Immunotherapy for Glioblastoma

Debebe Theodros, Dane Moran, Tomas Garzon-Muvdi and Michael Lim

Department of Neurosurgery, The Johns Hopkins University School of Medicine, The Johns Hopkins Hospital, The Johns Hopkins University, Baltimore, Maryland, USA

Corresponding author: Michael Lim, Department of Neurosurgery, Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Phipps Suite 123, Baltimore, MD 21287, USA, Tel: 410-502-1627; Fax: 410-502-4954; E-mail: mlim3@jhmi.edu

Received date: September 13, 2016; Accepted date: October 20, 2016; Published date: October 27, 2016

Glioblastoma; Immunotherapy; Microglia; Tumor; Vaccine

Abstract

Glioblastoma (GBM) is the most common primary malignant brain cancer with a dismal prognosis in spite of aggressive treatment options. Although once thought to be an “immune-privileged” site, recent advances have begun to highlight the complex interaction between the immune system and the central nervous system. Thus, great interest has emerged in the ability of immunotherapy to potentially prolong the survival of patients suffering from GBM. Indeed, numerous clinical trials have demonstrated durable responses in late stage disease, as well as, among patients with brain metastasis. A variety of approaches to modulating the immune system exist and their efficacy are currently being investigated in various clinical trials. Here we provide a brief overview of neuroimmunology and explore the various approaches towards priming the immune system against GBM.

Keywords: Glioblastoma; Immunotherapy; Microglia; Tumor; Vaccine

Introduction

Recent scientific advances have solidified the role of the immune system in maintaining central nervous system (CNS) homeostasis. New insight into the dynamic interogration of the CNS by the immune system reveals a dynamic interaction contrary to previously held notions that the brain is an immune sanctuary [1-4]. Significant advances using preclinical models of CNS autoimmune disease or infection have revealed clues as to the extent of immune surveillance occurring within the CNS. As such, new efforts are currently underway to better understand the immune response to primary and metastatic malignancy of the CNS. Indeed, a number of preclinical models suggest immunotherapy represents a potentially promising treatment modality for patients suffering from primary brain cancer [5-8]. Immunotherapeutic strategies to overcoming immunosuppression within the tumor microenvironment (TME) and restoring cytotoxic CD8+ T-cell responses include vaccine therapies, adoptive cell therapy, and immune checkpoint blockade among others. Here, we present a brief overview of CNS immunology, strategies to implementing immunotherapy as a treatment modality for GBM and future directions.

Glioblastoma

Glioblastoma is the most prevalent adult malignant brain tumor with a median survival of less than two years and a 5-year overall survival of less than 10% [9-11]. Current standard of care (SOC) includes maximal-safe resection, chemotherapy and radiation therapy [11]. Furthermore, GBM is an inherently heterogeneous disease associated with extensive infiltration making complete cure challenging as patients ultimately succumb to recurrence [12]. However, the limits of conventional therapies may be overcome by modulating the host immune response to cancer. Great strides have been made towards re-purposing the immune system to eliminate CNS malignancy.

Central Nervous System Immunology

Immune cells of the CNS

The healthy CNS parenchyma is home to only one immune cell population, the microglia, which are highly specialized macrophages [13]. Microglia are distinct from peripheral monocytes or macrophages as they originate from a yolk sac progenitor and are maintained via local proliferation without reconstitution from the bone marrow [14,15]. However, myeloid cells are present within the CNS as well, specifically within the meninges, choroid plexus (CP), and perivascular spaces and are maintained by peripheral blood monocytes [14-16]. Despite the lack of resident T cells within the CNS parenchyma, the cellular composition of CSF is overwhelmingly lymphocytic, with ~90% of cells within circulating CSF being T cells. Moreover, the CD4+ to CD8+ ratio is 3.5 to 1 with the vast majority of CD4+ cells being central or effector memory T cells [17-19].

“Immune-privilege”

Nearly a century of work suggested the CNS is a site of “immune privilege,” a term first coined by Billingham and Boswell, which was a concept based up the observation that direct administration of antigens does not elicit an adaptive immune response [20,21]. However, the precise definition of “immune privilege” decayed with time and was recently re-defined [21]. CNS immune privilege is compartmentalized to the parenchyma, as intracerebroventricular (ICV) injection of various antigens results in generation of both humoral and cytotoxic T-cell responses [22]. Similarly, innate immune responses in the CNS are limited to the ventricles as well as the CP, and meninges [23]. Drainage of interstitial fluid to the cerebrospinal fluid (CSF) provides meningeal, perivascular and choroid plexus macrophages the ability to constantly survey potential antigens present within the parenchyma [24]. Furthermore, recent work clearly
demonstrates direct connections between the CNS and deep cervical lymph nodes via lymphatic drainage creating the ability to generate immune responses peripherally [1,2]. Thus, the CNS is an immunologically active organ displaying the necessary anatomical structures to undergo immunosurveillance and potentially benefit from immunotherapy.

