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Immunotherapy for Glioblastoma

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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 interrogation 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 over 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 chemoattraction to 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/BSA^2/day for 7 days a week throughout radiation, followed by six cycles of adjuvant TMZ dosed 150-200 mg/m^2 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 isocitrate 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.

NCT number	Title	Agent	Phase	Outcome measures
NCT00643097	Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme	PEP-3 vaccine + sargramostim + Temozolomide	Phase 2	Humoral and Cellular Immune Response | Clinical Efficacy of Vaccination, in Terms of Progression-free Survival (PFS) | Response to Vaccination | Toxicity to PEP-3 Vaccine Immunization
NCT00639639	Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme	tetanus toxoid + therapeutic autologous dendritic cells and therapeutic autologous lymphocytes	Phase 1	Feasibility and safety of vaccination with cytomegalovirus pp65-LAMP mRNA-loaded dendritic cells (DCs) with or without autologous lymphocyte transfer | Humoral and cellular immune responses | Time to progression | Differential ability of indium In-111-labeled DCs to track to the inguinal lymph nodes under different skin preparative conditions | Differential ability of indium

				In-111-labeled DCs to track to lymph nodes on the tumor bearing and non-tumor bearing side of the cervical lymph nodes | Immunologic cell infiltrate in recurrent tumors | Evidence of antigen-escape outgrowth in recurrent or progressive tumors
NCT02772094	Dendritic Cell-Based Tumor Vaccine Adjuvant Immunotherapy of Human Glioblastoma Multiforme (WHO Grade IV Gliomas)	Dendritic Cell Vaccine	Phase 2	Overall survival with measures of medium survival period (in days) and annual survival rates (in %) | Adverse effects, acute and chronic, assessed according to NCI CTCAE Version 3 | Disease progression-free period
NCT00626483	Basiliximab in Treating Patients With Newly Diagnosed Glioblastoma Multiforme Undergoing Targeted Immunotherapy and Temozolomide-Caused Lymphopenia	RNA-loaded dendritic cell vaccine + basiliximab	Phase 1	Functional capacity of $CD4^+$,$CD25^+$, $CD127^-$ T-regulatory cells | Safety
NCT00890032	Vaccine Therapy in Treating Patients Undergoing Surgery for Recurrent Glioblastoma Multiforme	BTSC mRNA-loaded DCs	Phase 1	Feasibility and safety | Humoral and cellular immune responses
NCT01290692	Study To Test the Safety and Efficacy of TVI-Brain-1 As A Treatment for Recurrent Grade IV Glioma	TVI-Brain-1	Phase 2	Progression Free Survival | Overall Survival | Quality of life | Toxicity |Time to progression| Objective response rate | Cancer immunogenicity
NCT01204684	Dendritic Cell Vaccine for Patients With Brain Tumors	autologous tumor lysate-pulsed DC vaccination ± 0.2% resiquimod ± adjuvant polyICLC	Phase 2	Most effective combination of DC vaccine components | Time to tumor progression and overall survival
NCT01522820	Vaccine Therapy With or Without Sirolimus in Treating Patients With NY-ESO-1 Expressing Solid Tumors	DEC-205/NY-ESO-1 Fusion Protein CDX-1401+ Sirolimus	Phase 1	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 | NY-ESO-1 specific cellular immunity | NY-ESO-1 specific humoral immunity
NCT00458601	Phase II Study of Rindopepimut (CDX-110) in Patients With Glioblastoma Multiforme	CDX-110 with GM-CSF + temozolomide	Phase 2	Progression-free survival status | Safety and tolerability characterized by adverse events (term, grade, frequency). | Safety and tolerability characterized by physical examinations. | Safety and tolerability characterized by hematologic and metabolic panel (including CBC with differential, electrolytes, BUN, Cr, liver associated enzymes). | Safety and tolerability characterized by urinalysis. | Safety and tolerability characterized by vital signs. | Immune response; T-cell response to vaccine. | Immune response; antibody response to vaccine. | Immune response; HLA typing. | Overall survival.
NCT00045968	Study of a Drug [DCVax®-L] to Treat Newly Diagnosed GBM Brain Cancer	Dendritic cell immunotherapy	Phase 3	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. | The secondary objective is to compare overall survival and time to disease progression between DCVax-L treated and control patients.
NCT01400672	Imiquimod/Brain Tumor Initiating Cell (BTIC) Vaccine in Brain Stem Glioma	Tumor Lysate Vaccine + Imiquimod + Radiation therapy	Phase 1	Dose-limiting toxicity | Time to Tumor Progression | Drop-out rate
NCT01498328	A Study of Rindopepimut/GM-CSF in Patients With Relapsed EGFRvIII-Positive Glioblastoma	Bevacizumab+ Rindopepimut (CDX-110) with GM-CSF	Phase 2	Groups 1 and 2: Progression-free survival rate | Group 2C: Objective Response Rate | Safety and Tolerability | Anti-tumor activity | EGFRvIII-specific immune response
NCT01480479	Phase III Study of Rindopepimut/GM-CSF in Patients With Newly Diagnosed Glioblastoma	Rindopepimut (CDX-110) with GM-CSF | Drug: Temozolomide | Drug: KLH	Phase 3	Overall Survival | Progression-free survival | Safety and Tolerability
NCT01222221	Vaccine Therapy, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme	Glioblastoma multiforme multipeptide vaccine IMA950 + sargramostim + temozolomide + radiation therapy	Phase 1	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 | 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 | Progression-free survival (PSF) at 6 and 9 months post-surgery as assessed by the Macdonald criteria

