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Immunotherapeutic Approaches for Glioma

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Abstract

The development of effective immunotherapy strategies for glioma requires adequate understanding of the unique immunological microenvironment in the central nervous system (CNS) and CNS tumors. Although the CNS is often considered to be an immunologically privileged site and poses unique challenges for the delivery of effector cells and molecules, recent advances in technology and discoveries in CNS immunology suggest novel mechanisms that may significantly improve the efficacy of immunotherapy against gliomas. In this review, we first summarize recent advances in the CNS and CNS tumor immunology. We address factors that may promote immune escape of gliomas. We also review advances in passive and active immunotherapy strategies for glioma, with an emphasis on lessons learned from recent early-phase clinical trials. We also discuss novel immunotherapy strategies that have been recently tested in non-CNS tumors and show great potential for application to gliomas. Finally, we discuss how each of these promising strategies can be combined to achieve clinical benefit for patients with gliomas.

Keywords

glioma; cancer vaccines; antibody; adoptive transfer; T cells; antigen-presenting cells; tumor immunity

I. INTRODUCTION

Malignant gliomas are the most common type of primary brain tumor and a major unsolved public health problem, with more than 12,000 new cases diagnosed each year in the United States.¹

On the basis of the World Health Organization (WHO) classification, the four main types of gliomas are astrocytomas, oligodendrogliomas, ependymomas, and mixed gliomas (usually oligo-astrocytomas). Astrocytomas are typically classified as pilocytic (grade I), diffuse (grade II), anaplastic (grade III), or glioblastoma multiforme (GBM) (grade IV) in order of increasing anaplasia. Categorization of astrocytomas as low (I and II) or high (III and IV) grade is generally dependent on nuclear atypia, mitotic activity, microvascular proliferation, and focal necrosis. GBM is by far the most common and most malignant glial tumor. Composed of poorly differentiated neoplastic astrocytes, glioblastomas primarily affect adults, and they are located preferentially in the cerebral hemispheres.

Patients with GBM have a median survival of approximately 15 months, whereas those with anaplastic astrocytoma (AA) have a median survival of 24 to 36 months. For patients with recurrent malignant gliomas, the median time to further tumor progression, even with therapy,

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is only 8 weeks.² In addition, low-grade gliomas often progress to more malignant gliomas when they recur.² Despite extensive research, treatment options for these tumors remain limited.³ No significant advancements in the treatment of glioblastoma have occurred in the past 25 years except for chemotherapy with Temozolomide (TMZ) combined with radiotherapy, which has demonstrated a limited prolongation (approximately 3 months, compared with radiotherapy only) of patients' survival.^{4,5} The primary reason that no current treatment is curative is that the tumor is beyond the reach of local control when it is first detected clinically or radiologically. Clearly, there is an unmet clinical need for further improving treatment outcomes for patients with malignant gliomas. Recent advancements in our understanding of glioma biology should be applied to the development of effective combinational strategies, including molecularly targeted immunotherapy.

Immunotherapy, on the basis of the idea of taking advantage of the body's physiological mechanisms to defend itself, may develop as an effective and safe treatment modality for gliomas. However, the immunological microenvironment of the CNS and tumors arising in the CNS is still believed to be suboptimal for sufficient anti-tumor immune responses to mediate clinically meaningful changes *in situ* (reviewed in Refs. 6, 7).

In this review, we first discuss recent advances in the CNS and CNS tumor immunology, with particular attention to factors that may promote immune escape of gliomas. We also review advances in passive and active immunotherapy strategies for glioma, as well as novel immunotherapy strategies that have been recently tested in non-CNS tumors with great potential to treat gliomas. Finally, we discuss how each of these promising strategies can be combined to achieve clinical benefits for patients with glioma.

II. TUMOR IMMUNOLOGY AND THE CENTRAL NERVOUS SYSTEM

A. CNS-Immune System Interactions

For many decades, the brain has been referred to as an immune-privileged site (1) because it has been thought to lack dendritic cells (DCs) and lymphatics, (2) because of the presence of a blood brain barrier (BBB), and (3) on the basis of studies claiming a lack of allograft rejection in the brain.⁸ At the time, it remained a mystery as to whether "immunosurveillance,"⁹ the idea that thymus-dependent T cells constantly survey tissues for transformed cells, occurs in the brain. More recent work has described the CNS as "immune specialized,"¹⁰ after strong evidence of immune-CNS interactions in diseases such as multiple sclerosis (MS), experimental autoimmune encephalitis (EAE), and brain tumors. This section focuses on three key issues of CNS immunology, including how immune cells (1) are restricted from the brain, (2) can be activated against CNS antigens, and (3) traffic to and enter the brain. This discussion provides a framework to understand current treatment strategies harnessing the immune system to treat brain tumors.

1. How Immune Cells Are Restricted from the Brain—CNS cells are known to be extremely sensitive to the toxic effects of exogenous substances, making regulation of homeostasis and proper restriction of entry to the CNS crucial. To this end, the neurovasculature has evolved specialized mechanisms to control both molecular and cellular migration into (and out of) the CNS parenchyma and cerebral spinal fluid (CSF). The CNS capillary endothelial cells are termed the BBB due to their ability to restrict passive diffusion and maintain low pinocytotic activity.¹¹ Neuroimmunologists synonymously use the term BBB to describe both the capillary and postcapillary vessels, the latter of which is the site of T-cell migration into the brain. Restricting passive diffusion is accomplished through close cell-cell interactions known as tight junctions (TJs).¹⁰ The lack of fenestrations and the tight junctions prevent paracellular transport of large hydrophilic molecules (i.e., peptides and proteins) and cells.¹² Under low magnification electron microscopy (EM), TJs appear to be

fusions between the plasma membrane of juxtaposed cells, demonstrating how close these cell interactions are. However, high magnification reveals that in reality, TJs are the very close contact of cell membranes¹³ stabilized by specific proteins.

Many extracellular proteins have been studied as TJ proteins, primarily the occludin, claudin, and junctional adhesion molecule (JAM) families. Experimental characterization of each shows that mice carrying a null mutation in the occludin gene develop normal TJs, whereas claudins have been shown to be independently sufficient for TJ formation,¹⁴ suggesting the importance of claudins in TJ formation and regulation. Additionally, i.v. injection of monoclonal antibodies (MAbs) blocking JAM into mice has been shown to inhibit leukocyte accumulation in CSF and brain parenchyma,¹⁴ presumably by blocking leukocyte transmigration at the BBB.

The BBB does not function in isolation, and current research focuses on the BBB in the context of the neurovascular unit (NVU), which is composed of the BBB, pericytes, parenchymal membrane, and astrocytic feet.¹⁵ We know that of these NVU cells, astrocytic feet cover greater than 80% of the abluminal side of the CNS vessels.¹⁴ One study demonstrated astrocytic feet as the specific site of water entry to the brain during edema, presumably through aquaporin-4 channels.¹⁶ These astrocytes also contribute to the glia limitans, creating an added layer of protection for the brain or a barrier for immune cells. A more recent study has demonstrated that during cerebral ischemia, astrocytic swelling and detachment from the NVU results in increased permeability at the BBB.¹⁷ Thus, it seems that the BBB is just one layer of CNS capillary protection and that the capillaries are bound by a system rather than a static barrier. It is therefore important that *in vitro* experiments examining BBB behavior account for the function of the entire NVU.

The BBB in patients with GBM appears to be compromised. Davies¹⁸ has illustrated many variations in the capillaries of GBM microenvironments.¹⁸ In particular, GBMs secrete high levels of vascular endothelial growth factor (VEGF), leading to the upregulation of aquaporin-4 and decreased occludin and claudin-1, which correlates to edema. Furthermore, EM reveals breaks in TJs and increased pinocytosis at the TJs in GBM tissues.¹⁸ Others have reported alterations, including increased fenestrations, permeability, and a decrease in BBB-associated pericytes.¹⁹ The decrease in pericytes may be caused by endothelial cytoskeletal disorganization and a lack of cell adhesion, associated with increased edema and pericyte swelling.²⁰ These disruptions may affect immune-cell migration by allowing for GBM-specific methods of extravasation of immune cells into the parenchyma.

Despite the barriers in place to protect the brain, it is now well understood that immune cells do enter the brain, and activated T cells have been shown to cross intact BBB.²¹ Additionally, in GBM, the possibility exists that damage to the BBB may prove useful and may be harnessed to promote a strengthened immune response at the tumor site.

2. How Cells Are Activated to Neuroantigens—The classic paradigm of specific immune activation is achieved through antigen uptake by antigen-presenting cells (APCs), which migrate to the lymph nodes via draining lymphatics where APCs subsequently activate T cells. In the systemic immune system, DCs are considered to be the most potent APCs. In the CNS, a variety of cell populations have been postulated as primary CNS APCs, including vascular endothelial cells, smooth muscle cells, astrocytes, perivascular macrophages, choroid plexus epithelial cells, neurons, and DCs.²² Although microglia have also been proposed to be the primary resident APCs in the CNS, their capability for stimulating cellular immune responses is controversial.^{23–32} CD11c⁺ cells resembling classical bone marrow-derived DCs appear to be involved in murine infectious diseases³³ and autoimmune encephalitis.³⁴ However, in the glioma microenvironment, infiltrating microglia/macrophages fail to

upregulate MHC class II in response to IFN- γ .³⁵ Recent work focusing on possible CNS APCs examined microglia, macrophages, and DCs (F4/80-CD45^{hi}CD11c⁺) isolated from the peak of acute-phase EAE in mice. These cells were subsequently co-cultured with myelin proteolipid protein (PLP)_{139–151}-specific naive CD4⁺ transgenic (Tg⁺) T cells in the presence or absence of exogenous PLP_{139–151} peptide. All APCs with exogenously loaded peptides induced proliferation and IL-2 production from the T cells, with DCs being the most potent. However, with only endogenously processed peptide (no added peptide), microglia failed to induce proliferation. Macrophages induce proliferation only at very high APC:T-cell ratios, and only CNS DCs induced both proliferation and IL-2 production from the CD4⁺ Tg⁺ T cells,³⁶ demonstrating weak capabilities of microglia and macrophages as the primary CNS APCs. A recent study by Miller and colleagues³⁷ has further demonstrated that plasmacytoid DCs (pDCs), the major population of CNS DCs in EAE, seem to have a regulatory (rather than an activating) role for T cells. In fact, depletion of pDCs during different phases of EAE leads to increased disease severity and enhanced CNS CD4⁺ T-cell activation.³⁷ Thus, distinguishing between the myeloid DC and pDC role in T-cell activation is crucial.

It should be noted that antigen presentation has been hypothesized to occur through multiple mechanisms.⁷ Hypothesized mechanisms include the following: (1) DC uptake of antigen within the CNS, which is followed by migration and antigen presentation at lymph nodes. (2) Alternatively, antigen could drain to lymph nodes where they are then taken up by DCs processed and presented. (3) Cells that express the antigen may directly drain to lymph nodes and present their own antigen (direct presentation as opposed to cross presentation by DCs). Further studies are warranted to fully elucidate mechanisms by which cells and antigens leave the CNS and trigger systemic immune responses.

As mentioned previously, intratumorally injected DCs migrate to the cervical lymph nodes (CLNs). Furthermore, Cserr et al. have shown that, of radioactive-labeled antigen injected into various parts of rabbit brain, 18%–47% could be identified in the CLNs (as reviewed in Ref. 22). In additional studies on the progression of demyelinating brain diseases, autoantigens from brain lesions have been shown to drain to CLNs in both primate models of EAE and in human MS.³⁸ Notably, we and others have suggested that tumor-specific T cells can be primed in CLNs in murine GBM models.^{39–42} Surprisingly, Cserr and colleagues⁴¹ demonstrated that ovalbumin injected into the CNS parenchyma resulted in a greater humoral response than extracerebral injections of the same antigen in locations that also drain to CLNs. Presumably, there is an extra processing system or other specific drainage mechanisms in the CNS, which lead to an effective immune response.

The mechanism through which these antigens or DCs traffic to CLNs in humans is still very much unknown. In rabbit models, it has been shown by Cserr and others that the pathway of intracerebrally injected antigen is through sub-arachnoid space, along cranial nerves, crossing the cribriform plate entering the nasal mucosa and finally draining into the lymphatic system.²² While this is a mechanism in rodent models such as rabbits, the human system may be drastically different. CSF drains through the nasal mucosa in both rodents and humans. However, human CSF drains directly into venous blood through arachnoid villi and granulations, whereas in rabbits, over 50% drains into lymph nodes.⁴³ Although less understood, antigens may also travel via a pathway similar to interstitial fluid (ISF) clearance, a pathway unique to that of CSF. Not surprisingly, CNS ISF drainage also follows a mechanism distinct from ISF drainage from the periphery, and only 10%–15% of ISF gets transported through CSF in humans. Following endogenously produced amyloid-B, which is derived from the brain of patients with Alzheimer's disease, a mechanism could be found in humans similar to that in mice.⁴³

It is important to note that these experiments only represent one pathway for APC activation and that alternative pathways may exist. Further investigation is necessary in order to fully understand how antigens reach APCs and finally activate T cells.