**Immune Evasion**

Despite the clear role of immunosurveillance in maintaining and preserving normal brain architecture and function, multiple mechanisms exist within the tumor microenvironment (TME) to stifle an effective immune response. These mechanisms include the hypoxic microenvironment itself, the ability of tumor cells to secrete highly immunosuppressive factors, decreased expression of major histocompatibility complex (MHC) upon various APC subsets, inhibition of lymphocyte activity through increased surface expression of co-inhibitory immune checkpoint molecules, and recruitment of immunosuppressive cells to the TME. Here, we briefly review the known mechanisms of immunosuppression within the GBM TME.

The relative importance of immunosuppressive cells within GBMs is becoming rapidly apparent. One such population includes regulatory T cells (Tregs) commonly defined as CD4+FoxP3+CD25+ T cells, which are crucial under homeostatic conditions for maintaining tolerance; however, have been readily identified in human GBM samples [25]. These Tregs seem to be thymic-derived; however, the blockade of the CC chemokine receptor 4 (CCR4), a major chemoattractant receptor, does not completely deplete Treg infiltration within the TME, suggesting other mechanisms of Treg chemotraction into the TME [26,27]. Furthermore, abundance of Tregs within the TME has been shown to be associated with a poor prognosis [28-30]. Another cellular subset playing a role in maintaining a highly immunosuppressive TME are innate immune cells constituting tumor-associated macrophages (TAMs) and microglia. Factors such as colony-stimulating factor 1 (CSF-1), transforming growth factor-β (TGF-β), macrophage inhibitory cytokine-1 (MIC-1) and IL-10 recruit macrophages to the TME and shift polarization of recruited macrophages towards an M2 phenotype, decreasing phagocytosis while inhibiting cytotoxic T cell activity and enhancing Treg immunosuppression [31-35]. Additionally, TAMs and microglia influence GBM angiogenesis, growth, and invasion via secretion of endothelial growth factor (EGF), TGF-β, IL-6, CSF-1 and matrix metalloproteinases [32,36-39].

The TME itself is a highly immunosuppressive environment capable of inhibiting anti-tumor immune mediated responses through a variety of mechanisms. One such mechanism is the production of immunosuppressive cytokines, which induce immunosuppressive responses within the TME. One potent cytokine produced by GBM cells is IL-10, which enhances tumor growth while decreasing interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and MHC II expression, stifling anti-tumor immune responses [40-44]. Additionally, intense neovascularization, abnormal blood flow, and preferential oxygen consumption by rapidly proliferating tumor cells results in a hypoxic TME and activation of the STAT-3 inhibitory pathway within immune cells. Hypoxia induces numerous changes within the TME including the expansion of M2 TAMs and Tregs, which induce further vascularization and tumor cell invasion in a feed-forward manner as a result of STAT-3 mediated hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) expression [45].

**Immunotherapy Approaches**

The SOC, dubbed the “Stupp Protocol,” involves radiotherapy plus concomitant daily Temozolomide (TMZ) at 75 mg/m2/day for 7 days a week throughout radiation, followed by six cycles of adjuvant TMZ dosed 150-200 mg/m2 for 5 days during each 28-day cycle based upon the landmark study by Stupp et al. [11]. This study demonstrated a significant increase in median survival from 12.1 months to 14.6 months with the addition of temozolomide to radiation therapy. Additionally, the two-year survival rate following radiation with temozolomide versus radiation alone was 26.5% vs. 10.4%, respectively. However, the vast majority of patients ultimately succumb to disease. Neoplastic invasion of glioma stem cells beyond radiographically defined tumor margins and present after gross total resection undergo selection for alkylating/radiation-resistant clones following SOC [46,47]. Furthermore, the immense heterogeneity of glioma stem cells as illustrated by the capability to differentiate into various cell types, as well as, unique molecular profiles such as presence of mutations to iso-ocitrate dehydrogenase (IDH), O6-methylguanine-DNA methyltransferase (MGMT), and EGFR status further dictate response to treatment and prognosis. Thus, there is growing interest in novel treatments for GBM. Immunotherapy represents a potentially promising modality as early success has been demonstrated in a variety of solid malignancies [48,49].

**Vaccine Therapy**

GBM heterogeneity necessitates the need for patient-specific, anti-tumor immunotherapies with minimal toxicity. Strategies involving vaccination against tumor-associated antigens (TAA) have yielded success, as demonstrated by the FDA approved Gardasil™ (Merck, NJ, USA) for cervical cancer and sipuleucel-T (Provenge; Dendreon, WA, USA) for hormone-resistant metastatic prostate cancer [50]. Extensive efforts are underway to understand the potential role of vaccine therapy for GBMs (Table 1). Here we discuss the various types of vaccines and their efficacy for GBMs.

