits-all solution. Rather, dynamic, tumor-specific interactions within the tumor microenvironment continually shape the immunologic balance between tumor elimination and escape. High-grade gliomas are a particularly fascinating example. These aggressive, universally fatal tumors are highly resistant to radiotherapy and chemotherapy and inevitably recur after surgical resection. Located in the immune-privileged central nervous system, high-grade gliomas also use an array of defenses that serve as direct impediments to immune attack. Despite these challenges, vaccines have shown activity against high-grade gliomas, and anecdotal, preclinical, and early clinical data bolster the notion that durable remission is possible with immunotherapy. Realizing this potential, however, will require an approach tailored to the unique aspects of glioma biology. *Clin Cancer Res*; 20(14); 3651–9. ©2014 AACR.

### Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults, with an incidence of 2 to 3 per 100,000 (1). Despite recent advances in chemotherapy, radiotherapy, and surgical resection, GBM remains a devastating diagnosis with a median survival duration of 14.6 months (2). Although GBM exploits many of the same molecular pathways that drive aggressive behavior in other solid tumors, several characteristics of GBM deserve special consideration. In most solid tumors, metastasis is a sentinel event in cancer progression and a frequent harbinger of incurable disease. Multifocal GBM, however, is atypical in that it remains unclear whether a multifocal disease pattern represents disease spread or recurrent *de novo* tumor development. Furthermore, metastasis outside the central nervous system (CNS) has been reported (3), but is infrequent and not a primary cause of morbidity and mortality.

Despite the ability to reliably achieve gross total resection with modern surgical techniques, neoplastic infiltration

beyond the radiographically defined tumor margins leads to inevitable recurrence. Adjuvant therapy with radiation and alkylating chemotherapeutic agents, such as temozolomide and carmustine (2, 4), may delay disease progression, but outgrowth of resistant clones limits response durability. Mechanisms underlying resistance to radiochemotherapy are a topic of intense investigation with attention particularly focused on glioma stem cells (5), which are characteristically enriched for activity of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT; ref. 6), and have demonstrated resistance to alkylating agents (7) and ionizing radiation (8). In addition, glioma stem cells are remarkably malleable, as illustrated by the finding that this cell population can differentiate into pericytes and vascular endothelium (9, 10). Such plasticity also potentiates the characteristic molecular heterogeneity reflected in the distinction "multiforme" (11). Accordingly, recent work has parsed high-grade gliomas into subclasses (12) and identified molecular profiles, such as isocitrate dehydrogenase mutations (IDH1/IDH2; ref. 13), MGMT methylation status (14), and EGFR amplification (12) with clear prognostic significance. Even these more discriminating classification schemes, however, are incomplete as they fail to account for intratumoral heterogeneity, which may present a more significant therapeutic challenge (15). One strategy for circumventing the lack of a clearly targetable molecular signature is to intervene in a process that is presumably critical to all cells comprising the tumor mass, such as angiogenesis. However, GBM cells have demonstrated a remarkable capacity to escape angiogenesis

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inhibitors through several mechanisms, including enhanced migratory behavior via upregulation of matrix metalloproteinases (16).

Immunotherapy has recently emerged into the clinical mainstream with the approval of the first antigen-specific agent, sipuleucel-T, for castrate-resistant prostate cancer in 2010 (17), and the approval of an immune checkpoint inhibitor, ipilimumab, for metastatic melanoma in 2011 (18). Historically, immunotherapy for GBM has afforded valuable insights, but failed to generate comparable clinical results with melanoma, renal cell carcinoma, and prostate cancer. There are several fundamental reasons for this discrepancy. Unlike prostate cancer, which expresses reasonably well-characterized tumor-restricted antigens, and melanoma, which is clearly immunogenic, GBM expresses relatively few known tumor-restricted antigens and has been classically considered nonimmunogenic (19). These characteristics, coupled with location in the immunologically privileged brain—which is, in turn, confined within the edema-intolerant cranial vault—have likely tempered enthusiasm for the application of immunotherapy to GBM. Yet, recent successes indicate that immunotherapy for GBM may be effective and well tolerated, with several immunotherapy regimens currently in clinical trials.