3. T-Cell Trafficking to the Brain—As discussed earlier in this section, it is not well understood how lymphocytes traffic to the glioma sites. However, one model explains how lymphocytes translocate from high-velocity circulation into the parenchyma. This is a “multistep” model, characterized by 4 stages: (1) tethering/rolling, (2) activation, (3) adhesion, and (4) transmigration.^{14,44} Briefly, interactions between the carbohydrates on leukocytes and adhesion molecules on endothelial cells (usually selectins) slow down the leukocytes to a rolling phase along the endothelial walls. At a reduced velocity, the leukocytes sense chemokines on the endothelial cells, and through G-protein signaling, become activated, upregulating integrins such as very late antigen (VLA)-4.⁴⁵ Lymphocyte function-associated molecule (LFA) on lymphocytes allows for a stable interaction with their ligands VCAM and ICAM-1, respectively, on endothelial cells. Finally, with this tight interaction in place, cells may transmigrate into the parenchyma.

Expression of tissue-specific homing molecules directs antigen-experienced T cells to particular peripheral tissues. It is therefore essential to gain understanding of pivotal homing receptors that dictate CNS tumor homing of T cells. Calzascia et al.⁴⁶ defined *in vivo* imprinting of distinct homing phenotypes of T cells responding to antigens expressed by tumors in intracerebral, subcutaneous, and intraperitoneal sites with efficient VLA-4-mediated brain-tropism of CD8 T cells cross-primed in CLNs. Interestingly, multiple and distinct imprinting programs could occur simultaneously in the same LN when tumors were present in more than one site. Their study supports the concept that this critical functional parameter is dictated upstream of CLNs at the site of antigen capture by cross-presenting APCs; but the identity of the LN is not paramount in determining the homing phenotype.

In our own studies with murine brain tumors, we have demonstrated that type-1 cytotoxic T lymphocytes (Tc1), but not the type-2 counterpart (Tc2), can efficiently traffic to the CNS tumor site.⁴⁷ VLA-4 and CXCR3, a chemokine receptor for type-1 chemokines CXCL9–11, were uniquely upregulated on Tc1 compared with Tc2, and these receptors are critical for efficient CNS tumor homing of Tc1.^{45,47,48} On the basis of these original data, our current translational efforts are directed towards the development of vaccine strategies that promote expression of VLA-4 and CXCR3 on vaccine-induced effector T cells. Further information on mechanisms underlying efficient CNS tumor homing of T cells should be gained for the development of truly effective immunotherapy strategies for CNS tumors, including GBMs.

B. Interaction between the Immune System and Gliomas

Although GBMs are potentially immunogenic tumors,^{49–51} spontaneous clearance of established tumors by endogenous immune mechanisms is negligible. As is the case in other cancers, gliomas develop diverse strategies that escape tumor-specific immunity. A comprehensive overview of glioma development and progression suggests that the process is influenced by intrinsic properties of the glioma cells, as well as by microenvironmental factors. The glioma microenvironment is composed of tumor cells, endothelial cells, intermingling glia, neurons, a variety of leukocyte subsets, extracellular matrix fibers, and soluble mediators. An intricate interplay between the cellular and extracellular components determines the success or failure of tumor progression.^{52–54} This section outlines the role of different elements and the reciprocal interactions that exist between them, and sheds light on their potential involvement in glioma development and progression.

1. Impaired Immune Function in Patients Harboring Malignant Gliomas—

Previously characterized impairments in immunity of glioma patients have included low peripheral lymphocyte counts, reduced delayed-type hyper-sensitivity reactions to recall antigens, and impaired mitogen-induced blastogenic responses by peripheral blood mononuclear cells (PBMCs).^{55–64} The modulation and downregulation of T-cell function by immunosuppressive cytokines such as transforming growth factor- β (TGF- β) and interleukin 10 (IL-10) elaborated by malignant gliomas has been previously characterized.⁶⁵ TGF- β and IL-10 are known potent inhibitors of macrophage function⁶⁶ and of microglia production of proinflammatory cytokines.^{27,67,68} One of the most important prerequisites for the immune activation of effector T-cell responses is the presence of functional APCs that process and present tumor antigens, secrete specific cytokines and chemokines, and abundantly express costimulatory molecules essential for initiating and/or propagating tumor-directed T cells.⁶⁹

2. Glioma-Derived Immunosuppressive Factors

a. Soluble Immunosuppressive Factors Derived from Gliomas: Gliomas have been shown to synthesize and secrete multiple factors that are capable of inhibiting T-cell responsiveness. These include TGF- β 1, -2, and -3^{70–81}; PGE2^{77,82,83}; IL-10^{84–87}; and gangliosides (GANGs).^{88–90}

Transforming Growth Factor (TGF)- β : The immunosuppressive effects of TGF- β are multiple and complex. They include the inhibition of maturation and antigen presentation by DCs or other APCs, inhibition of T-cell activation, and differentiation towards effector cells (either cytotoxic cells expressing perforin or Th1 or Th2 cells).^{91–94} Inhibiting TGF- β *in vivo* in experimental tumor models has produced mixed results, probably because the effects of this cytokine are not only on the immune system, but also on the tumor cell, which may be protected from Fas (CD95)-mediated apoptosis in some circumstances.^{95,96} A recent study with glioma-cell clones that are resistant to the cytotoxic effects of allogeneic CTLs shows that TGF- β , but none of the other immunomodulatory molecules examined, is upregulated in these resistant glioma cells, pointing to the significance of TGF- β in glioma immune-escape mechanisms.⁹⁷

Prostaglandin (PG)E2: PGE2 is a product of arachadonic acid metabolism. It is produced at sites of inflammation or tissue damage, where it exerts many effects, including the enhancement of vascular permeability. PGE2 has been shown to have profound modulatory effects on T-cell activation, and gliomas synthesize PGE2. Thus, PGE2 could be associated with the suppressed T-cell function observed in patients with gliomas.^{77,82,83,98–100} However, the concentration of PGE2 observed in glioma-cell supernatants (GCS) does not correlate with the concentration of PGE required to inhibit T-cell proliferation.⁸³ Moreover, the T-cell unresponsiveness caused by the addition of GCS to mitogen-stimulated T-cell cultures is not altered when PGE2 inhibitors, such as naproxen or ibuprofen, are used during the generation of GCS.¹⁰⁰ Further characterization of the roles of PGE2 in glioma-induced immunosuppression is warranted.

Interleukin (IL)-10: The expression of IL-10 mRNA increases significantly with tumor grade; 87.5% of WHO grades III–IV astrocytomas expressed IL-10 mRNA versus 4% of grade II astrocytomas.⁸⁶ Gliomas exhibiting a high degree of brain invasiveness express IL-10 at higher levels than more localized gliomas.⁸⁴ In addition, glioma patient-derived monocytes, but not normal donor monocytes, produce IL-10 after *in vitro* stimulation with T-independent B-cell mitogen *Staphylococcus aureus* Cowen I strain (SAC).¹⁰¹

Gangliosides (GANGs): Since GANGs are produced by gliomas and they can modulate lymphocyte responsiveness, it is interesting to speculate that immunosuppression by GANGs

might account for some of the dysfunctions observed in glioma patients. The brain contains the highest concentrations of GANGs,¹⁰² which are highly immunosuppressive moieties affecting both APC and T-cell function.^{102–107} Concentrations as low as 1 mM of all brain-derived GANGs tested cause significant inhibition of normal T-lymphocyte proliferation *in vitro*.¹⁰² GANGs have been shown to inhibit the expression of CD4 on human and murine T cells as well as inhibit the generation of both cytotoxic T lymphocytes (CTL) and NK-cell activity^{105,108,109} and induce the death of T cells.¹¹⁰

Macrophage Chemoattractive Protein (MCP)-1 (also CCL2): Macrophage chemoattractive protein (MCP)-1 (also CCL2) was first purified and identified in 1989 from a human glioma.¹¹¹ MCP-1 is secreted by a variety of glioma cell lines and is expressed in glioblastomas.^{112–115} MCP-1 can directly promote angiogenesis *in vitro*¹¹⁶ and may also increase the levels of angiogenic factors *in vivo* through the recruitment of tumor-associated macrophages.¹¹⁷ MCP-1 has been shown to be critical for glioma tumor-cell proliferation¹¹⁸ and cancer-cell metastasis^{119,120} and is an important determinant of tumor aggressiveness.^{121–124} However, the MCP-1 chemokine has multifunctional activities that appear to have opposing effects on tumor biology. MCP-1 is a potent leukocyte chemoattractant and may play an important role in the host antitumor immune response. MCP-1 has been shown to promote *in vitro* chemotaxis of both CD8⁺ and CD4⁺ cells.^{125–127} Levels of tumor-derived MCP-1 often correlate with macrophage infiltration of malignant tissue. Vα24-Jβ18-invariant NK T cells have also been shown to traffic to and infiltrate neuroblastoma tumors in an MCP-1-dependent manner.¹²⁸ It has also been demonstrated that MCP-1 increases the efficiency by which adoptively transferred *ex vivo*-expanded T cells home to sites of tumor burden.¹¹⁴

b. Surface Immunosuppressive Molecules Expressed on Glioma Cells: Fas-FasL:

Malignant brain tumors, including GBM, express Fas, a receptor that mediates apoptotic signals upon ligation to its ligand Fas-L. Fas ligation also induces expression of proinflammatory and angiogenic mediators in malignant glioma cells,^{129,30} which can protect tumor cells from Fas-mediated cell death or support tumor growth by promoting angiogenesis.¹³¹ Moreover, some malignant tumors also express FasL, which induces apoptotic cell death of immune cells infiltrating into tumors and evade the host immune system, known as “counterattack.”^{132–135} Therefore, the roles of the FasL-Fas system in malignant brain tumors are complex: first, the expression of FasL by malignant tumors is a mechanism by which these cells evade the host immune system by inducing apoptosis of Fas-positive T cells that infiltrate into the tumor tissue; and second, Fas ligation on glioma cells can provoke apoptotic and/or proliferative responses.^{129,131} Microenvironmental factors will certainly influence the consequences of FasL expression by tumor cells; for example, tumors co-expressing both FasL and TGF-β may be particularly well adapted to combat CTL effector mechanisms.^{136,137}

B7-Homologue 1 (B7-H1), Also Known As Programmed Death Ligand-1 (PD-L1): The B7 family consists of activating and inhibitory co-stimulatory molecules that positively and negatively regulate immune responses. Recent studies have shown that human and rodent cancer cells, and stromal cells and immune cells in the cancer microenvironment, upregulate expression of inhibitory B7 molecules, and that these contribute to tumor immune evasion.

B7-homologue 1 (B7-H1), a recently identified homologue of B7.1/2 (CD80/86) and also known as programmed death ligand-1 (PD-L1), has been described to exert costimulatory and immune-regulatory functions. PD-1, a receptor for B7-H1, contains an immune receptor tyrosine-based inhibitory motif, and coligation of PD-1 and the T-cell receptor leads to rapid phosphorylation of Src homology region 2-containing protein tyrosine phosphatase-2, a phosphatase suggested to attenuate T-cell receptor signaling.¹³⁸ Wintterle et al.¹³⁹ investigated the expression and the functional activity of B7-H1 in human glioma cells *in vitro* and *in vivo*. They found that all 12 glioma cell lines, although lacking B7.1/2 (CD80/86),

constitutively expressed B7-H1 mRNA and protein. Exposure to IFN- γ strongly enhanced B7-H1 expression. Moreover, immunohistochemical analysis of malignant glioma specimens revealed strong B7-H1 expression in all 10 samples examined, whereas no B7-H1 expression could be detected on normal brain tissues. B7-H1 expressed on glioma cells was identified as a strong inhibitor of CD4⁺ as well as CD8⁺ T-cell activation as determined by increased cytokine production (IFN- γ , IL-2, and IL-10) and expression levels of the T-cell activation marker (CD69) in the presence of a neutralizing antibody against B7-H1 (mAb 5H1). B7-H1 expression may thus significantly influence the outcome of T-cell/tumor-cell interactions and represents a novel mechanism by which glioma cells evade immune recognition and destruction.