<table>
<thead>
<tr>
<th>NCT number</th>
<th>Title</th>
<th>Agent</th>
<th>Phase</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00643097</td>
<td>Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme</td>
<td>PEP-3 vaccine + sargamostim + Temozolomide</td>
<td>Phase 2</td>
<td>Humoral and Cellular Immune Response</td>
</tr>
<tr>
<td>NCT00639639</td>
<td>Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme</td>
<td>tetanus toxoid + therapeutic autologous dendritic cells and therapeutic autologous lymphocytes</td>
<td>Phase 1</td>
<td>Feasibility and safety of vaccination with cytomegalovirus pp65-LAMP mRNA-loaded dendritic cells (DCs) with or without autologous lymphocyte transfer</td>
</tr>
<tr>
<td>Study ID</td>
<td>Title</td>
<td>Phase</td>
<td>Key Information</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>NCT02772094</td>
<td>Dendritic Cell-Based Tumor Vaccine Adjuvant Immunotherapy of Human Glioblastoma Multiforme (WHO Grade IV Gliomas)</td>
<td>Phase 2</td>
<td>Overall survival with measures of medium survival period (in days) and annual survival rates (in %)</td>
<td>Adverse effects, acute and chronic, assessed according to NCI CTCAE Version 3</td>
</tr>
<tr>
<td>NCT00626403</td>
<td>Basiliximab in Treating Patients With Newly Diagnosed Glioblastoma Multiforme Undergoing Targeted Immunotherapy and Temozolomide-Caused Lymphopenia</td>
<td>Phase 1</td>
<td>Functional capacity of CD4+ , CD25+, CD127+ T-regulatory cells</td>
<td>Safety</td>
</tr>
<tr>
<td>NCT00890032</td>
<td>Vaccine Therapy in Treating Patients Undergoing Surgery for Recurrent Glioblastoma Multiforme</td>
<td>Phase 1</td>
<td>Feasibility and safety</td>
<td>Humoral and cellular immune responses</td>
</tr>
<tr>
<td>NCT01290692</td>
<td>Study To Test the Safety and Efficacy of Tvi-Brain-1 As A Treatment for Recurrent Grade IV Glioma</td>
<td>Phase 2</td>
<td>Progression Free Survival</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>NCT02080694</td>
<td>Dendritic Cell Vaccine for Patients With Brain Tumors</td>
<td>Phase 2</td>
<td>Most effective combination of DC vaccine components</td>
<td>Time to tumor progression and overall survival</td>
</tr>
<tr>
<td>NCT01522820</td>
<td>Vaccine Therapy With or Without Sirolimus in Treating Patients With NY-ESO-1 Expressing Solid Tumors</td>
<td>Phase 1</td>
<td>Incidence of adverse events in patients receiving the DEC-205/NY-ESO-1 fusion protein CDX-1401 with and without sirolimus, as evaluated according to the NCI CTCAE scale version 4.0</td>
<td>NY-ESO-1 specific cellular immunity</td>
</tr>
<tr>
<td>NCT00458601</td>
<td>Phase II Study of Rindopepimut (CDX-110) in Patients With Glioblastoma Multiforme</td>
<td>Phase 2</td>
<td>Progression-free survival status</td>
<td>Safety and tolerability characterized by adverse events (term, grade, frequency).</td>
</tr>
<tr>
<td>NCT00045968</td>
<td>Study of a Drug [DCVax®-L] to Treat Newly Diagnosed GBM Brain Cancer</td>
<td>Phase 3</td>
<td>The primary objective of this study is to compare progression free survival from time of randomization between patients treated with DCVax-L and control patients.</td>
<td>The secondary objective is to compare overall survival and time to disease progression between DCVax-L treated and control patients.</td>
</tr>
<tr>
<td>NCT01400672</td>
<td>Iriniquimod/Brain Tumor Initiating Cell (BTIC) Vaccine in Brain Stem Glioma</td>
<td>Phase 1</td>
<td>Dose-limiting toxicity</td>
<td>Time to Tumor Progression</td>
</tr>
<tr>
<td>NCT01498328</td>
<td>A Study of Rindopepimut/GM-CSF in Patients With Relapsed EGFRvIII-Positive Glioblastoma</td>
<td>Phase 2</td>
<td>Groups 1 and 2: Progression-free survival rate</td>
<td>Group 2C: Objective Response Rate</td>
</tr>
<tr>
<td>NCT01480479</td>
<td>Phase III Study of Rindopepimut/GM-CSF in Patients With Newly Diagnosed Glioblastoma</td>
<td>Phase 3</td>
<td>Overall Survival</td>
<td>Progression-free survival</td>
</tr>
<tr>
<td>NCT01222221</td>
<td>Vaccine Therapy, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme</td>
<td>Phase 1</td>
<td>Causality of each adverse event (AE) to glioblastoma multiform multi-antigen vaccine IMA950 and GM-CSF and AE severity according to NCI CTCAE Version 4.0</td>
<td>Total number of patients showing patient-individual T-cell responses against a single or multiple tumor-associated peptides (TUMAP) contained in the study vaccine IMA950 at one or more post-vaccination time points by HLA multimer analysis</td>
</tr>
<tr>
<td>Study ID</td>
<td>Study Title</td>
<td>Group Description</td>
<td>Design/Phase</td>
<td>Key Outcomes</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>NCT00323115</td>
<td>Phase II Feasibility Study of Dendritic Cell Vaccination for Newly Diagnosed Glioblastoma Multiforme</td>
<td>Autologous Dendritic Cell vaccine + Temozolomide + Radiotherapy</td>
<td>Phase 2</td>
<td>Tumor-specific Cytotoxic T-cell Response</td>
</tr>
<tr>
<td>NCT0106044</td>
<td>Efficacy &amp; Safety of Autologous Dendritic Cell Vaccination in Glioblastoma Multiforme After Complete Surgical Resection</td>
<td>Autologous dendritic cells</td>
<td>Phase 2</td>
<td>Evaluation of the treatment impact on progression-free survival</td>
</tr>
<tr>
<td>NCT00626015</td>
<td>Chemotherapy, Radiation Therapy, and Vaccine Therapy With Basiliximab in Treating Patients With Glioblastoma Multiforme That Has Been Removed by Surgery</td>
<td>PEP-3-KLH conjugate vaccine + dacarbazine + temozolomide</td>
<td>Phase 1</td>
<td>Functional suppressive capacity of CD4^*CD25^*CD127^- T-regulatory cells</td>
</tr>
<tr>
<td>NCT00576537</td>
<td>Tumor Lysate Pulsed Dendritic Cell Immunotherapy for Patients With Brain Tumors</td>
<td>Dendritic Cell Vaccine Immunotherapy</td>
<td>Phase 2</td>
<td>Evaluate the safety/toxicity of subcutaneous injections of autologous dendritic cells</td>
</tr>
<tr>
<td>NCT00905060</td>
<td>HSPPC-96 Vaccine With Temozolomide in Patients With Newly Diagnosed GBM</td>
<td>HSPPC-96</td>
<td>Phase 2</td>
<td>To evaluate the safety profile of HSPPC-96 administered concurrently temozolomide in patients with newly diagnosed GBM</td>
</tr>
<tr>
<td>NCT01081223</td>
<td>Phase I/II Study To Test The Safety and Efficacy of TVI-Brain-1 As A Treatment For Recurrent Grade IV Glioma</td>
<td>Cancer vaccine plus immune adjuvant, plus activated white blood cells</td>
<td>Phase 1</td>
<td>Relative toxicity</td>
</tr>
<tr>
<td>NCT00846456</td>
<td>Safe Study of Dendritic Cell (DC) Based Therapy Targeting Tumor Stem Cells in Glioblastoma</td>
<td>Dendritic cell vaccine with mRNA from tumor stem cells</td>
<td>Phase 1</td>
<td>Adverse events</td>
</tr>
<tr>
<td>NCT00576641</td>
<td>Immunotherapy for Patients With Brain Stem Glioma and Glioblastoma</td>
<td>autologous dendritic cells</td>
<td>Phase 1</td>
<td>Evaluate safety/toxicity of Dendritic cell vaccine, Monitor survival and time to progression and monitor the cellular immune responses.</td>
</tr>
<tr>
<td>NCT01213407</td>
<td>Dendritic Cell Cancer Vaccine for High-grade Glioma</td>
<td>Trivax, Temozolomide, Surgery, Radiotherapy</td>
<td>Phase 2</td>
<td>Progression free survival</td>
</tr>
<tr>
<td>NCT00612001</td>
<td>Vaccine Therapy in Treating Patients With Malignant Glioma</td>
<td>Glioma-associated antigen peptide-pulsed autologous dendritic cell vaccine</td>
<td>Phase 1</td>
<td>Dose-limiting toxicity and maximum tolerated dose of autologous dendritic cells pulsed with synthetic glioma-associated antigen</td>
</tr>
<tr>
<td>NCT0069940</td>
<td>Vaccine Therapy and Sargramostim in Treating Patients With Sarcoma or Brain Tumor</td>
<td>Sargramostim + telomerase + 540-548 peptide vaccine</td>
<td>Phase 1</td>
<td></td>
</tr>
<tr>
<td>NCT0003185</td>
<td>Biological Therapy in Treating Patients With Glioblastoma Multiforme</td>
<td>Autologous tumor cell vaccine + sargramostim + tumor-draining lymph node lymphocyte therapy + cyclophosphamide + conventional surgery</td>
<td>Phase 2</td>
<td></td>
</tr>
<tr>
<td>NCT01171469</td>
<td>Vaccination With Dendritic Cells Loaded With Brain Tumor Stem Cells for Progressive Malignant Brain Tumor</td>
<td>Dendritic Cells + Imiquimod</td>
<td>Phase 1</td>
<td>Maximum Tolerated Dose</td>
</tr>
</tbody>
</table>
Table 1: Vaccine-based clinical trials for GBM. Source: clinicaltrials.gov.