### Redefining CNS Immune Privilege

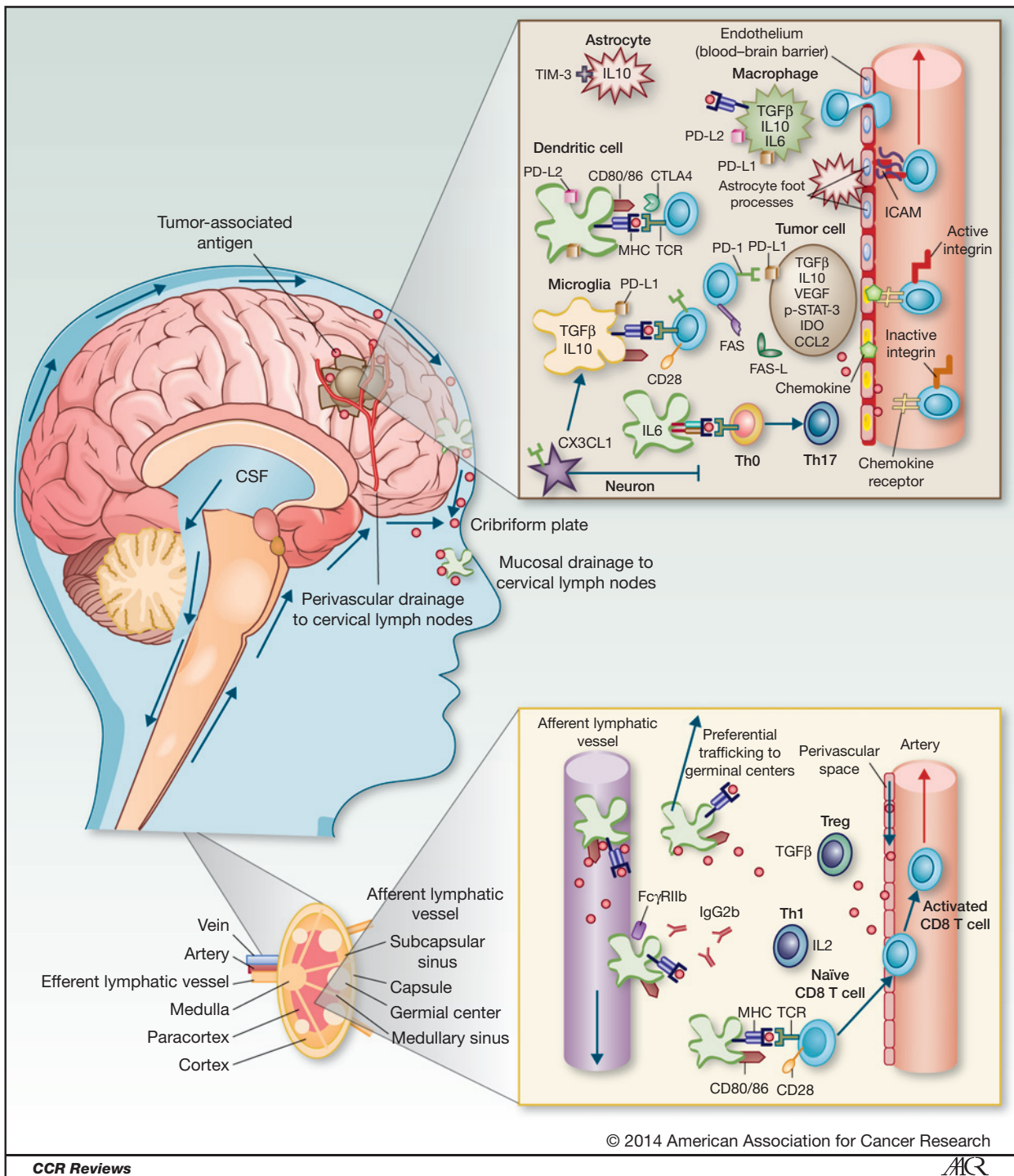
Classification of the CNS as an immunologically privileged site originated with the observation that tissues engrafted into the brains of experimental animals were rejected more slowly than tissues transplanted to other sites (20). Subsequent work characterizing the blood–brain barrier (BBB), an absence of conventional lymphatic structures, a paucity of professional antigen-presenting cells (APC) within the brain parenchyma, low levels of major histocompatibility complex (MHC) molecule expression, and the constitutive expression of immunosuppressive cytokines, such as IL10 and TGF $\beta$ , have established the CNS as immunologically distinct. The notion of immunologic privilege, however, is inconsistent with several clinical observations. For example, recent evidence suggests that downregulation of HLA class I expression corresponds with poor prognosis in GBM (21) and low CD4 counts in patients receiving standard therapy for high-grade gliomas portend shorter survival (22). Taken together, these observations suggest that a T-cell response to GBM could potentially modulate outcome.