B7-H1 expression can be induced or maintained by many cytokines,^{140–142} of which IFN- γ is the most potent. In light of its stimulatory effect on B7-H1 expression, IFN- γ could thus be a “double-edged sword” in tumor immunity. Whereas IFN- γ could increase antigen processing and presentation by up regulating the expression of major histocompatibility complex (MHC) molecules and components of the antigen-processing machinery,¹⁴³ the effects of IFN- γ on B7-H1 expression might downregulate T-cell immunity. This could explain, at least partially, why IFN- γ has not been effective as a therapeutic agent for most human cancers.

In human glioma, loss of phosphatase and tensin homolog (PTEN) and activation of the phosphatidylinositol-3-OH kinase (PI3K) pathway induce expression of the gene encoding B7-H1 increases posttranscriptionally.¹⁴⁴ In primary GBM tissues, levels of B7-H1 protein correlated with PTEN loss, and tumor-specific T cells lysed human glioma targets expressing wild-type PTEN more effectively than those expressing mutant PTEN, demonstrating a novel mechanism linking loss of the tumor suppressor PTEN with immunoresistance, mediated in part by B7-H1.¹⁴⁴

Receptor-Binding Cancer Antigen Expressed on SiSo Cells (RCAS1): Receptor-binding cancer antigen expressed on SiSo cells (RCAS1), a membrane molecule strongly expressed in aggressive human cancer cells, has also been reported to participate in tumor immune escape in certain types of cancer. It acts as a ligand for a receptor present on normal peripheral lymphocytes, such as T, B, and NK cells, and induces apoptotic cell death.¹⁴⁵ Nakabayashi et al.¹⁴⁶ demonstrated that RCAS1 overexpression significantly correlates with high histological grade and poor prognosis in 57 glioma cases. Furthermore, reduced infiltration and increased apoptosis of tumor-infiltrating lymphocytes (TILs) were observed in RCAS1-positive regions. Thus, RCAS1 expression in gliomas may play roles in tumor progression and tumor immune escape.

Human Leukocyte Antigen (HLA)-G: A non-classical MHC class I molecule, HLA-G, expressed on astrocytoma cells has been proposed to have immunosuppressive potential¹⁴⁷ and to suppress NK- and T-cell immune responses, but this is controversial.^{148,149} Regarding astrocytomas, a proportion of astrocytoma cell lines and tumor biopsies expressed HLA-G protein, and inhibition of CD4 and CD8 T-cell responses was demonstrated *in vitro*, but this was only tested after incubation of cell lines with high concentrations of IFN- γ (500 U/mL), or after gene transfer of HLA-G into glioma lines.

CD70: CD70 is a CD27 ligand and a member of the tumor necrosis factor (TNF) family. Neoplastic glial cells often express CD70 *in vivo*, mediating immune escape of human malignant gliomas.^{150,151} CD70-positive GBM cell lines induce apoptosis in T cells.¹¹⁰ Of the four GBM cell lines that induced T-cell death, three highly expressed CD70. Two nonapoptogenic GBM lines (CCF3 and U138), on the other hand, had only minimally detectable CD70 expression. Neutralization of CD70 but not TNF- α or FasL is responsible for initiating T-cell

death via the receptor-dependent pathway. CD70 is also expressed on activated T and B cells, with roles in regulating immune responses via interaction with CD27 expressed on lymphoid cells.¹⁵²

3. Glioma-Associated Immunosuppressive Leukocytes—A large number of observations suggest that certain types of tumor microenvironmental immune cells are not innocent bystanders at brain tumor sites, and that they actively affect tumor development and progression. Inflammatory cells, primarily macrophage/microglia and regulatory T cells, may affect these processes via their ability to express a large variety of factors, including immunoregulatory cytokines. These cytokines may be secreted not only by inflammatory cells but also by the tumor cells and stroma cells, together establishing a network of factors that significantly affects brain tumors.

Macrophages/Microglia: In the CNS, macrophage/microglial cells constitute the first line of cellular defense against a variety of stressors, participating in the regulation of innate and adaptive immune responses in human and rat gliomas.²⁹ Many human gliomas exhibit a prominent macrophage/microglia infiltrate. Glioma-infiltrating macrophages (GIMs) can account for as much as 30% of the tumor mass.¹⁵³ GIMs represent the largest sub-population infiltrating human gliomas from postoperative tissue specimens of glioma patients.¹⁵⁴

With regard to the distinction between resident microglia (CD11b⁺/CD45^{dim}) and macrophages (CD11b⁺/CD45^{high}), most studies have demonstrated that glioma-infiltrating CD11b⁺ cells are mostly CD45^{high} macrophages.³² Indeed, it has been described that “microglia” in human gliomas appears mostly amoeboid and morphologically distinct from the resting microglia present in the intact brain.^{155,156}

Intratumoral macrophage/microglia density is higher than in the normal brain, and the abundance of microglia correlates with the grade of malignancy. In patients with gliomas, the number of macrophages in glioblastomas (grade IV) is higher than that in grades II or III gliomas, and it is closely correlated with vascular density in the tumors.^{157,158}

It is postulated that the defense functions of macrophage/microglia against glioma are compromised in the tumor microenvironment. These GIMs expressed substantial levels of Toll-like receptors (TLRs), which are critical components for APCs to mediate innate immune responses to any infectious or traumatic challenge and activate adaptive immune responses. However, GIMs did not appear to be stimulated to produce proinflammatory cytokines (TNF- α , IL-1, or IL-6), and *in vitro*, lipopolysaccharides could bind TLR-4 but could not induce GIM-mediated T-cell proliferation.¹⁵⁷ Moreover, it has been found that these GIMs, in addition to decreased surface expression of MHC class II,^{23,35} lack expression of the costimulatory molecules CD86, CD80, and CD40, which are critical for T-cell activation. They are thereby unable to activate T cells properly *ex vivo*.¹⁵⁴ Macrophages/microglia can release many factors, including extracellular matrix proteases and cytokines, which may directly or indirectly influence tumor migration/invasiveness and proliferation.^{159–161} In Boyden chamber assays, glioma cell migration is stimulated by the presence of macrophage/microglia or macrophage/microglia-conditioned medium.¹⁶² It has been recently demonstrated in an organotypic brain culture that the invasive potential of glioblastoma was lower in macrophage/microglia-depleted slices, but the addition of microglial cells to the microglia-depleted slices restored the invasiveness.¹⁶³ These findings suggest that macrophage/microglia in human gliomas may promote and support the invasive phenotype of these tumors.

Regulatory T Cells (Tregs): The suppressive activity of regulatory T cells (Tregs) has been implicated as an important factor limiting immune-mediated destruction of tumor cells. An increased FoxP3⁺ Treg to CD4⁺ T cells ratio correlates with impairment of CD4⁺ T-cell

proliferation in peripheral blood specimens obtained from patients with GBM.¹⁶⁴ In this referenced study, *in vivo* depletion of Tregs led to glioma rejection in murine model systems. Other studies have shown that an immunosuppressive population of Tregs is present within the GBM microenvironment.^{154,165} Moreover, it has been demonstrated that Tregs are not present in normal brain tissue and were very rarely found in low-grade gliomas and oligodendrogliomas.¹⁶⁶ The same study also observed that Treg infiltration differed significantly in the tumors according to lineage, pathology, and grade. Tregs seemed to have the highest predilection for gliomas of the astrocytic lineage (over oligodendroglioma) and specifically in the high-grade gliomas, such as GBM. In both univariate and multivariate analyses, the presence of Tregs in GBMs seemed to be prognostically neutral.¹⁶⁶ However, in a study with a mouse GL261 glioma model,¹⁶⁷ treatment of glioma-bearing mice with anti-CD25 mAb delayed the tumor growth and prolonged the survival of the mice, suggesting that CD4⁺CD25⁺ Treg cells play an important role in suppressing the immune response to CNS tumors.¹⁶⁸

Furthermore, Grauer et al.¹⁶⁹ demonstrated a time-dependent accumulation of CD4⁺FoxP3⁺ Tregs in brain tumors with a syngeneic murine glioma GL261 model. They observed that the expression of CD25, CTLA-4, GITR, and CXCR4 on intratumoral CD4⁺FoxP3⁺ Treg during tumor growth is upregulated in a time-dependent manner. They also demonstrate that treatment with anti-CD25 MAbs significantly provokes a CD4 and CD8 T-cell-dependent destruction of glioma cells. Moreover, combining Treg depletion with administration of blocking CTLA-4 mAbs further boosted glioma-specific CD4⁺ and CD8⁺ effector T cells, as well as antiglioma IgG2a antibody titers, resulting in complete tumor eradication. This study illustrated that intratumoral accumulation and activation of CD4⁺FoxP3⁺ Tregs act as a dominant immune-escape mechanism for gliomas and underline the importance of controlling tumor-infiltrating Tregs in glioma immunotherapy.¹⁶⁹

III. IMMUNOTHERAPY APPROACHES FOR GLIOMAS

A. Passive Immunotherapy

Passive immunotherapy consists of therapeutic modalities that utilize immune-effector cells and/or a variety of molecules, including MAbs and cytokines.

The efficient use of MAbs against brain tumors presents unique challenges. First, the passage of therapeutic agents from the circulation through the BBB favors small, uncharged, lipid-soluble molecules. The uniquely large size of antibodies as macromolecules favors novel delivery strategies to administer antibodies directly to the brain tumors. Indeed, an IgG antibody has a molecular weight of approximately 150 kDa, whereas many chemo-therapeutic agents have a molecular weight on the order of 1 kDa.¹⁷⁰ Although most small molecule drugs rely on diffusion as a mode of transport through tissue, antibodies must rely on bulk fluid flow (convection) in which antibodies flow down their pressure gradient. It has been shown that interstitial pressure in a solid tumor mass is elevated above the interstitial pressure of the surrounding normal tissue.^{170–172} This increased pressure maybe the result of a less-developed lymphatic system, which is thereby less able to drain interstitial fluid. Also, increased cell density could be a contributing factor to this phenomenon.^{171–173}

The binding site barrier is another challenge to antibody penetration. At equilibrium, the concentration of antibodies in normal tissue is negligible compared with the interface at the tumor site.¹⁷⁰ For transport into the core of a tumor mass, free antibody must pass from the low concentration region of target-free normal tissue, past a boundary layer of the tumor (the binding site). This boundary layer contains a high concentration of tumor-bound antibody. To diffuse into the tumor core, the antibody would have to flow against a significant concentration gradient and would do so inefficiently, leaving the interior of a solid tumor ineffectively treated.

Because of the limitations presented by the binding site barrier and the penetrance of large molecule therapeutics through brain tissue, it is an attractive option to deliver drugs in concert with a complete surgical resection—an integral part of the current standard of care.

Convection Enhanced Delivery (CED) has emerged as an attractive drug-delivery method that may be instrumental to the development of novel therapeutic modalities. CED is suitable for enhancing the delivery of both large and small molecules by physical-pressure processes. In CED, the therapeutic agent is infused at high pressure through an intracranial catheter.¹⁷⁴ As opposed to diffusion, drug delivery using CED relies on bulk flow. A number of passive immunotherapy clinical trials have utilized CED as the method of drug delivery, many of which are discussed in the following sections. However, a major challenge with CED remains in that the bulk flow produced may be slow and insufficient to deliver IgG-based molecules throughout a typical treatment volume.^{174,175} Other delivery techniques, including intraventricular infusion, intratumoral and intracavitary injections, and biodegradable polymers (such as BCNU Gliadel wafers), rely on diffusion.¹⁷⁵

1. Antibodies: Inhibition Strategies

Anti-EGFR Antibodies: An attractive candidate for IgG-based inhibition has been epidermal growth factor receptor (EGFR). EGFR is overexpressed on 40%–50% of tumors.¹¹¹ EGFR overexpression is associated with increased tumor growth rate and shorter survival.^{176,177} EGFR, a transmembrane receptor tyrosine kinase, binds its ligands epidermal growth factor (EGF) and transforming growth factor TGF- α . EGFR activation generates gene transcription modulations, resulting in stimulated proliferation, angiogenesis, and metastasis.¹⁷⁸ A mutation of EGFR, termed EGFR variant III (EGFRvIII), is frequently expressed in glioblastoma and enhances tumorigenicity.¹⁷⁹

Cetuximab (Imclone, Bristol Meyers Squibb, New York, NY) has been shown to enhance the antitumor effects of chemotherapy and radiotherapy, inhibiting the EGFR pathway. EGFRvIII can also be bound by cetuximab, and it has been suggested that cetuximab has antitumor efficacy against EGFRvIII⁺ glioma cells.¹⁸⁰ Cetuximab targets the extracellular domain (ECD) of EGFR.¹⁸¹ In 2006, Belda-Iniesta et al.¹⁸¹ reported three patients with recurrent EGFR-expressing GBM who responded to treatment with a single agent, cetuximab.