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Description</th>
<th>Abbreviation</th>
<th>Phase</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT0014573</td>
<td>Chemotherapy and Vaccine Therapy Followed by Bone Marrow or Peripheral Stem Cell Transplantation and Interleukin-2 in Treating Patients With Recurrent or Refractory Brain Cancer</td>
<td>a) Chemotherapy + interleukin-2 + vaccine + autologous tumor cell vaccine + fligrastim + sargramostim + therapeutic autologous lymphocytes + cisplatin + cyclophosphamide + paclitaxel + autologous bone marrow transplantation + conventional surgery + peripheral blood stem cell transplantation</td>
<td>Phase 2</td>
<td>Safety and maximum tolerated dose</td>
</tr>
<tr>
<td>NCT00293423</td>
<td>GP96 Heat Shock Protein-Peptide Complex Vaccine in Treating Patients With Recurrent or Progressive Glioma</td>
<td>HSPPC-96</td>
<td>Phase 1 Phase 2</td>
<td>Dose Limiting Toxicity</td>
</tr>
<tr>
<td>NCT00068510</td>
<td>Vaccine Therapy in Treating Patients With Malignant Glioma</td>
<td>Therapeutic autologous dendritic cells</td>
<td>Phase 1</td>
<td>Immune response (Phase I)</td>
</tr>
<tr>
<td>NCT00004024</td>
<td>Biological Therapy Following Surgery and Radiation Therapy in Treating Patients With Primary or Recurrent Astrocytoma or Oligodendroglioma</td>
<td>Aldelesinukin + autologous tumor cell vaccine + muronomab-CD3 + sargramostim + therapeutic autologous lymphocytes + surgical procedure + radiation therapy</td>
<td>Phase 2</td>
<td>Antitumor immune-mediated response</td>
</tr>
</tbody>
</table>