Preclinical data further elucidate these inconsistencies by describing a highly dynamic immunologic compartment in the CNS. The BBB is relatively impenetrable to circulating immune cells and antibodies in the quiescent state; however, transcription of inflammatory chemokines is upregulated in the presence of danger signals, such as pathogen-associated molecular patterns. Transcription of the IFN $\gamma$ -inducible chemokines CXCL9, CXCL10, and CXCL11 subsequently potentiates immune cell incursion (23, 24) via interactions with CXCR3, resulting in lym-

phocyte arrest and binding of cell adhesion molecules (CAM) to  $\alpha$ 4 and  $\beta$ 1 integrins (25). Taken together, these data describe a well-orchestrated mechanism for potential lymphocyte egress into an evolving CNS tumor. Thus, the BBB, which was once thought to be a major impediment to immune cells accessing the CNS, may play little or no role in limiting active T-cell responses.

Adaptive immune responses are driven by professional APCs presenting antigen to T and B lymphocytes in a lymph node. Two distinct pathways for antigen egress from the CNS have been described: (i) antigens and antigen-loaded APCs are conveyed in the cerebrospinal fluid, pass through the cribriform plate, and drain to cervical lymph nodes via the nasal mucosa (26); (ii) interstitial fluid drains in a parallel pathway along capillary wall basement membranes and between smooth muscle cells in the tunica media of muscular arteries to reach the cervical lymph nodes (27). The relative role of each of these routes in tumor antigen presentation is unknown and deserves further study. Once an antigen reaches the cervical lymph nodes, however, it is likely that immune responses to CNS antigens may be constrained by the same mechanisms associated with mucosal tolerance induction, including TGF $\beta$  production by T regulatory cells (Treg; ref. 28), production of antibodies that signal through inhibitory Fc receptors like CD32 (FcRIIB; ref. 29), and preferential presentation in germinal centers, resulting in skewing toward B-cell predominant responses (30).

Microglia, the resident tissue macrophages of the CNS, are a particularly important component of this system. Microglia are of myeloid origin and migrate to the brain during development, but are phenotypically distinct from macrophages in peripheral tissues (31). The interplay between peripheral and local myeloid cell populations is illustrated by the finding that circulating monocytes readily repopulate the microglia niche in CD11b-herpes simplex virus thymidine kinase (CD11b-HSVTK) transgenic mice receiving intraventricular injection of ganciclovir (32). However, resident microglia are confined to the CNS, and whether cells that express dendritic cell (DC) markers and originate in the CNS have access to peripheral tissues remains a focus of investigation (33). In addition to their prominent role in innate immunity, which is reviewed in detail elsewhere (34), microglia play a crucial, albeit more nebulous role in adaptive immunity by representing antigen to activated lymphocytes, a critical process for preventing deletion of lymphocytes that enter the brain parenchyma (31). Microglia may work in concert with local DCs, which migrate in from the peripheral circulation and are also found in the meninges and choroid plexus (35), although the relative contributions of resident and migrating APCs in shaping the adaptive immune response is another aspect of CNS immunology that is poorly understood. In summary, CNS antigens may be presented in draining lymph nodes, by local microglia, and/or by immigrating DCs. A number of tolerogenic mechanisms have been identified in each of these pathways, which are depicted in Fig. 1.