Nimotuzumab (h-R3, YM Biosciences, Mississauga, ON, Canada), which similarly targets the ECD of EGFR, has been used in Phase II trials.¹⁸² Ramos et al.¹⁸² observed a 17.5-month MST for GBM patients. In high-grade glioma patients, there was a 37.9% objective-response rate and stable disease in another 41.4% of patients.

A variety of other anti-EGFR antibodies, including Panitumumab, Matuzumab, and Zalutumumab, have been applied to other EGFR⁺ cancers (such as colorectal cancer) but have not been applied to malignant gliomas in the clinical setting at this time, to the best of our knowledge.¹¹¹

Daclizumab (Zenapax, Roche, Basel, Switzerland), a MAb against IL-2R, is primarily an immunosuppressant but may inhibit the recovery of Tregs after chemotherapy-induced lymphocytopenia. Ongoing clinical trials are investigating daclizumab along with peptide/autologous lymphocyte treatment following temozolomide therapy (NCT00626015 on www.clinicaltrials.gov).

The use of antibodies as inhibitors has the advantage of being precise and specific, but the challenges of delivery and penetrance remain. As cancer biology and the mechanisms of tumor-cell proliferation and immune escape are increasingly understood, more candidates for therapeutic inhibition will be found.

2. Radioimmunotherapy—Radionucleotides conjugated to MAbs have tremendous potential for both diagnostic imaging and for targeted radiotherapy. The paradigm of radioimmunotherapy (RIT) is the targeting of tumor-associated antigens by a radioisotope-conjugated antibody, which binds at the tumor site and delivers a local, lethal dose of radioactivity to the tumor site. Cytotoxicity from radiation is the result of the ionization of an emitted particle from a disintegration reaction as it deposits energy into the cell, irreparably damaging DNA.¹⁷⁰ It is advantageous to concentrate radioactivity at the tumor site, thus sparing normal brain tissue.¹⁸³ There has been less success with bulky solid tumors, and this approach is ideally used in combination with gross total resection, with minimum residual disease. Delivery can be focused within the surgical cavity in efforts to access the tumor cells invading at the surgical margin.¹⁸³

Dosimetry of RIT is challenging because the absorbed radioactive dose is dependent on many factors, including the tissue geometry and properties, and the radiopharmaceutical clearance rate of the individual glioma patient. Many algorithms are based on absorbed dose calculated by the MIRD schema, developed by the Medical Internal Radiation Dose Committee.¹⁷⁰ This schema employs a series of three-dimensional Monte Carlo calculations applied to idealized models of tissue properties specific to the choice of radioisotope and target organ.¹⁷⁰ Calculations of the biological effective dose (BED) have since become more advanced, taking into account the clearance rate, interaction between absorption in the cortex and medulla of the kidney (S value), and red marrow dosimetry.¹⁸⁴

Two common types of radionucleotides used in RIT emit α particles, helium nuclei, or β -particles, expelled from the nucleus with the mass of an electron. There exists an inherent tradeoff in a selection of either an α -emitting radionucleotide such as ^{211}At or a β -emitting radionucleotide such as ^{131}I . Although the range of most β -emitters is on the order of 1 mm, many α -emitters have a range of just a few cell diameters, with a higher linear energy transfer.¹⁸³ This means that the α particles released from a disintegration reaction travel a shorter distance but deposit energy more intensely along their path. This implies greater cytotoxicity and specificity and therefore a higher allowable dose to the tumor site. However, given the heterogeneity of malignant glioma, the “cross-fire” effect of longer range β -emitters may be advantageous to kill antigen-negative tumor cells. This heterogeneity also implies that a multiantigen approach may be more effective than a single-target approach.

Halogens, which are β -emitters, can be directly attached to antibodies. This is an advantage over some other radionucleotides, including ^{111}In and ^{90}Y , which must be linked through intermediate agents. In light of the relatively short half-lives of many of these radioisotopes, speed and simplicity of generation are essential to on-site or near-site production.

Antitenascin: Tenascin has been perhaps the most-studied target for glioma RIT. Tenascin is a glycoprotein expressed ubiquitously on the surface of several types of tumors, including high-grade gliomas. Tenascin expression may be correlated with disease progression, with 90% of GBMs exhibiting high levels of tenascin.¹⁸⁵

The antitenascin antibody 81C6 was developed at Duke University and has been shown to uptake in tumor tissue with high specificity.^{186,187} MAb 81C6 has since been used extensively in RIT studies, typically conjugated to ^{131}I , and has shown improved survival of glioma patients over historical controls as well as a reduction in toxicity relative to stereotactic radiotherapy.¹⁸⁸ Trials of ^{131}I -81C6 administered to the tumor-resection cavity at the time of surgery demonstrated median survival time (MST) of 12–13 months for patients with recurrent gliomas and up to 20 months in newly diagnosed GBM patients.^{189–192} On the basis of these studies, an optimal radiation dose of 44 Gy was chosen to balance the risk of radiation necrosis and tumor recurrence.¹⁸⁵

The group at Duke University has also developed a ^{211}At -labeled chimeric antitenascin therapy. The choice of an α -emitting radioisotope is thought to allow for a much greater specificity for the delivery of radiation of a tumor. Indeed, in a Phase I trial by Zalutsky et al.,¹⁸⁵ more than 95% of the radiation was localized to the tumor resection cavity. While maintaining a 0.01 Gy exposure to normal tissue (including tenascin + liver and spleen), an average radiation dose of 2986 Gy was able to be delivered to the resection site.¹⁸⁵ In addition, by replacing murine 81C6 with chimeric ch81C6, considerably higher *in vivo* stability can be achieved.¹⁸⁵ No dose-limiting toxicity has been reported.¹⁹³ The overall median survival was 57 weeks, and MST for GBM patients was 52 weeks; however, 2 of 14 patients survived for almost 3 years.¹⁹³

Tumor Pretargeting: Another group from the European Institute of Oncology has used a different antibody to tenascin, BC-2 and BC-4.¹⁹⁴ With ^{131}I -BC-2/4, Riva et al.¹⁹⁵ observed no significant toxicity, a MST of 16 months, and 3 of 17 patients with complete remissions.¹⁹⁵ A follow-up report combining data from 111 patients in a Phase I/II trial showed a MST of 20 months.¹⁹⁶

Paganelli and colleagues^{197,198} have developed the method of tumor pretargeting using BC-4 MAb against tenascin. In this three-step targeting approach, high-affinity avidin-biotin binding is exploited. Biotinylated antibody is injected into the patient, and on the basis of kinetic studies, unbound antibody clears the body within 48 hours.^{197,198} During the second step, streptavidin is injected. Biotinylated radionucleotides are administered several hours later, as the third and final step.¹⁹⁷ The biotinylated radioisotopes bind to the equilibrated antibody-avidin complex that is theoretically bound specifically to the tumor. Since the biotinylated radioisotope is much smaller than the bulky IgG molecule that binds directly to the tumor site, this smaller effector molecule equilibrates and migrates to the tumor site much faster than directly conjugated IgG, increasing the possible radioactive dose to the tumor site without increasing the normal tissue dose. In a clinical trial utilizing ^{90}Y and the three-step biotin-avidin method administered intravenously, 12 of 48 patients demonstrated a reduction in tumor (17% maintained reduction after 1 year), another 52% had stable disease, and three experienced complete remissions.¹⁹⁹ In a subsequent study applying the same strategy, 37 patients with grades III/IV glioma were treated. Patients with grade III tumors had a reported progression-free survival (PFS) of 56 months and 82% (9/11) survival at 60 months. Thirty-eight percent of GBM patients survived with a MST of 33.5 months compared with 0% survival of control patients (MST = 8.5 months) treated with the standard care.¹⁹⁷

In later studies, pretargeted RIT was delivered “locoregionally” by direct infusion into the surgically created cavity placed during a second surgery in recurrent GBM patients. The average absorbed dose to normal brain was minimal. AA patients had a MST of 19 months from the second surgery (53 months overall). GBM patients had a MST of 11.5 months from the second surgery (20 months overall). Forty-four percent of GBM patients had significant delay in tumor growth, prolonged survival, and improvement in quality of life.²⁰⁰ Pretargeted RIT was also examined in combination with chemotherapy (temozolomide, TMZ). Patients with recurrent GBM were once again treated with a second surgery to administer pretargeted ^{90}Y -antitenascin every 2 months, with or without TMZ. Patients receiving RIT alone demonstrated a 5-month PFS and 17.5-month survival compared with patients who received RIT and TMZ who demonstrated a 10-month PFS and 25-month MST. No increased neurological toxicity was seen in patients treated with combination therapy versus monotherapy. A 75% objective-response rate was reported.²⁰¹

DNA/Histone HI Complex: Phase I and II clinical trials have been completed with ^{131}I -chTNT-1/B MAb (Cotara, Peregrine Pharmaceuticals, Tustin, CA). ^{131}I -chTNT-1/B MAb is a radioiodine-conjugated MAb specific to the DNA/histone HI complex.²⁰² This intracellular

marker is only exposed to the interstitial space in the necrotic core of the tumor, where a population of tumor cells spontaneously dies and releases DNA/histone complex. The use of the β -emitting ^{131}I radionuclide is appropriate to generate a significant amount of midrange cytotoxicity and also affects the invasive glioma cells that are not marker specific, but with a reasonable radiation range cutoff to provide an extent of protection to the surrounding normal tissue.²⁰³ In clinical trials, ^{131}I -chTNT-1/B MAb has been administered via CED to achieve maximal tumor penetrance.²⁰² It was reported that the majority of patients received infusions within 10% of the prescribed radioactive dose to the targeted region, and the therapy was well tolerated.²⁰² These trials demonstrated safety and feasibility of ^{131}I -chTNT-1/B MAb administered via CED. Data is insufficient to determine efficacy, but in 28 patients with recurrent GBM, the median time to progression and MST were 8.4 and 23 weeks, respectively, which is in line with historical controls.²⁰² However, in a “normalized” subset of patients receiving total radioactive dose in the therapeutic window, MST was reported as 37.9 weeks (203).²⁰² Several patients demonstrated objective responses, and 25% of recurrent GBM patients survived for greater than 12 months.²⁰³

Other Targets: Other studies have targeted the extra domain B (ED-B) of fibronectin, a marker of tumor angiogenesis^{204,205}; the α chain of the IL-2 receptor²⁰⁶; and EGFR²⁰⁷ in the preclinical setting. Rosenkranz et al.²⁰⁷ have developed a ^{211}At -conjugated internalizable ligand to EGFR. The application of ^{125}I -anti-EGFR MAb following surgical resection and external beam radiotherapy (EBRT) was reported to demonstrate significant improvement in survival, although this study did not include a control group.²⁰⁸ In a small sample of patients, simultaneous ^{125}I -anti-EGFR MAb and EBRT failed to improve survival or PFS compared with EBRT alone.²⁰⁹ Alternative radionuclides, including ^{225}Ac , ^{213}Bi , ^{212}Bi , ^{224}Ra , ^{212}Pb , and ^{149}Tb have been investigated preclinically, as well.^{210,211} The chemistry of chelating radionuclides is yet to be fully elucidated. For example, ^{225}Ac undergoes four disintegration reactions with intermediates ^{221}Fr , ^{217}At , and ^{213}Bi emitting α particles during each event.²¹⁰ To develop one chelating agent to bind all intermediates of multistep disintegrations to IgG would not be feasible, and the clinical consequences of the release of intermediates has not been demonstrated.²¹⁰

3. Boron Neutron Capture Therapy—Boron Neutron Capture Therapy (BNCT) is a strategy in which an agent delivers stable isotope boron-10 selectively to the tumor site. Upon exposure to an external epithermal neutron beam, the boron disintegrates into stable lithium and emits an α particle with a short path length (9 μm) and high-linear-energy transfer.²¹² A variety of nonimmunological boron carriers have been used with encouraging results in clinical trials. Most notably, recent clinical trials using boronophenylalanine (BPA) or sodium borocaptate (BSH) as boron carriers have reported promising results, with MST of 13–20 months.^{212–218}

The potential to use novel targeting methods, including the use of MAb, could dramatically improve the specificity and efficacy of this therapy.²¹² In a preclinical study, Yang et al.²¹⁹ explored the use of a mixture of boronated antibodies targeted against EGFR (cetuximab) and EGFRvIII (L8A4) to treat composite F98_{EGFR}/F98_{EGFRvIII} tumors in rats. It was observed that the use of a combination of targets was significantly more effective than the use of either boronated antibody alone.²¹⁹ EGFR is amplified in 57% of GBMs, and EGFRvIII is amplified in 36% of GBMs.²²⁰ On the basis of this heterogeneity, it is likely that BNCT will be most effective clinically, using a combination of specific boron carriers and also in combination with other therapeutic modalities.