**Peptide vaccines**

Peptide vaccines represent a platform of immunotherapy consisting of TAAs in combination with an adjuvant to prime T cells to mount an anti-tumor immune-mediated response. TAAs are uptaken by antigen-presenting cells (APCs), internally processed and mounted on MHC I or II and ultimately recognized by the cognate T cell receptor on CD8+ or CD4+ T cells, respectively [51]. Thus, identification of unique TAA and not over-expressed endogenous peptides predicts the success of potential peptide vaccines. Despite the identification of multiple TAAs such as, HER-2, gp-100, MAGE-1, AIM-2, and IL-13Rα2 in a variety of tumors, endogenous expression of these targets explains the presence of non-reactive T cells in patients [52]. One promising target, aberrant EGF receptors (EGFR) has been shown to regulate cell proliferation, differentiation, survival and invasiveness in multiple tumor types, including GBM [53-58]. One such variant, EGFRvIII, is selectively expressed on 27-67% of GBMs, representing a potential target for peptide vaccine therapy [58,59].

Based upon the EGFRvIII discovery, a Phase II multicenter trial termed the ACTIVATE trial was initiated. The ACTIVATE trial involved use of the PEPvIII-KLH peptide in combination with granulocyte macrophage-colony stimulating factor (GM-CSF) without pulsed autologous DCs. The ACTIVATE trial enrolled 19 patients with newly-diagnosed, EGFRvIII positive GBMs who underwent gross total resection and standard of care radiation and TMZ treatment. Patients underwent three biweekly intradermal injections at the upper thigh followed by monthly injections until radiographic progression or death. The median time-to-progression (TTP) was 12 months vs. a TTP of 7.1 months for historical controls (p=0.0058). Furthermore, ex vivo analysis demonstrated humoral responses as well as antigen-specific responses to PEPvIII and EGFRvIII which predicted median OS. The median time-to-progression (TTP) was 12 months, (p=0.0058). Pathological samples obtained from recurrent tumors were negative for EGFRvIII via immunohistochemical staining (IHC) in 82% of samples, which the authors attributed to immunoediting following vaccination [60].