				from conventional gadolinium-enhanced MRI and clinical assessment | Correlation between steroid levels and observed T-cell responses | Correlation between O6-methyl-DNA-methyltransferase (MGMT) promoter methylation status in tumor tissue using methylation-specific polymerase chain reaction and clinical benefit (PFS at 6 months and 9 months) | Kinetics of vaccine-induced TUMAP responses including summary descriptions of the time of onset, sustainability, and magnitude of the observed response
NCT00323115	Phase II Feasibility Study of Dendritic Cell Vaccination for Newly Diagnosed Glioblastoma Multiforme	Autologous Dendritic Cell vaccine + Temozolomide + Radiotherapy	Phase 2	Tumor-specific Cytotoxic T-cell Response|Feasibility and Toxicity Profile of Intra-nodal DC/Tumor Lysate Vaccination|Progression Free Survival (PFS)and Overall Survival (OS) Comparison to Prognostic Matched Historical Controls|Immunological Parameters With PFS vs Overall Survival|Radiological Response When There is Residual Enhancing Tumor at Baseline MRI
NCT01006044	Efficacy & Safety of Autologous Dendritic Cell Vaccination in Glioblastoma Multiforme After Complete Surgical Resection	Autologous dendritic cells	Phase 2	Evaluation of the treatment impact on progression-free survival | Safety evaluation | Evaluation of impact on other efficiency clinical parameters | Study of specific immune response and correlates with clinical outcome | Cell line characterization and correlate the final product with clinical efficacy
NCT00626015	Chemotherapy, Radiation Therapy, and Vaccine Therapy With Basiliximab in Treating Patients With Glioblastoma Multiforme That Has Been Removed by Surgery	PEP-3-KLH conjugate vaccine + daclizumab + temozolomide	Phase 1	Functional suppressive capacity of $CD4^{+}CD25^{+}CD127^{-}$ T-regulatory cells | Comparison of proliferative T-cell response to phytohemagglutinin (PHA) among treatment groups (with versus without daclizumab/basiliximab)
NCT00576537	Tumor Lysate Pulsed Dendritic Cell Immunotherapy for Patients With Brain Tumors	Dendritic Cell Vaccine Immunotherapy	Phase 2	Evaluate the safety/toxicity of subcutaneous injections of autologous dendritic cells
NCT00905060	HSPPC-96 Vaccine With Temozolomide in Patients With Newly Diagnosed GBM	HSPPC-96	Phase 2	To evaluate the safety profile of HSPPC-96 administered concurrently temozolomide in patients with newly diagnosed GBM. | Survival Time | To evaluate the immunologic response to vaccine treatment | Progression free survival from date of surgical resection
NCT01081223	Phase I/II Study To Test The Safety and Efficacy of TVI-Brain-1 As A Treatment For Recurrent Grade IV Glioma	Cancer vaccine plus immune adjuvant, plus activated white blood cells	Phase 1 | Phase 2	Relative toxicity | Progression free survival | Immunogenicity | Overall survival
NCT00846456	Safe Study of Dendritic Cell (DC) Based Therapy Targeting Tumor Stem Cells in Glioblastoma	Dendritic cell vaccine with mRNA from tumor stem cells	Phase 1 | Phase 2	Adverse events | Evaluation of immunological response, time to disease progression and survival time
NCT00576641	Immunotherapy for Patients With Brain Stem Glioma and Glioblastoma	autologous dendritic cells	Phase 1	Evaluate safety/toxicity of Dendritic cell vaccine, Monitor survival and time to progression and monitor the cellular immune responses.
NCT01213407	Dendritic Cell Cancer Vaccine for High-grade Glioma	Trivax, Temozolomide, Surgery, Radiotherapy	Phase 2	Progression free survival | Quality of Life | Progression free survival at 18 and 24 months | Overall survival
NCT00612001	Vaccine Therapy in Treating Patients With Malignant Glioma	Glioma-associated antigen peptide-pulsed autologous dendritic cell vaccine	Phase 1	Dose-limiting toxicity and maximum tolerated dose of autologous dendritic cells pulsed with synthetic glioma-associated antigen (GAA) peptides | Survival | Tumor progression
NCT00069940	Vaccine Therapy and Sargramostim in Treating Patients With Sarcoma or Brain Tumor	Sargramostim + telomerase + 540-548 peptide vaccine	Phase 1	
NCT00003185	Biological Therapy in Treating Patients With Glioblastoma Multiforme	Autologous tumor cell vaccine + sargramostim + tumor-draining lymph node lymphocyte therapy + cyclophosphamide + conventional surgery	Phase 2	
NCT01171469	Vaccination With Dendritic Cells Loaded With Brain Tumor Stem Cells for Progressive Malignant Brain Tumor	Dendritic Cells + Imiquimod	Phase 1	Maximum Tolerated Dose | Time to Tumor Progression