4. Coupled Targeted Toxins—A number of cytokine receptors have been observed to be upregulated in glioma, including IL-4R and IL-13R α 2. By harnessing these and other receptor-

ligand interactions that are up-regulated in GBM, targeting of tumor cells can be achieved, and by fusing cytokines with toxins, selective cytotoxicity can be achieved.^{221,222}

IL4-PE: Interleukin-4 receptors (IL-4R) are upregulated in glioma cells relative to normal tissue. In fact, 83% of GBM tumors and 86% of astrocytoma tumors were found to be moderately to highly positive for IL-4R *in situ* by Joshi et al.²²³ Puri et al.²²⁴ have developed a chimeric fusion protein, including domains of IL-4 and *Pseudomonas* exotoxin (PE), which is produced by expressing chimeric gen in *E. coli* and purifying the protein using inclusion bodies. Phase I studies have been executed to determine safety and tolerability.^{225,226} It was also reported that in one preliminary study, 6 out of 9 patients with recurrent malignant glioma demonstrated tumor necrosis after receiving IL4-PE via CED with multiple catheters, as evidenced by gadolinium-enhanced MR images. One patient remained disease free for greater than 18 months.²²⁷

IL13-PE: Interleukin-13 receptor (IL-13R) is also found to be overexpressed on a majority of glioma cell lines and resected GBM specimens.²²⁸ A mutated form of PE fused to human IL-13, named IL-13PE38QQR, or cintredekin besudotox (CB), has been developed and has been shown to effect specific cytotoxicity on glioma cell lines.^{228,229} CB has been studied in a number of Phase I clinical trials to investigate dosimetry and catheter positioning and is reportedly more active against glioma cell lines than IL-4-targeted toxins *in vitro*.²²⁹ Among Phase I trials, a total of 51 GBM patients have been treated with CB, with a MST of 42.7 weeks (55.6 weeks for the subset of patients with two or more catheters for drug delivery).²³⁰ A Phase III study compared the efficacy of CB to Gliadel wafers. PFS was longer (17.7 versus 11.4 weeks) in patients treated with CB, but there was no significant difference in the primary end point, MST, between BC and Gliadel wafers.²²⁹ Kioi et al.²³¹ developed a strategy in which PE is conjugated to a smaller scFv anti-IL13R human antibody. Overall, IL-13-based toxins have the potential to be utilized as an adjuvant therapy for malignant glioma pending further positive clinical studies.

Transferrin-Diphtheria Toxin: Another candidate for targeted toxin therapy has been transferrin (Tf). The receptors of this molecule (TfR) are upregulated in GBM and other rapidly dividing cells.²³² Tf-CRM107 is a conjugate of human transferrin and modified diphtheria toxin (DT), which inhibits protein synthesis. The subunit of DT responsible for toxin binding is mutated to generate a 200,000-fold specificity for tumor cells, while maintaining the function of the toxin.²³³ A Phase I study was executed to determine safety and dosing as a primary objective for Tf-CRM107 treatment of patients with progressive glioma after receiving standard care. During this trial, a greater than 50% reduction in tumor size by MRI was achieved in 9 of 15 (60%) of evaluable patients, and a MST of 58.8 weeks was observed.²³³ A multicenter Phase II study followed, and 44 patients with HGG who failed to respond to standard therapy were treated with 1–2 infusions of Tf-CRM107. Complete or partial responses were seen in 35% of patients, and the MST was 37 weeks.²²⁶ Phase III studies of Tf-CRM107 are ongoing (NCT0083447, NCT00088400, NCT87230).

TGF α -PE: As mentioned previously, EGFR is an overexpressed surface molecule in GBM, making it an attractive target for specific therapies. TP-38 (IVAX, Inc., Miami, FL) is a composite of the EGFR-binding ligand TGF- α and a form of PE modified to eliminate nonspecific binding. Twenty patients (19 with HGG) were treated with 1–4 μ g of TP-38. The overall MST was 23 weeks after TP-38 administration. The maximum tolerated dose was not reached.²³⁴ One patient was enrolled after a variety of chemotherapeutic treatments failed to stabilize disease. She has had a remarkable response and has survived greater than 5 years from her initial diagnosis of GBM.²³⁵

B. Adoptive T-Cell Therapy

Adoptive transfer of tumor-reactive autologous CTLs may hold promise as an attractive future immunotherapeutic intervention against malignant glioma. The earlier form of this therapeutic approach was mostly used to treat malignant melanoma, in which autologous lymphocytes infiltrating tumor nodules were first isolated and expanded *in vitro* in the presence of IL-2 and subsequently returned to the patients.²³⁶ In glioma, *ex vivo*-activated lymphokine-activated killer (LAK) cells have been applied as an adjunct to surgery, often in combination with low-dose IL-2.^{237–242} Although clinical responses have been observed in some cases, this approach relies upon innate immune effector cells (i.e., LAK cells), whose killing activity may not be tumor specific. In contrast, antigen-specific CD8⁺ CTLs survey the CNS parenchyma and selectively kill astrocytes that express a model antigen hemagglutinin (HA) without collaterally damaging neurons and oligodendrocytes or myelin. This was demonstrated in an elegant study using HA-specific T cells obtained from a TCR transgenic mouse line and recipient transgenic mice expressing HA in their astrocytes.²⁴³ Importantly, i.v.-injected tumor-specific CTLs have established their anti-tumor potency in syngeneic rodent models of glioma.²⁴⁴ Antigen-nonspecific LAK cells, in contrast, fail to eradicate tumors in most of these experimental models.²⁴⁵ Collectively, these studies demonstrate that CTLs have the capacity to migrate into brain parenchyma.

Since then, the approach has been vastly improved by the use of recent advances in several areas of human T-cell biology, including *in vitro* human T-cell culture and *ex vivo* genetic manipulation. Although adoptive T-cell therapy remains the experimental therapy for limited types of cancers (mainly malignant melanoma), there have been increasing attempts to widen the use of adoptive T-cell therapy to treat other types of tumors, including malignant glioma. This section focuses on recent technological advances in adoptive T-cell therapy as well as current and future adoptive T-cell therapy for malignant glioma, with an emphasis on recent perspectives from human studies because there are several excellent recent reviews on this subject^{246–249} that dedicate large sections to detailed discussions of adoptive T-cell therapy in various mouse tumor models.

1. Fundamentals of Adoptive T-Cell Therapy—Contrary to an active or direct immunization approach, adoptive T-cell therapy involves passive infusion or transfer of autologous CTLs specific for tumor antigens to the host. There are at least two sources of the tumor-specific CTLs. One is PBMCs from the patient. These cells can be expanded *in vitro* through multiple cycles of antigenic stimulation, and, subsequently, cells with a monoclonal specificity to the particular tumor-associated antigen (TAA) will be generated. In theory, infusion of a clonal population of the TAA-specific CD8⁺ T cell to the patient results in the accumulation of TAA-specific CD8⁺ T cells to tumor nodules where they recognize TAA and begin exerting cytotoxic functions on tumor cells. Evidence supporting the effectiveness of this approach came from early studies of adoptive therapy using MART-1, melanoma-associated antigen specific CD8⁺ T cells.²⁵⁰ After infusion, *in vitro*-expanded MART-1-specific CD8⁺ T-cell clones from a patient with metastatic melanoma localized to melanoma and normal skin tissues where substantial destruction of melanocytes and other cells expressing MART-1 were observed. The later study using low-dose IL-2 administration, in addition to infusion of MART-1 and gp100-specific CTL clones, for refractory, metastatic melanoma showed that low-dose administration of IL-2 enhanced infiltration and localization of MART-1 and gp100-specific CD8⁺ T-cell clones preferentially to the tumor tissues and had favorable antitumor responses in 8 out of 10 patients out to 21 months follow-up.²⁵¹

Another important source of the TAA-specific CTLs is the tumor nodule. The tumor nodule typically contains tissue-infiltrating TAA-specific CTLs that could be first polyclonally expanded in *ex vivo* tissue culture in the presence of IL-2 and later selected for the TAA

specificity.²⁴⁷ The use of *ex vivo*-expanded TILs has been central to adoptive T-cell therapy for malignant melanoma. Infusion of these *ex vivo*-expanded TILs to treat an advanced stage melanoma initially met challenges, such as low response rates associated with poor persistence of infused TILs in patients.²⁵² However, the later modification, lymphodepletion prior to the infusion of TILs, produced a vast improvement in the efficacy of TIL-based adoptive T-cell therapy for metastatic melanoma, as demonstrated by 50% objective response rate reported recently.²⁵³ The mechanism by which lymphodepletion prior to TIL infusion dramatically improves therapeutic effects is not fully understood. It has been suggested that lymphodepletion in the absence of myeloablation removes immunoregulatory elements (e.g., regulatory T cells) and increases production of lymphokines, such as IL-21, and physical spaces in secondary lymphoid organs, allowing infused TIL to expand with less competition from pre-existing lymphocytes.

2. Genetic Manipulation of TAA-Specific CD8⁺ T Cells *Ex Vivo*—Similar to other experimental tumor immunotherapies, adoptive T-cell therapy faces several technical hurdles. However, unlike other tumor immunotherapies, for adoptive therapy, a majority of such hurdles could be overcome by *ex vivo* genetic manipulation of T cells prior to transfer. For instance, the majority of TAAs are poorly immunogenic, and it is often difficult to raise CTLs that possess T-cell receptors (TCRs) that have sufficiently high avidity for use in adoptive therapy. One of the ways to solve this problem is to systematically search TAA-specific CD8⁺ T cells for clone(s) with higher TCR avidity, clone TCR α and β genes, and exogenously express the high-avidity TCR in bulk CD8⁺ T cells. Li et al.²⁵⁴ recently utilized phage display to systematically search for a high-avidity TCR against an HLA-A0201-restricted epitope in a TAA NY-ESO-1.²⁵⁵ Moreover, adoptively transferred high-avidity TCR transgenic T cells demonstrated sustained high levels of circulating, engineered cells at 1 year after infusion in 2 of 15 patients, both of whom demonstrated objective regression of metastatic melanoma lesions,²⁵⁶ suggesting the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

An interesting alternative to the expression of high-avidity TCR-transgenic T cells is to express a chimeric molecule that has antigen-binding domains of a MAb fused with a signal-transduction domain of CD3,²⁵⁷ namely, chimeric antigen receptors (CAR). A significant advantage of CARs over TCRs is that the antigen recognition is not restricted by the expression of certain MHC class I molecules, and, recently, CARs have been used to treat malignant gliomas,²⁵⁸ ovarian cancer,²⁵⁹ pediatric neuroblastomas,²⁶⁰ and renal carcinoma,²⁶¹ among others. A recent promising development with a slight variation to this approach is a single-chain CAR that targets CD19²⁶² and a single-chain chimeric TCR-IL-2 signal-transduction domain.²⁶³

An underlying theme of these modern adoptive T-cell approaches is to design a surface receptor that has defined antigen specificity and is able to trigger T-cell activation but overcomes several limitations of using transgenic TCRs. However, overexpression of high-avidity TCR or nonphysiological expression of CAR may have unwanted consequences. Frequent exposure to TAAs may render these engineered T cells functionally unresponsive, similar to the unresponsive CD8⁺ T cells specific for antigens derived from viruses that establish persistent infection. In fact, there is an accumulating line of evidence suggesting that functional unresponsiveness of the tumor-specific CTL is mediated by the expression of PD-1.^{264–269} It is possible that lengthy *ex vivo* expansion induces high levels of PD-1 expression on a majority of TAA-specific CD8⁺ T cells. However, it may also be possible to overcome this problem by administering blocking anti-PD-1 or anti-PD-L1 antibodies, as reported in a case for correcting unresponsive HIV-1-specific CD8⁺ T cells in persistent HIV-1 infection.²⁷⁰ The use of humanized anti-PD-1 antibody is currently being tested in a randomized Phase I clinical trial (272).²⁷¹

Another related issue is whether a majority of *ex vivo*-activated cells for adoptive transfer become enriched in an effector T-cell subset (T_E) during *ex vivo* expansion. T_E are generally considered to be terminally differentiated CTLs that have the highest cytotoxic capacity but lack appreciable proliferating capacity.²⁷² A currently prevailing notion is that because these cells are terminally differentiated, they will not be able to establish a long-term persisting population of TAA-specific CTLs. Clearly, there is a significant association between clinically favorable responses and the persistence of *ex vivo*-expanded melanoma-specific CTL clones after infusion.²⁷³ Moreover, persisting melanoma-specific CTL clones expanded *ex vivo* prior to transfer appear to have relatively shorter telomere length, indicating that the higher replicating capacity of transferred CTLs is a prerequisite for their persistence.²⁷⁴ Interestingly, these *ex vivo*-expanded CTLs ($CCR7^-CD27^-CD45RA^+CD28^-$) clearly indicate the T_E phenotype. However, these cells appear to re-express an immature differentiation marker, CD27, after infusion. It is known that once $CD8^+$ T cells lose expression of these markers as a part of the differentiation process, they do not re-express such markers *in vivo*.²⁷⁵ This apparent contradiction is most likely due to the fact that *ex vivo* expansion involves a milieu of cytokines exogenously added at nonphysiological concentrations. In fact, it has been shown that re-expression of CCR7 on CMV-specific $CD8^+$ T cells, a prototypical T_E ($CD27^-CD45RA^+$), is only induced in the presence of high levels of IL-15 after a week of antigenic stimulation.²⁷⁶ Furthermore, exposure to a high level of IL-2 restored CD28 expression on otherwise $CD28^-$ CMV-specific and HIV-1-specific $CD8^+$ T cells.²⁷⁷ Restoration of CD27 and CD28, but not CCR7 and CD62L expression, is also seen on melanoma-specific $CD8^+$ T cells 2 months after transfer.²⁷⁸ The gain of CD27 expression by transferred CTLs appears to be particularly important because CD27 is the critical molecule for long-term persistence²⁷⁹ and expansion²⁸⁰ of transferred $CD8^+$ T cells.