Following the adoption of the Stupp protocol as SOC, the ACTIVATE (ACT) II trial was initiated. The ACT II trial enrolled 21 patients with EGFRvIII positive GBMs to receive CDX-110 (rindopepimut and GM-CSF) within 6 weeks of completion of SOC radiation and chemotherapy, followed by an additional two doses at two week intervals, then monthly vaccination until disease progression. Despite Grade 2 TMZ-related lymphopenia, similar clinical benefits were observed with a median TTP of 15.2 months and an OS of 23.6 months [61,62]. The ACT III trial, a multicenter, single-arm, phase II study, sought to confirm the results in a large, multicenter study. A total of 65 patients were enrolled and received Rindopepimut following SOC Stupp protocol. The median OS was 21.8 months with a 36-month OS of 26%, confirming the results of the previous trials [63]. With encouraging results, the ACT IV trial was initiated. This randomized, double-blind phase IV study enrolled 745 patients to either SOC and rindopepimut with GM-CSF versus SOC and KLH injection alone. Despite promising results in previous trials, the ACT IV trial was discontinued in March, 2016 based upon preliminary results revealing the control arm significantly outperforming the vaccine arm (hazard ratio=0.99, median OS: Rindopepimut 20.4 months vs. control 21.1 months). The ReACT trial, is a Phase II, randomized, double-blind trial currently underway.
evaluating Rindopepimut/GM-CSF vaccine therapy and bevacizumab treatment in currently 125 EGFRvIII positive recurrent GBM patients (NCT01498328). Results revealed in November 2015 demonstrated a significant benefit in OS with 25% of patients in the rindopepimut arm alive at 2 years versus 0% in the control arm.

**Dendritic cell (DC) vaccines**

Dendritic cells, termed “professional” APCs function as critical mediators of immune surveillance, antigen presentation, and cross talk between the innate and adaptive immune system. Recognition of pathogen-associated molecular patterns (PAMPs) results in internalization of foreign proteins/peptides, internal processing and extracellular presentation in the context of MHC I or II and migration/adverse event greater than Grade II, with ex vivo analysis by antigen pulsation. was not an inclusion criterion; yet, twelve patients received three equal intradermal doses every two weeks. No patient demonstrated increased immune cell infiltration in post-vaccine tumor resection samples as well [76], Bloch et al. conducted a Phase II study evaluating tumor antigenic peptides in the context of HSP-96 for recurrent GBMs. The study enrolled 41 patients with a median PFS of 19.1 weeks and median, 6-month and 12-month OS were 42.7 weeks, 90.2% and 29.3%, respectively. Lastly, a higher absolute lymphocyte count (ALC) was found to correlate with improved survival [77]. Currently, multi-center, single arm Phase II trials evaluating the efficacy of HSPPC-96 in newly diagnosed GBMs (NCT00905060) as well as recurrent/progressive GBMs (NCT00293423) have completed accrual and are currently in follow-up phase with another phase II trial evaluating HSPPC-96 with or without bevacizumab in recurrent GBMs (NCT0181413) currently recruiting patients.

**Adoptive Cell Therapy (ACT)**

The elucidation of the function of T lymphocytes in the 1960’s followed by the discovery of IL-2 in 1976 represented the foundation through which adoptive cell therapy (ACT) could thrive [78,79]. Furthermore, success using IL-2 for patients with metastatic melanoma and renal cell carcinoma revealed the ability to induce an endogenous host immune response against cancer [80]. The observation that tumor specimens were heavily infiltrated by lymphocytes and that ex vivo expansion and adoptive transfers in murine models could establish regression of established tumors provided proof of principle followed by human studies resulted in objective responses, albeit for short durations [80-82].

Cytotoxic T lymphocytes (CTLs) represent an important component of host immune responses to cancer. Indeed, infiltrative tumor-reactive CTLs recognize non-self epitopes with specificity via the interaction of the T-cell receptor (TCR) with peptide in the context of MHC I or II.
Here, we discuss ACT in the context of glioma treatment.

Adoptive cell therapy (ACT) involves ex vivo autologous culture of tumor infiltrating lymphocytes in the presence of IL-2 and passive transfer following selection for lymphocytes with high-avidity for tumor epitopes. ACT is associated with numerous advantages relative to other cancer immunotherapies. These include the ability to expand large quantities of TILs in vitro, bypassing immunosuppressive environments seen in vivo. Lastly, host TME manipulation prior to ACT affords the ability to optimize the efficacy of transferred cells [83]. Here, we discuss ACT in the context of glioma treatment.

**Lymphokine-activated killer (LAK) cells**

Lymphokine-activated killer (LAK) cells represent a population of peripheral blood mononuclear cells (PBMCs) with IFN-β from tumor specimens, large scale production and financial challenges/burdens. Many of these challenges are being overcome by the development of genetically engineered T cells derived from patients with transgenic T cell receptors (TCRs) or chimeric antigen receptors (CARs) derived from high-affinity antibodies capable of being designed with specificity to a variety of antigens. Indeed, these CAR T-cells have resulted in impressive clinical responses in hematological malignancies [94,95]. To date, the majority of CAR based studies have focused upon B-cell malignancies where CD19 or CD20 CARs have consistently demonstrated significant clinical responses [94-97]. Based on these successes, CAR therapies with specificity to the EGFRVIII protein are currently under active investigation for GBM. Indeed, the therapeutic potential of CAR therapy for GBM has been demonstrated [98-102].