NCT00014573	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	aldesleukin + autologous tumor cell vaccine + filgrastim + sargramostim + therapeutic autologous lymphocytes + cisplatin + cyclophosphamide + paclitaxel + autologous bone marrow transplantation + conventional surgery + peripheral blood stem cell transplantation	Phase 2	
NCT00293423	GP96 Heat Shock Protein-Peptide Complex Vaccine in Treating Patients With Recurrent or Progressive Glioma	HSPPC-96	Phase 1 | Phase 2	Safety and maximum tolerated dose | Frequency of gp96 heat shock protein-peptide complex vaccine (Phase I [closed to accrual as of 7/25/2007]) | Toxicity (Phase I [closed to accrual as of 7/25/2007]) | Progression-free survival at 6 months (Phase II) | Immunological response (Phase I [closed to accrual as of 7/25/2007]) | Safety (Phase II) | Tumor response as measured by neuro-imaging and neurologic exam (Phase II) | Survival (Phase II) | Immunological response (Phase II)
NCT00068510	Vaccine Therapy in Treating Patients With Malignant Glioma	Therapeutic autologous dendritic cells	Phase 1	Dose Limiting Toxicity | Time to tumor progression, overall survival and cellular immune responses in brain tumor patients injected with tumor lysate pulsed dendritic cells
NCT00004024	Biological Therapy Following Surgery and Radiation Therapy in Treating Patients With Primary or Recurrent Astrocytoma or Oligodendroglioma	Aldesleukin + autologous tumor cell vaccine + muromonab-CD3 + sargramostim + therapeutic autologous lymphocytes + surgical procedure + radiation therapy	Phase 2	

Table 1: Vaccine-based clinical trials for GBM. Source: clinicaltrials.gov.

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/activation of DCs to local draining lymph nodes and initiation of an adaptive immune response. Thus, enhanced priming of $CD4^+$ and $CD8^+$ T cells using DC vaccine platforms represent another interesting avenue of cancer immunotherapy.

The VICTOR I trial was a Phase I study with 12 patients vaccinated with autologous DCs pulsed with Rindopepimut (CDX-110; Celldex Therapeutics, MA, USA), a PEPvIII peptide conjugated to keyhole limet hemocyanin (KLH). Of note, expression of EGFRvIII expression was not an inclusion criterion; yet, twelve patients received three equal intradermal doses every two weeks. No patient suffered any serious adverse event greater than Grade II, with *ex vivo* analysis demonstrating evidence of antigen-specificity and a humoral response. The median progression free survival (PFS) was 10.2 months with an overall survival (OS) of 22.8 months. Despite a statistically insignificant increase in survival, the results of the VICTOR I trial provided evidence that a peptide-based vaccine may prove beneficial in patients with GBMs [60].

The ICT-107 vaccine, developed by Immunocellular Therapeutics Ltd. (CA, USA) is an autologous DC vaccine with activity against six antigens including AIM-2, GP100, IL13Rα2, HER2, MAGE-1 and TRP-2 and demonstrated clinical activity in a Phase I trial. The phase I trial consisted of 21 patients (17 newly-diagnosed GBM patients) with a PFS of 16.9 months and median OS of 38.4 months [52]. A Phase II, randomized, double-blind study of ICT-107 failed to meet the primary OS survival of 2-3 years among the ICT-107 group but did meet the secondary PFS outcome of 2-3 years. Based upon this work, a Phase III trial is currently underway actively recruiting patients (*NCT02546102*). Additionally, a Phase I trial investigating the therapeutic potential of ICT-121 (Immunocellular Therapeutics, Ltd.) in recurrent GBM is also underway actively recruiting patients (*NCT02049489*).