3. Adoptive T-Cell Therapy for Glioblastoma: Present and Future—Although a number of clinical trials using adoptive T-cell therapy for malignant glioma are very limited when compared to clinical trials using active immunization strategies, there are currently at least three active clinical trials for GBM using adoptive T-cell transfer according to the NIH clinical trial database (www.clinicaltrials.gov). Interestingly, two of such clinical trials involve *ex vivo* expansion of autologous $CD8^+$ T cells that are specific for a CMV antigen (refer to Section III.C.1).

Apparently, adoptive T-cell therapy for malignant gliomas needs to incorporate recent advances in the repertoire of target antigens and the engineering of T cells. First, one of the most important features missing in current clinical trials for malignant gliomas is use of the glioma-associated antigen (GAA)-derived epitopes to generate and expand $CD8^+$ T cells (refer to Section III.C.1). These currently include HLA-A*02-restricted IL-13R α 2,²⁸¹ TRP-2,²⁸² AIM-2,²²² HER-2, gp100, MAGE-1,²⁸³ EGFRvIII,²⁸⁴ EphA2,²⁸⁵ and EphB6.²⁸⁶ These GAA epitopes have been preclinically validated, and a majority of these peptides have been used in various clinical trials of DC-mediated active immunization against malignant gliomas.

As the list of GAA epitopes grows, it is necessary to generate a library of human CTL clones against GAAs and test their TCR avidities from which high-avidity CTL clones will be selected and further expanded. As previously discussed, sources of CTL clones could be TILs isolated from tumor biopsies and PBMCs. Once a high-avidity CTL clone is selected and established, TCR usage can be identified and subsequently used to build a library of high-avidity TCR gene pairs from which transgenic CTLs (expressing cloned high-avidity TCRs tailored to the particular GAA epitopes) could be generated. In this context, it will be of interest to identify long-term controllers²⁸⁷ or active DC immunization responders who may naturally possess high-avidity $CD8^+$ T cells specific for GAAs and characterize TCR usage of GAA-specific CTLs from such individuals. Alternatively, CTL clones could be generated from transgenic mice expressing human HLA molecules upon antigenic challenge. An advantage of this

approach is that mouse T cells naturally have relatively higher avidity for epitopes derived from human antigens,²⁸⁸ and appear to form a functional signal-transduction complex with human CD3.²⁸⁹ A major caveat of this approach is that mouse proteins are naturally immunogenic; the extent to which expression of such mouse, or even humanized, TCRs affect persistence of transferred transgenic T cells in humans is unknown. Moreover, human T cells bearing transgenic high-avidity mouse TCRs may run the risk of losing effector functions and become functionally unresponsive upon tumor infiltration, as discussed previously. In fact, a recent observation shows that high-avidity mouse CTLs differentiate into effectors in the draining lymph nodes and become functionally unresponsive after tumor infiltration.²⁹⁰ Thus, CTLs expressing either endogenous or exogenous high-avidity TCRs may not maintain functionality *in vivo*.

4. Summary—Adoptive T-cell therapy may emerge as one of the most promising cancer immunotherapy modalities. Today's adoptive T-cell therapy employs cutting edge methodologies involving *ex vivo* expansion as well as genetic manipulation of TAA-specific T cells prior to the transfer of such cells. Despite a promising future, adoptive T-cell therapy has to be further refined to overcome several of the major problems, including T-cell suppression by Tregs, inadequate persistence of transferred T cells, and undirected migration of T cells. Adoptive T-cell therapy for malignant gliomas is no exception to these concerns. In fact, it faces many similar and more difficult challenges. In particular, the microenvironment of malignant glioma poses a significant problem, such as the presence of the BBB and the production of a variety of immunosuppressive substances. Nevertheless, adoptive T-cell therapy is better suited to circumventing these problems than active immunotherapy because adoptive therapy allows direct *ex vivo* manipulations of TAA-specific CTLs to give these cells enhanced or novel functions that are otherwise impossible *in vivo*. Thus, it may be possible to redirect trafficking of transferred CTLs to the site of gliomas simply by exogenously expressing chemokine receptors (e.g., CXCR3)⁴⁷ and integrin receptors (e.g., very late antigen [VLA]4)^{45,48} that dictate efficient homing of T cells to gliomas. It should also be mentioned that adoptive T-cell therapy is not limited to the transfer of CD8⁺ T cells. There are NK cells and TAA-specific CD4⁺ T cells that can be isolated, expanded, and transferred back to the autologous cancer patient. It may be possible to overcome some of these problems discussed previously by the simultaneous transfer of TAA-specific CD4⁺ T cells, providing necessary helper functions to TAA-specific CTLs. Historically, therapeutic values of TAA-specific CD4⁺ T cells in human cancer treatment have been long neglected until recently shown by a study demonstrating beneficial clinical responses to malignant melanoma by the transfer of *ex vivo*-expanded melanoma-specific CD4⁺ T cells.²⁹¹ Considering recent developments, the future of adoptive T-cell therapy against malignant gliomas appears promising. With further innovation and refinement of *ex vivo* T-cell manipulation, adoptive T-cell therapy may become a mainstream therapeutic intervention for malignant gliomas.

C. Active Immunotherapy (Tumor Vaccines)

1. T-Cell Epitopes Derived from Glioma-Associated Antigens—There is a large body of reported studies that have demonstrated the safety and preliminary efficacy of whole glioma cell-based vaccine approaches.^{292–295} However, the use of whole glioma cell-derived antigens, such as glioma lysate, may limit the feasibility and safety of the approach because of the cumbersome preparation procedures and theoretical concerns of autoimmune encephalitis.²⁹⁶ Therefore, the effectiveness and safety of T-cell-mediated immunotherapy of glioma depends on the proper selection of the targeted antigens—that is, GAAs and, more specifically, the CTL epitopes in GAAs. In addition, the use of “off-the-shelf” synthetic peptides coding GAA-derived CTL epitopes may be feasible, especially for multicenter clinical trials. Table 1 lists GAA epitopes known to elicit T-cell responses. The following section discusses selected human GAA-derived epitopes that appear to be promising on the basis of relatively restricted

expression (compared with the normal brain) and biological significance in gliomas, as well as well-characterized immunogenicity. The review primarily focuses on HLA-class I-restricted CTL epitopes. However, the growing body of knowledge on other CTL and helper T-cell epitopes will expand our future options to encompass more efficient immunotherapy for glioma.

IL-13R α 2: IL-13R α 2 is a membrane glyco-protein that is overexpressed by >80% of malignant gliomas but is not expressed in normal brain tissues at detectable levels or at high levels in other normal organs, except for testes.³⁰⁹ Although the function of IL-13R α 2 has not been fully elucidated,^{310,311} IL-13R α 2 has attracted significant attention as a target for glioma therapy.²⁵⁸ We recently found that an analogue peptide of natural IL-13R α 2₃₄₅₋₃₅₃,²⁸¹ in which the first and ninth amino acid residues, tryptophan and isoleucine, have been replaced by valine and alanine, respectively, can elicit a greater CTL response against HLA-A2⁺, IL-13R α 2⁺ glioma cells compared to the natural peptide (IL-13R α 2_{345-353:1A9V}).³¹² Recently, an HLA-A24-restricted CTL epitope was identified in IL-13R α 2, expanding the target patient population for vaccines targeting IL-13R α 2.²⁹⁷

EphA2: EphA2 is a tyrosine kinase receptor that plays a role in carcinogenesis.^{313,314} In normal cells, EphA2 localizes to sites of cell-to-cell contact,^{315,316} where it may negatively regulate cell growth. EphA2 is frequently overexpressed and often functionally dysregulated in advanced cancers, contributing to their malignant characters.³¹⁷ We have reported that EphA2₈₈₃₋₈₉₁ can elicit an HLA-A2-restricted CTL response against glioma cell lines.²⁸⁵ Recent studies by us²⁸⁵ and others³¹⁸ have revealed that a majority of malignant gliomas express high levels of EphA2. Furthermore, EphA2 mRNA overexpression was found to correlate inversely with survival in a panel of 21 GBMs.³¹⁹ These findings support the idea that targeting of EphA2 by immunotherapy may provide a major impact on controlling tumor growth and prolonging patients' survival.

Survivin: Survivin is an apoptosis inhibitor protein overexpressed in most human cancers, and inhibition of its function results in increased apoptosis.³²⁰ The induction of cytotoxic immunity against Survivin may, therefore, be an attractive strategy.³²¹ Survivin has multiple T-cell epitopes, including Survivin₉₆₋₁₀₄ as an HLA-A2-restricted CTL epitope.^{299,300} Moreover, vaccination of a pancreatic cancer patient with a modified Survivin epitope-peptide, Survivin_{96-104:2M}, in which the second residue threonine was replaced by methionine, induced complete remission of liver metastasis.³²² Further, vaccinations using DCs loaded with Survivin₉₆₋₁₀₄ induced positive IFN- γ ELISPOT responses in four out of five patients with advanced melanoma.³²³ A recent immunohistochemical study demonstrated that 100% of astrocytoma specimens (n = 29; grades II–IV), but not normal brain tissues, contain Survivin-positive cells.³⁰¹ The mean percentage of immunoreactive cells in each specimen was 70.0 in grade II, 81.3 in grade III, and 85.0 in grade IV. Interestingly, high-level expression of Survivin was correlated with poor prognosis in patients with grade II or III astrocytomas.³⁰¹

Wilm's Tumor (WT)1: WT1 is the product of the Wilm's tumor gene. WT1 is a transcription factor oncogene, overexpressed in various types of leukemia and solid tumor cells.^{304,324} Inhibition of WT1 in leukemic cell lines led to a decrease in proliferation and an increase in apoptosis of tumor cells,³²⁵ implying that the elimination of tumor cells that overexpress WT1 may allow efficient control against tumor growth. Two 9-mer HLA-A2-binding WT1-derived peptides (WT1₁₂₆₋₁₃₄ and WT1₁₈₇₋₁₉₅) have been demonstrated to sensitize CD8⁺ antigen-specific CTLs.³⁰³ Also, HLA-24-restricted epitopes have been characterized and tested in cancer vaccine trials.³²⁶ In human astrocytomas, 48 of 51 primary GBM tissue samples showed immunohistochemical staining for the WT1 protein.³²⁷ A recent quantitative evaluation of WT1 in grades I–IV astrocytomas showed that WT1 protein was expressed in 5 of 6 low-grade (grades I–II) and in 18 of 18 high-grade (grades III–IV) astrocytomas, with a

trend of higher expression levels in high-grade astrocytomas. The WT1 protein was not detected in the normal glial cells contained in the tumor specimens.³⁰² Furthermore, a recent Phase II study of vaccinations using an HLA-A24-restricted WT1 peptide in patients with recurrent GBM has demonstrated complete or partial response in 2 of 21 (9.5%) patients and median PFS at 20 weeks.³²⁸