**Immunologic Pharmacodynamic Parameters For The PD-1 Checkpoint Therapy**

Among the most exciting immunotherapeutic modalities, immune checkpoint blockade has garnered FDA approval for a variety of malignancies including melanoma, squamous and non-squamous non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and classical Hodgkin lymphoma (CHL). The amplitude and quality of T cell responses is initiated by TCR engagement and fine-tuned by co-stimulatory and co-inhibitory immune checkpoints. These co-stimulatory and co-inhibitory molecules maintain self-tolerance under normal conditions; however, a variety of malignancies expression checkpoint molecules in an effort to induce tolerance [103]. As a result, intense efforts focus upon the utilization of co-stimulatory agonist and co-inhibitory antagonist monoclonal antibodies as an additional approach to restore anti-tumor immune function for a variety of malignancies including GBMs (Table 2).

<table>
<thead>
<tr>
<th>NCT number</th>
<th>Title</th>
<th>Agent</th>
<th>Phase</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02798406</td>
<td>Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects</td>
<td>DNX-2401 + pembrolizumab</td>
<td>Phase 2</td>
<td>Objective response rate (ORR)</td>
</tr>
<tr>
<td>NCT02852655</td>
<td>A Pilot Surgical Trial To Evaluate Early Immunologic Pharmacodynamic Parameters For The PD-1 Checkpoint Inhibitor, Pembrolizumab (MK-3475), In Patients With Surgically Accessible Recurrent/Progressive Glioblastoma</td>
<td>Drug: MK-3475</td>
<td></td>
<td>Tumor Infiltrating T Lymphocyte (TIL) Density</td>
</tr>
</tbody>
</table>

**Natural killer (NK) cells**

NK cells, identified as CD56+ lymphocytes, represent a subset of cytotoxic lymphocytes capable of non-specific anti-viral and anti-tumor activity. Ligation of killer inhibitory receptors (KIRs) on NK cells with MHC I molecules inhibits the tyrosine-kinase-based cytolytic activity of NK cells [89]. Advantages to NK ACT include the short period of time needed to undergo NK cell expansion. Additionally, because NK cells kill in a non-specific manner, tumor specimens are not needed. Epigenetic alterations resulting in gain-of-function mutations promote natural killer (NK) cell-mediated lysis [90]. However, the immunosuppressive glioma TME results in decreased IL-2 and IFN-γ production, which is critical for NK activity, representing a potential challenge to NK ACT.

Ishikawa et al. performed adoptive transfers of autologous NK cells derived from peripheral blood mononuclear cells (PBMCs) with IFN-β for patients suffering from recurrent high-grade gliomas. A total of 9 patients underwent 16 courses of ACT. Of those 9 patients, 3 experienced partial responses, 2 experienced a minor response, 4 experience no change in disease, and 7 experienced progressive disease with no signs of severe neurological toxicity [91]. This study highlighted the feasibility and safety of NK ACT for malignant gliomas.

**Chimeric Antigen Receptor (CAR) T Cells**

Significant advances over the past few decades have revolutionized the use of adoptive T-cell transfer and demonstrated clear durable responses in a variety of aggressive and metastatic diseases [92,93]. However, formidable challenges still abound regarding adoptive T-cell transfer, including technical challenges related to isolation of T cells from tumor specimens, large scale production and financial challenges/burdens. Many of these challenges are being overcome by the development of genetically engineered T cells derived from patients with transgenic T cell receptors (TCRs) or chimeric antigen receptors (CARs) derived from high-affinity antibodies capable of being designed with specificity to a variety of antigens. Indeed, these CAR T-cells have resulted in impressive clinical responses in hematological malignancies [94,95]. To date, the majority of CAR based studies have focused upon B-cell malignancies where CD19 or CD20 CARs have consistently demonstrated significant clinical responses [94-97]. Based on these successes, CAR therapies with specificity to the EGFRVIII protein are currently under active investigation for GBM. Indeed, the therapeutic potential of CAR therapy for GBM has been demonstrated [98-102].

**Immune Checkpoint Therapy**

Among the most exciting immunotherapeutic modalities, immune checkpoint blockade has garnered FDA approval for a variety of malignancies including melanoma, squamous and non-squamous non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and classical Hodgkin lymphoma (CHL). The amplitude and quality of T cell responses is initiated by TCR engagement and fine-tuned by co-stimulatory and co-inhibitory immune checkpoints. These co-stimulatory and co-inhibitory molecules maintain self-tolerance under normal conditions; however, a variety of malignancies expression checkpoint molecules in an effort to induce tolerance [103]. As a result, intense efforts focus upon the utilization of co-stimulatory agonist and co-inhibitory antagonist monoclonal antibodies as an additional approach to restore anti-tumor immune function for a variety of malignancies including GBMs (Table 2).
Cytotoxic T lymphocyte antigen-4 (CTLA-4), an inhibitory checkpoint and member of the B7 family, was the first clinically targeted inhibitory checkpoint. While CTLA-4 binds B7-1 or B7-2 and serves as an inhibitory signal following TCR ligation with cognate antigen in the context of MHC, CD28 also binds B7-1 or B7-2 providing co-stimulation following TCR ligation [104-108]. Despite expression on CD8⁺, the role of CTLA-4 expression on CD4⁺ T helper cells and Tregs appears to play the dominant physiological role. Moreover, CTLA-4 serves to dampen CD4⁺ T helper cells while engagement on Tregs enhances suppressive activity [109-111]. The biological significance of CTLA-4 is highlighted by the lethal intense autoimmune phenotype demonstrated by Cfa-4⁻/⁻ mice [112,113].