**Figure 1.** Summary of adaptive immune response activation and tolerance mechanisms against CNS tumor antigens. Antigens drain to cervical lymph nodes along two distinct pathways: (i) soluble antigens and activated APCs pass through the cribriform plate and are conveyed to cervical lymph nodes via lymphatic pathways draining the nasal mucosa, (ii) soluble antigens in interstitial fluid drain along capillary wall basement membranes and between smooth muscle cells in the tunica media of muscular arteries to reach the cervical lymph nodes. In the cervical lymph nodes, CNS APCs preferentially migrate to germinal centers, stimulating B-cell predominant responses. The presence of CD32 (Fc $\gamma$ RIIb) and Tregs producing TGF $\beta$ , further blunt the immune response. Activated T cells acquire CNS tropism via expression of  $\alpha$ 4 and  $\beta$ 1 integrins. In the CNS vasculature, these integrins bind CAMs expressed on the surface of vascular endothelial cells, mediating T-cell egress. Once in the tumor microenvironment, expression of immune checkpoint ligands on a variety of cell types indicates that immune checkpoint signaling may play a central role in suppression of cytotoxic effector function. Inhibitory cytokines, including TGF $\beta$ , IL10 are also produced by a variety of cell types, including microglia, astrocytes, tumor cells, and immigrating macrophages. CX3CL1 (fractalkine) expressed by neurons inhibits activation of microglia. Furthermore, skewing toward Th17 differentiation limits maturation of CD8 T cells from Th0 cells entering the inflamed CNS. This combination of immunosuppressive mechanisms both intrinsic and extrinsic to the tumor cells themselves is likely to subserve blunted immune responses against glioma antigens. CSF, cerebrospinal fluid.



## Glioma Vaccines: Initiating an Antitumor Immune Response

Antitumor vaccines are the most established and well-studied immunotherapeutic modality. A target-specific antigen is administered in the context of danger signals (adjuvant), resulting in antigen uptake by APCs, expression of proinflammatory costimulatory molecules, such as CD80/CD86, and presentation of peptide fragments in the context of MHC molecules. Recognition of the antigen-MHC complex by specific CD4 or CD8 T cells in this milieu triggers T-cell activation, proliferation, tracking to the periphery, homing to sites of antigen expression, and effector function—in particular, lysis of antigen-expressing targets by CD8 T cells. This framework highlights two fundamental challenges in developing effective antitumor vaccines. First, unlike pathogenic microorganisms, which are molecularly distinct from host cells, tumor cells are derived from host tissues and, therefore, have similar molecular expression patterns. Second, many tumors have commandeered mechanisms of tolerance induction that have evolved in healthy tissues to protect against autoimmunity. Thus, a proinflammatory stimulus that might be sufficient to initiate a response against an invading organism is frequently inadequate to eliminate tumor cells.

Several known tumor-associated antigens are now being targeted in GBM, including HER-2, TRP-2, gp100, MAGE-1, IL13 $\alpha$ 2, and AIM-2 (36); however, the most extensively studied vaccine strategies have targeted the tumor-restricted neoantigen EGFR variant III (EGFRvIII; ref. 37). Mutated EGFRs are molecular drivers of malignant cell proliferation, differentiation, survival, invasion, and angiogenesis in many solid tumors, including GBM. EGFRvIII is a truncated, constitutively active EGFR variant expressed in 15% to 60% of GBMs, but only rarely expressed in healthy tissues (38–40). The first clinical trial targeting EGFRvIII in GBM involved administration of a 14-amino acid peptide (PEPvIII) conjugated to the foreign antigen keyhole limpet hemocyanin (KLH; ref. 41). This agent is also known as rindopepimut or CDX-110 (Cell-dex). Eighteen patients harboring tumors with confirmed EGFRvIII expression received the vaccine in combination with standard radiotherapy and chemotherapy. No significant adverse events were reported, and median survival among this cohort was 26 months. Interestingly, 82% of the tumors that eventually recurred in these patients did not express EGFRvIII. This finding is potentially encouraging in light of the immunoediting hypothesis, which postulates that tumors may lose proteins targeted by the immune system in an effort to "escape" immunologic pressure (42). However, caution is warranted, because the effects of radiotherapy and chemotherapy on EGFRvIII have not yet been fully characterized (43). Nevertheless, subsequent trials of peptide vaccines targeting EGFRvIII have been initiated and interim results generally support that the vaccine is immunologically active and has an acceptable safety profile.