Sry-Related High-Mobility Group Box (SOX): SOX is a family of transcriptional cofactors implicated in the control of diverse developmental processes and exhibits highly dynamic expression patterns during development of diverse tissues and cell types, especially during embryogenesis.³²⁹ In addition, SOX genes are amplified or up-regulated in different tumors and tumor cell lines (reviewed in Ref. 142). Indeed, SOX2, SOX5 (335),³³⁰ SOX6,³³¹ and SOX11³⁰⁶ were highly expressed in glioma cell lines and a majority of glioma tissues tested. SOX5 and SOX6 induced specific IgG responses in one third of the glioma patients.^{330,331} Patients with GBM who showed IgG responses against SOX5 exhibited significantly better survival periods than GBM patients without SOX5 antibodies.³³⁰ DNA vaccinations with SOX6 exerted protective and therapeutic antitumor responses in the glioma-bearing mice.³³² SOX2- and SOX11-derived peptides were able to elicit HLA-A2-restricted CTL responses against glioma cell lines.^{305,306} Their preferential expression in glioma and immunogenicity indicate that SOX proteins are attractive targets for immunotherapy. Furthermore, a recent study demonstrated that SOX2 was expressed in neural stem cells and GBM tumor-initiating cells, and it played a role in the maintenance of self-renewal capacity of neural stem cells, as well, when they acquired cancer properties,³³³ suggesting that the SOX2-derived CTL epitope may induce CTL responses against glioma-initiating cells, which are likely to be responsible for the malignant behavior of cancer due to resistance to chemotherapy and radiotherapy.
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HER-2/neu: HER-2/*neu* is a transmembrane glycoprotein and member of the EGFR family. HER-2/*neu* promotes tumor-cell proliferation, migration, adhesion, and angiogenesis, and decreases apoptosis.³³⁹ HER-2/*neu* is widely expressed in a variety of normal tissues, but selectively overexpressed in various tumors, such as breast, ovarian, colorectal, pancreatic, and renal-cell cancers.^{338,340–343} HER-2/*neu* protein expression was detected in 76% of GBM primary cell lines and an HLA-A2-restricted HER-2/*neu*-specific CTL clone recognized GBM cells.²⁸³ Moreover, Her-2/*neu* protein overexpression was found to correlate inversely with survival in a panel of 149 GBMs,³⁴⁴ suggesting that HER-2/*neu*-targeting immunotherapy for GBM may provide a major impact in controlling tumor growth and prolonging patients' survival.

EGFR vIII: The type III variant of the EGFR mutation (EGFR vIII) is present in 30%–50% of patients with GBM. Saikali et al.³⁴⁵ extensively evaluated the expression of nine tumor antigens in human GBM in both mRNA and protein levels in a series of 47 GBM cases. Among nine antigens, including ALK, EGFRvIII, GALT3, gp100, IL-13R α 2, MAGE-A3, NA17-A, TRP-2, and tyrosinase, it was found that EGFRvIII and IL-13R α 2 are expressed most frequently and restrictedly (vs. normal brain samples) in primary GBM cases. A peptide encoding the amino acid sequence for EGFRvIII (1–13) was shown to induce modest IFN- γ and CTL responses against HLA-A0201⁺ EGFRvIII-transfected U87 glioma cells.²⁸⁴

Squamous Cell Carcinoma Antigen Recognized by T Cells 1 (SART-1): SART-1 was identified as a gene-coding tumor antigen recognized by a squamous cell carcinoma-specific CTL line using the expression-gene cloning methods.³⁰⁸ SART-1 was expressed in various cancers, including glioma, but not in normal tissues, except for testes.³⁰⁷ A SART-1-derived peptide induced an HLA-A24-restricted CTL response against glioma cell lines. However, there is a paucity of information in the literature regarding the roles of SART-1 in neoplastic

cells and the association between SART-1 expression and the clinical features of glioma patients.

Cytomegalovirus (CMV) as a Potential Target: Cobbs et al.³⁴⁶ reported that a high percentage of malignant gliomas are infected with human CMV, suggesting that this virus plays an active role in glioma pathogenesis. Although there was some controversy over these observations, recent studies demonstrated the presence of CMV in GEM^{347,348} and even in low-grade gliomas.³⁴⁹ These studies suggest that the presence of human CMV in glial tumors could serve as an immunotherapeutic target in glioma. The facilitation of an immune response against viral antigens contrasts with the difficulty of immunization against self-antigens. It will be intriguing to introduce the CMV-derived epitope to multiepitope-based vaccines for glioma.

Although the list of available GAA-derived CTL epitopes is growing, it is also important to point out the possibility that immune responses to GAAs are not restricted to HLA-A2, A24, or other HLA-A alleles. Other HLA alleles, including HLA-B and HLA-C, clearly present GAA epitopes and these epitopes could potentially be more immunogenic than those presented by the HLA-A allele. There is limited but significant evidence indicative of this notion. Immune responses to HLA-B-restricted HIV-1 epitopes typically have greater magnitude than those restricted by HLA-A alleles.³⁵⁰ CD8⁺ T cells specific for HLA-B-restricted HIV-1 epitopes have greater polyfunctionalities than the ones specific for HLA-A-restricted HIV-1 epitopes.³⁵¹ Clearly, new GAA epitopes will be discovered in the future, although the safety and immunogenicity of these peptides must be further evaluated concurrently in the clinical trials.

2. Active Vaccination Strategies for Gliomas—Although numerous preclinical studies in mouse models have shown the efficacy of peripheral vaccinations against intracranial gliomas (reviewed in Ref. 255), therapeutic vaccines face a substantial challenge in glioma patients because they must overcome a variety of immunoregulatory mechanisms that have already established the immune escape of tumors, as discussed in the previous section. Nevertheless, a number of clinical trials have been attempted to generate therapeutic immune responses against gliomas and have shown some positive effects. Strategies that have been most studied are categorized as follows: (1) whole glioma-cell vaccines, (2) peptide-based vaccines targeting glioma-associated antigens, and (3) DC vaccines (Tables 2 and 3).

Whole Glioma-Cell Vaccines: Initial vaccination strategies for gliomas consisted of subcutaneous inoculations of irradiated, autologous,³⁷⁰ or allogeneic^{167,371} glioma cells. This type of vaccine has the advantage of providing a panel of multiple potential GAAs that are naturally expressed by glioma cells. Autologous glioma cells, in particular, should allow immunizations against the most relevant GAAs expressed in the patient's tumor (i.e., tailored medicine). Potential downsides of this approach, however, include (1) cumbersome procedures and quality control (QC)/quality assurance (QA) issues associated with large-scale cultures of autologous glioma cells (Table 2) and (2) theoretical risks of autoimmune encephalomyelitis (reviewed in Ref. 370). Nevertheless, this type of vaccine strategy has been carefully examined. Schneider et al.³⁵⁹ and Steiner et al.³⁶² recently reported pilot clinical trials using autologous glioma cells modified with Newcastle-Disease-Virus, which is known to serve as a vaccine adjuvant and, therefore, to improve the efficacy of glioma vaccines. More recently, Ishikawa et al.³⁶⁸ reported a Phase I clinical trial using formalin-fixed glioma tissues as a source of antigens. The advantage of this strategy is that formalin fixation preserves the specific antigenicity of glioma cells. These studies reported no major adverse events.

Peptide-Based Vaccines Targeting Glioma-Associated Antigens: In vaccines using synthetic peptides for shared GAA-epitopes, advantages and disadvantages are distinct from those in whole glioma-cell approaches. Although synthetic GAA peptide-based vaccines may not adequately target antigens in each patient's tumor, these vaccines have less concern for

autoimmunity and provide “off-the-shelf” feasibility. Indeed, a wide range of peptide-based vaccines have been evaluated. Yajima et al.³⁶⁶ reported a Phase I study of peptide-based vaccinations in patients with recurrent malignant gliomas. In this study, prior to the first vaccine, each patient’s PBMCs were evaluated *in vitro* for cellular and humoral responses against a panel of antigens, and peptides that induced a positive response were used for vaccinations. The regimen was well tolerated and resulted in an 89-week median survival of treated patients. However, there is little evidence that the antigens used in this study are expressed in gliomas at high levels. More recently, Izumoto et al.³²⁸ reported a Phase II clinical trial using a single WT1 peptide. In this study, they reported a median PFS of 20 weeks and a possible association between the WT1 expression levels and clinical responses. When single or oligo antigens are selected and targeted by vaccines, it also seems necessary to harness the concepts of epitope spreading to address the problems of tumor immune escape, while avoiding the augmentation of deleterious CNS autoimmune responses.³⁷²

DC Vaccines: DCs are the most potent APCs, driving the activation of T cells in response to invading microorganisms.³⁷³ The ability to culture DCs from human peripheral blood monocytes has generated significant interest in using DCs in novel cancer-vaccination strategies.³⁷⁴

Yu et al.³⁵⁶ reported a Phase I trial of vaccinations using DCs pulsed with peptides eluted from autologous glioma cells. Later, Liao et al.²⁹² also reported a Phase I trial in patients with newly diagnosed GBM using DCs pulsed with acid-eluted glioma peptides. In this study, the authors reported the median overall survival as 23.4 months and that the benefit of the vaccine treatment was more evident in the subgroup of patients with slowly progressing tumors and in those with tumors expressing low levels of TGF- β 2.

However, pulsing DCs with eluted peptides requires a large culture of autologous glioma cells and time-consuming procedures, for which QC/QA is not always feasible. To overcome this issue, glioma-cell lysate has been used to pulse DCs in a number of trials.^{294,295,360,363,365,375} Yamanaka et al.^{360,365} reported a Phase I/II study using DC pulsed with glioma lysate. Patients received either DCs matured with OK-432 or DCs without OK-432-mediated maturation. GBM patients receiving mature DCs had longer survival than those receiving DCs without OK-432-mediated maturation. Furthermore, patients receiving both intratumoral and intradermal DC administrations demonstrated longer overall survival than those with intradermal administrations alone.³⁶⁵ Recently, Wheeler et al.²⁹⁴ reported a Phase II clinical trial with lysate-pulsed DCs. IFN- γ production levels from postvaccine PBMC correlated significantly with patients’ survival and time to progression.

Fusion of glioma cells and DCs represents a unique cancer-vaccine strategy. Fused cells can effectively present antigens derived from tumor cells.³⁷⁶ Kikuchi et al.^{358,364} reported a study using DCs fused with glioma cells by polyethylene glycol, with or without concomitant administration of IL-12. Although there were no serious adverse effects, the study did not clearly demonstrate the effects of IL-12 administration on immunological and/or clinical response.³⁶⁴ Further trials, including the optimization of the IL-12 dosage, are needed to draw any conclusions.

Recently, a small subset of tumor cells in GBM has been identified by their unlimited self-renewal ability and remarkable *in vivo* tumor-forming capability.³⁷⁷ These brain tumor stem cells (BTSCs) or glioma stem cells (GSC), which have been isolated by the neural stem-cell marker CD133,³⁷⁸ are believed to play a major role in tumor growth as well as resistance against radiation³³⁵ and chemotherapy.³⁷⁹ However, their susceptibility to immunotherapeutic attack has not been extensively tested. Immunologic targeting of the GL261 murine glioma using DCs pulsed with lysate derived from *in vitro*-cultured GL261

stem cells was more efficient than DCs pulsed with lysate of conventional tumors.³⁸⁰ Thus, identification of antigens expressed in GSCs and development of immunotherapies targeting GSCs is a promising approach.

To date, no consensus exists on the optimal DC subtypes, the optimal conditioning and activation stimuli, the optimal route of administration, and the optimal dose and frequency of DC vaccinations.³⁸¹ We recently reported a pilot vaccine study using autologous type 1-polarized DCs (α DC1s) with IL-4-transfected fibroblasts.²⁹³ This strategy is based on the previous studies demonstrating that (1) α DC1s produce high levels of IL-12 and promote type 1 adaptive immunity,^{382,383} and (2) local paracrine production of IL-4 in vaccine sites promotes the accumulation and maturation of IL-12-secreting tumor-infiltrating DCs.³⁸⁴ Because this was a pilot study with only 1×10^6 α DC1/vaccination, there were no detectable immunological or clinical responses. However, fostered by the feasibility data for production of α DC1 in vaccine trials, we recently implemented a Phase I/II trial in patients with recurrent WHO grades III or IV glioma to evaluate the safety and induction of anti-GAA immunity of a vaccine strategy that employs intranodal injections of α DC1s ($1-3 \times 10^7$) loaded with 4 human GAA peptides (EphA2₈₈₃₋₈₉₁, IL-13R α ₂₃₄₅, 1A9V, YKL-40₂₀₁₋₂₁₀ and GP100₂₀₉₋₂₁₇:M2) in combination with intramuscular injections of poly-ICLC (20 μ g/kg; twice per week) (University of Pittsburgh Cancer Institute protocol #05-115). To date, we have enrolled a total 13 patients and completed 4 scheduled vaccinations per patient in 10 participants. No CTCAE Grade 3 or higher adverse events were reported. None of the patients enrolled in this trial have demonstrated clinical indices, radiological signs, or laboratory data suggestive of auto immunity. Immune responses have been evaluated in 9 participants who received at least 4 vaccinations, and 7 participants demonstrated detectable positive responses against at least one of the GAAs in ELISPOT and/or tetramer assays. As type 1 immunity is the optimal adaptive immune response^{45,47,48} for CNS tumor immunotherapy, α DC1-based vaccine approaches seem to hold promise.