Despite initial concern over the potentially lethal ramifications of CTLA-4 blockade, Allison and colleagues revealed blockade of CTLA-4 did not result in overt immune toxicity in preclinical models and could endogenously anti-tumor responses [114,115]. By the early 2000s, two fully humanized antagonist CTLA-4 antibodies; ipilimumab (Bristol Meyer-Squibb) and tremelimumab (Pfizer) began clinical testing. Ipilimumab would ultimately go on to become the first therapy resulting in a survival benefit and increased overall survival for patients with metastatic melanoma and was ultimately approved by the Food and Drug Administration (FDA) in 2010 [49]. Efforts are underway to investigate the safety and dosage of ipilimumab with temozolomide in newly diagnosed GBM (NCT02319290) with another Phase II/III study of standard of care (SOC) temozolomide in combination with ipilimumab for newly diagnosed glioblastoma (RTOG 1125) [116].

Similar to CTLA-4, the programmed death 1 (PD-1) inhibitory immune checkpoint receptor represents another promising target. The major biologic role of PD-1 appears to be in limiting peripheral immune responses during inflammatory insults [117-121]. Following T cell activation, PD-1 surface expression increases and engagement of PD-1 with either programmed death ligand 1 (PD-L1, B7-H1 or CD274) or programmed death ligand 2 (PD-L2, B7-DC, CD273) inhibits TCR-mediated T cell activation [117,118,122,123]. Persistently high levels of PD-1 expression occur during chronic antigen exposure resulting in T cell exhaustion. Interestingly, the PD-1:PD-L1/L2 interaction upon T cell infiltrating lymphocytes (TILs), myeloid cells and tumor cells appears to be a major mechanism of immune evasion in cancer [124-131]. PD-L1 expression on GBM tumor cells increases with loss of phosphatase and tensin homolog (PTEN) and activation of the phosphatidylinositol-3-OH kinase (PI3K) pathway [89].

Mounting evidence suggests the PD-1:PD-L1 pathway may play a role in mediating immune evasion in high-grade glioma [132-134]. A number of therapeutic human antibodies targeting the PD-1 receptor have been developed including Pembrolizumab (Merck) and Nivolumab (BMS) to name a few. Despite initial concerns, antibodies targeting the PD-1 pathway may not result in unique CNS toxicity [135]. The majority of clinical data available regarding CNS malignancy has primarily focused upon investigating the efficacy of anti-PD-1 therapy for brain metastasis. A non-randomized Phase II trial investigated the efficacy of Pembrolizumab for patients with untreated melanoma or non-small cell lung cancer (NSCLC) brain metastasis revealed durable responses in 4 of 18 patients with melanoma and 6 of 18 patients with NSCLC [136]. Given recent data demonstrating PD-1 expression upon tumor-infiltrating lymphocytes, recent clinical trials determining the efficacy of anti-PD-1 or anti-PD-L1 therapy in primary brain tumors are under investigation [137,138]. A phase III trial comparing Nivolumab with bevacizumab and Nivolumab with or without Ipilimumab is currently recruiting patients although a small safety lead-in revealed an overall survival at 6 months of 76% (NCT02017717; Checkmate 143). A number of clinical trials involving anti-PD-1/L1 therapy for newly diagnosed or recurrent glioblastoma are currently underway (NCT02617589, NCT02667587, NCT02550249, NCT02319290, NCT02337491, NCT02337686, NCT02658279, NCT0236165).

**Conclusions & Future Directions**

Significant advances in the fields of neuro- and cancer immunology provide a compelling argument for the use of immunotherapy for CNS malignancies. Despite the devastating prognosis associated with GBM,
immunotherapy represents a novel anticancer modality with the ability to result in drastic responses in otherwise incurable diseases. A greater understanding of the mechanisms through which GBMs evade the immune system will aid in the development of strategic immunotherapy regimens tailored to each person's disease. Questions remain regarding the efficacy of immunotherapy in the context of the current SOC and how best to utilize immunotherapy. Future studies are necessary to explore the aforementioned questions; however, significant hope remains for the role of immunotherapy in the treatment of GBM.

References