Recently, a phase II clinical trial of rindopepimut plus granulocyte-macrophage colony-stimulating factor (GM-CSF; ACT III) in newly diagnosed GBM was completed and a phase II trial of rindopepimut plus GM-CSF in combination with bevacizumab in recurrent GBM (ReACT) reported interval results. ACT III included 65 patients with newly diagnosed GBM who received three biweekly intradermal injections followed by monthly injections until radiographic tumor progression, at which time patients received standard temozolomide. Reported median overall survival (OS; 21.3 months) compared favorably with historic controls (44). The ReACT trial is ongoing and involves randomization to bevacizumab, rindopepimut, or combination therapy. Interval results from ReACT reported a 12-month OS and 3.7-month progression-free survival with rindopepimut plus bevacizumab compared with 7.9 and 2.0 months, respectively, with bevacizumab alone (45). Several other studies are ongoing, notably, including a phase III study of rindopepimut plus GM-CSF in patients with newly diagnosed GBM (ACT IV).

Administration of tumor antigen-loaded DCs has the theoretical advantage of activating host lymphocytes while bypassing the requirement for antigen processing by host APCs, which are often dysfunctional in patients with cancer (46). Trials of DC vaccines targeting EGFRvIII have generated comparable data with peptide vaccine trials. An early feasibility study by Sampson and colleagues involving administration of PEPvIII-KLH-pulsed autologous DCs to 12 patients reported only mild toxicity and a median OS of 22.8 months (47). Polyvalent DC vaccines, including ICT-107 (Immunocellular Therapeutics) and the autologous antigen-based DC-Vax(R)-L Brain (Northwest Biotherapeutics), have also generated encouraging results, with phase I data indicating limited toxicity and median survival in the range of 30 to 40 months (36, 48). A phase II trial of ICT-107 is ongoing (NCT01280552) and a phase III trial of DC-Vax-L is currently recruiting (NCT00045968).

The seminal work in glioma vaccine development took place in an era of cancer immunotherapy dominated by peptide and DC vaccines. More recently, a new generation of vaccine platforms is demonstrating encouraging efficacy for targeted immunotherapy. One particularly promising vaccine platform is recombinant *Listeria monocytogenes*, a facultative intracellular pathogen that efficiently delivers antigens to the class I processing pathway (49). Live-attenuated listeria vaccines have demonstrated an impressive capacity to protect against tumor challenge and mediate regression of established tumors (50). Early trials in pancreatic cancer have confirmed that recombinant listeria vaccines can be administered safely and stimulate robust antitumor immunity (51). Clinical trials of CRS-207 (Aduro Biotech) are currently ongoing in pancreatic cancer (NCT1417000) and recruiting for mesothelioma (NCT01675765), as well as HPV-associated cancers (ADXS11-001; Advaxis, NCT01671488, NCT 01116245, NCT01598792, NCT01266460). A recombinant live-attenuated listeria vaccine expressing EGFRvIII and NY-ESO-1 has been constructed, and a phase I trial of this agent in patients with GBM (NCT01967758) is currently enrolling (Table 1).