Several pilot and Phase I/II clinical studies of active vaccination have been undertaken in patients with glioma. Despite the fact that feasibility and safety have been sufficiently documented in most studies, clinical efficacy has not yet been convincingly proven. Although some studies demonstrated improved survival of patients and objective clinical responses, the ultimate judgment for clinical activity has to be made by rigorous evaluation in randomized studies.

IV. CONCLUDING REMARKS

We reviewed recent progress in the field of brain and brain-tumor immunology. We also reported recent progress and current challenges in immunotherapeutic strategies for brain tumors. In the field of CNS and CNS-tumor immunology, in the last decade, novel immunoregulatory mechanisms, such as B7-H1, have been identified and added to the list of classical immunosuppressive molecules, including TGF- β and IL-10. It is clear that the CNS and CNS tumors are equipped with numerous and layered immunosuppressive and immune-escape mechanisms, perhaps including ones that we have not yet identified. These discoveries, however, allow us to develop strategies to overcome each of these mechanisms.

Remaining unique challenges against gliomas include relative difficulties in obtaining tumor tissues following immunotherapeutic treatments. Unlike other cancers, such as cutaneous melanoma, intracranial glioma tissues are not readily accessible following vaccine treatment. Designing neoadjuvant settings with vaccines is not always feasible because recurrent malignant gliomas, for which surgical resection is clinically indicated, typically do not allow us to wait for weeks before surgery and often require treatment with high-dose corticosteroids.

As reviewed in this article, the concept of immunotherapy has a diverse scope of strategies and target molecules. Extensive review of each field in this article has led us to identify the challenge for each strategy. Such challenges, however, may be overcome by appropriate combinations with other strategies. For example, adoptive-transfer strategies may need to be combined with appropriate adjuvants and/or vaccinations to promote long-lasting memory responses and antitumor immunosurveillance. Use of targeted-toxin therapy may facilitate the release of tumor antigens, thereby inducing T-cell immune responses against a variety of T-cell epitopes (i.e., epitope spreading). Such events may be harnessed by concurrent vaccinations against multiple epitopes and/or the blockade of appropriate immune-escape mechanisms. However, when each of these agents are owned by separate industries with intellectual properties, such creative combinatorial strategies may not be implemented as efficiently as we would wish. Although several early-phase clinical trials demonstrated promising therapeutic outcomes, to date, clinical trials of immunotherapy for gliomas have not yet demonstrated objective proof of clinical efficacy in rigorous Phase II and III studies. The eventual success of immunotherapies for brain tumors will be dependent upon not only an in-depth understanding of immunology behind the brain and brain tumors, but also the implementation of molecularly targeted trials that address multiple layers of challenges in gliomas.

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ABBREVIATIONS

AA	anaplastic astrocytoma
APCs	antigen-presenting cells
B7-H1	B7-homologue 1
BBB	blood brain barrier
BED	biological effective dose
BNCT	boron neutron capture therapy
BPA	boronophenylalanine
BTSCs	brain tumor stem cells
CAR	chimeric antigen receptor
CB	cintredekin besudotox
CED	

	convection-enhanced delivery
CLNs	cervical lymph nodes
CMV	cytomegalovirus
CNS	central nervous system
CSF	cerebral spinal fluid
CTL	cytotoxic T lymphocytes
DCs	dendritic cells
DT	diphtheria toxin
EAE	experimental autoimmune encephalitis
EBRT	external beam radiotherapy
ECD	extracellular domain
EGFR	epidermal growth factor receptor
EM	electron microscopy
GAA	glioma-associated antigen
GANGs	ganglio-sides
GBM	glioblastoma multiforme
GCS	glioma cell supernatants
GIM	glioma-infiltrating macrophages
GSC	glioma stem cells
HA	hemagglutinin

HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
ISF	interstitial fluid
JAM	junctional adhesion molecule
LAK	lymphokine-activated killer
LFA	lymphocyte function-associated molecule
MAb	monoclonal antibody
MCP-1	macrophage chemoattractive protein
MHC	major histocompatibility complex
MS	multiple sclerosis
MST	median survival time
NK	natural killer
NVU	neurovascular unit
PBMCs	peripheral blood mononuclear cells
PD-L1	programmed death ligand-1
PE	<i>Pseudomonas exotoxin</i>
PFS	progression-free survival
PG	prostaglandin
PI3K	phosphatidylinositol-3-OH kinase

PLP	myelin proteolipid protein
PTEN	phosphatase and tensin homolog
RCAS1	receptor-binding cancer antigen expressed on SiSo cells
RIT	radioimmunotherapy
SAC	<i>Staphylococcus aureus</i> Cowen I strain
SART-1	squamous cell carcinoma antigen recognized by T cells 1
SOX	Sry-related high-mobility group box
TAA	tumor-associated antigen
TCR	T-cell receptor
T_E	effector T-cell subset
Tf	transferrin
TGF	transforming growth factor
Tg⁺	transgenic
TILs	tumor-infiltrating lymphocytes
TJ	tight junction
TLR	Toll-like receptor
TMZ	temozolomide
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor
VLA	very late antigen

WHO

World Health Organization

WT

Wilm's Tumor

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Glioma-Associated Antigens

TABLE 1

Antigen peptide	Expression in cancers	Expression in normal Tissues	Roles in tumorigenesis	HLA restriction	Refs.
IL-13R+2	Gli, CC	Testis	ND	A*0201, A*2402	281,297,309,312
EphA2	Gli, CC, OC, Bla, GC, RCC, Pro, EC	Sites of cell-to-cell contact	Regulation of angiogenesis induced by tumors	A*0201	285,298,315
Survivin	Gli, Mel, PC, LC, CC, BC, Pro	Undetectable in most differentiated normal adult tissues	Apoptosis inhibition in tumor progression	A*0201, A*2402, A1, A3	299,300,301,320
WT1	Gli, AML, LC, BC	Kidney, bone marrow, pleura, testis, ovary, hemopoietic stem cells	Cell proliferation, apoptosis inhibition	A*0201, A*2402, A1	302, 303, 304, 324, 326
SOX2	Gli	Neural stem cells, fetal brain, testis	Proliferation of glioma-initiating cells	A*0201	305, 306, 334
SOX11	Gli	Fetal brain	ND	A*0201	306
HER2/neu	Gli, BC, CC, PC, OC, RCC, Medu	Ubiquitously expressed	Cell proliferation, apoptosis inhibition	13 HLA class I epitopes	283,340,341,342
EGFRvIII	Gli, LC, CC, OC, GC, EC, RCC, Bla, PC	No	Cell proliferation	A*0201	284
gp100	Gli, Mel	Melanocyte, retina	ND	16 HLA class I epitopes	283
MAGE-1	Gli, Mel, HCC, BC, RCC	Testis, placenta	ND	11 HLA class I epitopes	283
TRP-2	Gli, Mel	Melanocyte, retina	ND	A*0201	282
AIM-2	Gli, Mel, CC, BC, OC	Liver, testis	ND	A1	283
SART-1	Gli, EC, HCC, RCC, BC	Testis	ND	A*2402, A*2601	307, 308

Note: Gli = glioma, Mel = melanoma; CC = colorectal cancer; LC = lung cancer; GC = gastric cancer; BC = breast cancer; HCC = hepatocellular cancer; OC = ovarian cancer; PC = pancreatic cancer; EC = esophageal cancer; RCC = renal cell cancer; Pro = prostate cancer; Bla = bladder cancer; Medu = medulloblastoma; AML = acute myeloid leukemia.

TABLE 2

Published Glioma Vaccine Trials

Authors	Pts	Diagnosis	Study design	Type of vaccination	Outcome	Indicators for better results
Holladay et al. ³⁵²	15	GBM,AA	Phase I	Autologous irradiated glioma cells and BCG	7 PR	
Plautz et al. ³⁵³	10	GBM	Phase I	Autologous irradiated glioma cells and GM-CSF	2PR	
Plautz et al. ³⁵⁴	12	GBM,A, astrocytoma	Phase I	Autologous irradiated glioma cells and GM-CSF	4PR	
Wood et al. ³⁵⁵	9	GBM,AA	Pilot study	Autologous irradiated glioma cells and BCG	3PR	
Yu et al. ³⁵⁶	9	GBM,AA	Phase I	DCs pulsed with acid-eluted peptides		
Andrews et al. ³⁵⁷	12	GBM,AA	Pilot study	Autologous glioma cells treated with IGF-IR antisense oligo DNA	2CR, 4PR	
Kikuchi et al. ³⁵⁸	8	GBM,AA	Phase I	DC-glioma fusion cells by PEG	2 PR	
Schneider et al. ³⁵⁹	11	GBM	Phase I	Autologous glioma cells infected by NDV		
Yamanaka et al. ³⁶⁰	10	GBM,AA	Phase I/II	DCs pulsed with autologous glioma-cell lysate	2 MR	
Yu et al. ²⁹⁵	14	GBM,AA	Phase I	DCs pulsed with autologous glioma-cell lysate		
Caruso et al. ³⁶¹	9	Glioma	Phase I	DCs transfected with glioma-derived RNA	1 PR	
Steiner et al. ³⁶²	23	GBM	Pilot study	Autologous glioma cells infected by NDV	1 CR	Increased CTL activity
Ruthkowski et al. ³⁶³	12	Glioma	Phase I	DCs pulsed with autologous glioma cell lysate	1 PR	
Kikuchi et al. ³⁶⁴	15	GBM,AA	Phase I	DC-glioma fusion cells by PEG and IL-12	4 PR	IL-12 administration
Yamanaka et al. ³⁶⁵	24	GBM,AA	Phase I/II	OK-432-stimulated DCs pulsed with autologous glioma-cell lysate	1 PR, 3 MR	OK-432 stimulation: a combination of intradermal and intracranial DC injections
Liau et al. ²⁹²	12	GBM	Phase I	DCs pulsed with acid-eluted peptides	1 PR	Slowly progressing tumors; tumors producing TGF- β 2 at low levels
Yajima et al. ³⁶⁶	17	GBM,AA	Phase I	DCs pulsed with recombinant peptides	5 PR	
Sloan et al. ³⁶⁷	19	GBM,AA	Pilot study	Autologous irradiated glioma cells and GM-CSF	5 PR	

Authors	Pts	Diagnosis	Study design	Type of vaccination	Outcome	Indicators for better results
Ishikawa et al. ³⁶⁸	12	GBM	Pilot study	Formalin-fixed autologous glioma cells	1 CR, 1 PR, 2 MR	
Okada et al. ²⁹³	12	GBM, AA	Phase I	DCs pulsed with autologous glioma-cell lysate and IL-4-transfected fibroblasts	2 PR	
Izumoto et al. ³²⁸	21	GBM	Phase II	DCs pulsed with WT1 peptide	2 PR	
De Vleeschouwer et al. ³⁶⁹	72	HGG	Phase II	DCs pulsed with autologous glioma-cell lysate		Younger age, total resection of tumors
Wheeler et al. ²⁹⁴	34	GBM	Phase II	DCs pulsed with autologous glioma-cell lysate	3 CR, 4 PR	Higher responsiveness of IFN- γ in PBMCs

Note: GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; BCG = Bacille de Calmette et Guérin; PR = partial response; GM-CSF = granulocyte-macrophage colony-stimulating factor; CR = complete response; DCs = dendritic cells; PEG = pegylated; NDV = Newcastle disease virus; CTL = cytotoxic lymphocyte; HGG = high-grade glioma; MR = minor response.

TABLE 3

Pros and Cons of Each Vaccine Approach

	Pros	Cons
Whole-cell vaccine	Identification of glioma-associated Ags not needed All HLA types available Broad immune response Limiting risk of tumor immune escape	Quantity of antigenic materials needed Cumbersome for patient-specific vaccine preparation Variable vaccine composition Increased risk of autoimmunity
Peptide vaccine	“Off-the-shelf” feasibility Autologous tumor cells not needed Relative tumor specificity	Paucity of defined antigen for human gliomas Specificity for individual HLA types needed Increased risk for antigen-loss variant
DC vaccine	Arguably the most potent Ag-presenting cells	Cost for generating high-quality DCs

Note: Ags = antigens; DCs = dendritic cells.