**Table 1.** Selected trials of immunotherapy in GBM

Reference or NCCT identifier	Design	No. of Subjects	Phase	Results/endpoints
Completed (41)	PEPVIII-KLH was administered 2 weeks after surgery and once per month thereafter until radiographic progression.	18	II	PFS: 14.2 Months MS: 26 Months
(36)	21 patients (17 newly diagnosed, 3 recurrent, and 1 brainstem glioma) underwent leukapheresis; DCs were isolated and pulsed with tumor-associated antigen peptides administered at 3-week intervals.	21	I	PFS 16.9 months MS: 38.4 months
(48)	Glioma lysate-pulsed DCs and imiquimod or poly-ICLC adjuvant were administered every 3 months until tumor progression.	23	I	PFS: 15.9 months MS: 31.4 months
(44)	CDX-110 was administered in three biweekly intradermal injections followed by monthly injections until radiographic tumor progression; patients then received temozolomide.	65	II	MS: 21.3 months
Ongoing NCT01480479	A randomized trial of patients receiving standard-of-care surgery, radiation, and temozolomide with half of the patients randomized to receive CDX-110.	440	III	MS
NCT00045968	Randomized, controlled trial with participants in the placebo arm receiving autologous PBMC, while participants in the experimental arm will receive DCVax-L; treatment will be given at days 0, 10, and 20 and weeks 8, 16, 32, 72, 96, and 120.	300	III	PFS MS
NCT01280552	All patients receive standard of care surgery, radiation and temozolomide; after a 6-week washout period, patients undergo apheresis and DCs are purified, cultured, and pulsed with synthetic tumor antigens; pulsed DCs are then administered to the experimental group, while the placebo group receives unpulsed autologous DCs.	200	II	MS
NCT00458601	A multicenter trial of CDX-110 in combination with standard-of-care radiation, surgery, and temozolomide.	82	II	PFS
NCT01967758	Dose escalation study of live-attenuated <i>L. monocytogenes</i> expressing EGFRvIII peptide and full-length NY-ESO-1 will be administered to patients with recurrent EGFRvIII-positive high-grade gliomas; patients will be vaccinated up to four times separated by 21 days.	TBD	I	Safety Immunogenicity
NCT01952769	Patients with diffuse pontine gliomas or recurrent high-grade gliomas will receive CT-011 at 6 mg/kg every other week until disease progression or a serious adverse event occurs.	30	I-II	PFS at 6 months Toxicity
(85)	In development: a randomized two-arm study of ipilimumab.	TBD	II-III	TBD

Abbreviations: MS, median survival; PBMC, peripheral blood mononuclear cells; PFS, progression-free survival; TBD, to be decided.

## Immune Checkpoints: Objective Antitumor Responses in Multiple Cancers

Multiple mechanisms exist to protect healthy tissues against autoimmunity. In the earliest stages of lymphocyte development, T-cell receptors (TCR) that bind autoantigens are deleted during negative selection in the thymus. This process is imperfect, however, necessitating the evolution of mechanisms that inactivate autoreactive lymphocytes in the periphery. Immune checkpoints, a class of membrane-bound, inhibitory molecules expressed by activated and exhausted lymphocytes, play a critical role, as clearly evidenced in preclinical knockout models of one of the most extensively studied immune checkpoints, Cytotoxic T lymphocyte antigen-4 (CTLA-4). Mice lacking CTLA-4 develop lethal autoimmunity in multiple organ systems at an early age (52). Immune checkpoint molecules are often relatively overexpressed in the setting of advanced cancer, as tumors use immune checkpoint signaling to protect against immunologic elimination. The discovery of immune checkpoints and the development of agents targeting these pathways represent a major breakthrough in the field of cancer immunotherapy.

Immune checkpoint pathways seem to be nonredundant, with distinct signaling mechanisms (53) and locations of activity (54, 55). CTLA-4, for example, is expressed exclusively by T cells and serves as a major checkpoint at the level of initial T-cell activation in secondary lymphoid organs. Mechanistically, CTLA-4 and the activating T-cell molecule CD28 share the ligands CD80 and CD86 (56); however, CTLA-4 binds these ligands with higher affinity, sequestering CD80/CD86 and inhibiting T-cell activation (57). CTLA-4 binding further inhibits T-cell activation by dephosphorylating the CD3 $\zeta$  chain via activity of SHP2 and PP2A phosphatases (58). One of the important challenges to applying CTLA4 blockade for cancer therapy, however, is the lack of specificity associated with inhibiting a checkpoint that functions early in the process of lymphocyte activation. This prediction is clinically manifest in the development of immune-related adverse events, including colitis, hepatitis, dermatitis, and hypophysitis (59). Nevertheless, CTLA-4 blocking antibodies have demonstrated clinically meaningful activity against advanced cancers and the monoclonal antibody ipilimumab (Yervoy; Bristol-Myers Squibb) was the first immune checkpoint–targeted agent to receive the FDA approval based on a survival benefit noted in phase III trials in melanoma (60, 61).

Several recent studies have evaluated the activity of ipilimumab against brain metastases. Most notably, Margolin and colleagues (62) enrolled 72 patients with advanced melanoma and brain metastases. These patients were divided into two cohorts. Cohort A (51 patients) included asymptomatic patients not on steroids, while cohort B (21 patients) included symptomatic patients on a stable steroid regimen. Patients who had previously received immunomodulatory antibodies were excluded, and patients that had received previous radiotherapy were included only if they exhibited subsequent disease progression (63). Patients received one

dose of ipilimumab every 3 weeks for four doses and patients with stable disease at 24 weeks received one dose every 12 weeks. Twenty-four percent of patients in cohort A and 10% of patients in cohort B achieved stable or improved CNS disease burden, which was consistent with control of systemic lesions (27% in cohort A and 5% in cohort B). In contrast, recent retrospective data from Mathew and colleagues are less encouraging as they indicated no difference in local disease control in patients undergoing stereotactic radiosurgery alone versus stereotactic radiosurgery in combination with ipilimumab (64). These data should be interpreted with caution, however, as the retrospective nature of the study precluded precise timing and sequencing of radiotherapy and ipilimumab. The relative sequence of radiotherapy and immunotherapy has yet to be formally evaluated in patients with intracranial tumors, but is a fundamental component of combining radiotherapy with CTLA-4 blockade in other tumor models (65).

The precise location at which another major immune checkpoint, programmed cell death-1 (PD-1) encounters its ligand(s) PD-L1 and/or PD-L2 remains unclear; but, there is general agreement that the PD-1 pathway is involved in governing the activity of T cells in peripheral tissues (66). The PD-1 ligands, PD-L1 and PD-L2, are expressed on the surface of tumor cells (67–69) and tumor-associated macrophages (54), as well as supportive structures in tumor environment, such as vascular endothelial cells (70) and presumed bystanders, including neurons (71). Studies have suggested that expression of PD-L1 on the tumor cell surface is associated with a higher likelihood of response to PD-1 blockade (67). Several pharmaceutical entities are actively developing PD-1 (Merck; Bristol-Myers Squibb; Curetech), and PD-L1 (Medimmune; Roche) blocking antibodies. Two of these antibodies have achieved FDA designations that facilitate accelerated approval. Bristol-Myers Squibb's nivolumab has been granted fast-track status (72) and Merck's lambrolizumab was recently tabbed as a breakthrough therapy (73). Additionally, a phase I–II trial evaluating the effectiveness of PD-1 blockade with CT-011 (pidilizumab) in patients with recurrent high-grade gliomas opened in November 2013 and is currently recruiting participants (NCT01952769).

Other immune checkpoints that are being investigated for clinical translation include lymphocyte activation gene-3 (LAG-3) and T-cell immunoglobulin mucin-3 (TIM-3). LAG-3 is a CD4 homolog that binds MHC class II (74) and seems to play a role in controlling expansion of previously activated T cells rather than independent modulation of T-cell activity (75). This hypothesis has been borne out in preclinical models (76). TIM-3 is a glycoprotein with extracellular immunoglobulin and mucin domains that binds Galectin-9. Like LAG-3, TIM-3 seems to be principally involved in fine-tuning ongoing immune responses (77).

## Combination Immunotherapy

Studies in checkpoint signaling illustrate a general tenet of immune tolerance: It is generated, maintained, and



refined by multiple overlapping, yet nonredundant pathways, which operate in distinct anatomic and physiologic compartments. A striking clinical example comes from a recent clinical trial combining nivolumab and ipilimumab in patients with metastatic melanoma (78). The authors reported greater than 80% reduction in tumor burden in 53% of patients, albeit with a toxicity profile somewhat more pronounced than that observed using ipilimumab monotherapy. Furthermore, recent work by our group suggests that synergistic responses may be achieved by combining immunotherapy with radiotherapy in a glioma model (79). Several other candidate combinatorial strategies are being evaluated, and accumulating data suggest that intervention at multiple levels of the immune response will be required to achieve maximum antitumor activity.

We suspect that a combination immunotherapy regimen will be required to achieve meaningful responses in GBM. Specifically, such a regimen would involve activation of a systemic tumor antigen-directed response in addition to combinatorial checkpoint blockade and manipulation of the tumor microenvironment. Our focus on vaccines and checkpoint inhibitors in this review reflects an illustrative, though by no means exclusive, means of translating this strategy. This notion is supported by work in other tumors. GVAX has been safely administered in combination with CTLA-4 blockade (80), a combination that has demonstrated efficacy in preclinical models (65, 81) and combining GVAX with PD-1 blockade has been similarly promising (82). Although listeria vaccines are only beginning to be applied to GBM, this vaccine platform may prove particularly advantageous for combinatorial therapy as listeria vaccines can drive T-cell proliferation without PD-1 upregulation (83) and increase T effector to Treg ratios in the brain tumor microenvironment (Jackson, Lim, and Drake; unpublished data). In the process of translating these findings to a combinatorial clinical regimen, it should be noted that intracranial tumors might respond differently to listeria vaccination than peripheral tumors (84). Additional data from our group indicate that myeloid cells in the brain tumor microenvironment may modulate systemic immunologic responses to brain tumor-associated antigens differently than myeloid cells associated with otherwise equivalent non-CNS tumors (Jackson, Lim, and Drake;

unpublished data). Taken together, these concepts reinforce the status of the CNS as a unique immunologic compartment, which mandates special considerations for GBM immunotherapy.

## Conclusions

GBM remains a devastating clinical diagnosis and significant scientific challenge. Effectively developing immunotherapy against GBM will require combinatorial strategies that target multiple levels of immune tolerance. These efforts will likely be guided by successes in other tumors, but the unique aspects of GBM pathology must remain at the forefront. Many aspects of GBM tumor antigen presentation described in this review are extrapolated from studies conducted in autoimmune and infectious models. Although this framework is useful in considering general principles of CNS immunity, further investigation in neuro-oncologic models is clearly needed. The role of local myeloid cells in GBM pathogenesis and progression is also unclear, but may be critical to understanding the balance between tumor-mediated tolerance and persistent adaptive immune responses. Immune checkpoint blockade is dramatically changing the field of cancer immunotherapy, but effective implementation of checkpoint blockade in GBM will likely require a more detailed understanding of the role of these pathways both within the CNS and peripherally in this patient population. In summary, the unique and poorly understood interplay between peripheral and CNS immune responses in the setting of intracranial tumors makes the challenge of GBM immunotherapy singularly intriguing. Unlocking these connections will be critical to developing efficacious combination immunotherapy regimens for patients with high-grade gliomas.

## Disclosure of Potential Conflicts of Interest

M. Lim reports receiving commercial research grants from Accuray and Arbor Pharmaceuticals; speakers bureau honoraria from Accuray and Stryker CMF; and is a consultant/advisory board member for Stryker CMF. C.G. Drake reports receiving commercial research grants from Aduro Biotech, Bristol-Myers Squibb, and Janssen; has ownership interest (including patents) in Compugen; and is a consultant/advisory board member for Bristol-Myers Squibb, Compugen, Dendreon, and Roche/Genentech. No potential conflicts of interest were disclosed by the other author.

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