The DCVax platform (Northwest Biotherapeutics, Inc. MD, USA) is a DC-based vaccine platform currently in numerous trials for a variety of malignancies including GBM. Three different DCVax platforms exist, two involve purifying autologous DCs and *in vitro* differentiation by antigen pulsation. The third platform, DCVax-Direct, is derived from monocyte purification from leukopheresis followed by DC differentiation and *in vitro* stimulation with Calmette-Geurin to induce DC activation. The DCVax-Direct platform is used in cases of inadequate tumor sample/unresectable cases and is injected directly into tumors [64].

Phase I/II trials conducted out of the University of California, Los Angeles enrolled 39 patients (20 newly-diagnosed GBMs) revealed 33% of patients met or exceeded a median OS of 48.0 months and 27% exceeded a median OS of 72.0 months, with 2 patients alive greater than 10.0 years. Currently, a Phase III randomized, double-blind, multi-center trial investigating DCVax in newly diagnosed GBM patients is currently ongoing (*NCT00045968*) [65].

Heat shock protein (HSP) vaccines

Heat shock proteins (HSPs) represent a broad group of constitutively expressed proteins that function as intracellular molecular chaperones or proteases whose concentrations can rise dramatically in the setting of protein misfolding, unfolding, or aggregation [66-69]. These stress response proteins maintain protein architecture by responding to varying temperature, oxidative stresses, metabolic disturbances, exogenous chemical activity, viral infection, hypoxic conditions, and malignant transformations. Furthermore, soluble HSPs are capable of binding CD91 upon DCs leading to enhanced priming of $CD4^+$ and $CD8^+$ T cell responses.

Interestingly, immune responses are generated against peptide sequences associated with HSPs, while HSPs serve as adjuvants [70-72]. Furthermore, only HSP-peptide complexes are able to generate antitumor immune response [73]. One HSP of interest, GP96, released following cell death, has been shown to interact with Toll-like receptor 2 (TLR-2) and receptor 4 (TLR-4) on dendritic cells and macrophages. Binding of GP96 to TLR-2 or TLR-4 upon these cells increases expression of co-stimulatory molecules CD80, CD86, and CD40 as well as MHC II, IL-12, and TNF-α expression [74-76].

Crane et al. investigated the efficacy of HSP-96 for recurrent GBM in a phase I study involving 12 patients with autologous tumor-derived HSP peptide complex (HSPPC, Aegenus Incorporated). Eleven patients demonstrated specific peripheral immune responses and seven demonstrated increased immune cell infiltrate 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 resulting in robust activation, proliferation and effector molecule/cytokine production. Autologous CTLs from tumor samples can be expanded *in vitro* in the presence of IL-2 and stimulated with antibodies specific to the TCR and passively transferred into host recipients.

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 peripherally derived $CD8^+$ cells activated *in vitro* in the presence of IL-2 with non-specific tumoricidal activity. Furthermore, these cells are capable of lysing fresh, non-cultured, natural killer (NK) cell-resistant tumor cells. Adoptive transfer of LAK cells with recombinant IL-2 mediated regression of a variety of metastatic tumors in numerous murine models [84-87]. Hayes et al. reported their results treating 19 adult patients with recurrent malignant glioma with intra-cavitary autologous LAK cells plus IL-2 following re-operation. Of note, one patient with anaplastic astrocytoma experienced a complete response and one patient with GBM experienced a delayed complete response with two other patients with GBM experiencing partial responses. Furthermore, the median survival was 53 weeks following re-operation compared to 25.5 weeks for contemporary patients with GBM who underwent re-operation and chemotherapy. Interestingly, aspiration from the Ommaya reservoir revealed regional eosinophilia and an extensive lymphocytic infiltrate [88].

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).

NCT number	Title	Agent	Phase	Outcome measures
NCT02798406	Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects	DNX-2401 + pembrolizumab	Phase 2	Objective response rate (ORR) | Overall survival (OS) | Time to tumor response | Duration of response
NCT02852655	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	Drug: MK-3475		Tumor Infiltrating T Lymphocyte (TIL) Density | Incidence of Treatment-Emergent Adverse Events | Progression Free Survival

NCT02667587	Study of Temozolomide Plus Radiation Therapy With Nivolumab or Placebo, for Newly Diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer).	Nivolumab + Temozolomide + Radiotherapy	Phase 2	Overall survival defined as time from the date of randomization to the date of death. | Progression free survival, defined as the time from randomization to the date of the first documented tumor progression or death to any cause.
NCT02617589	Study of Nivolumab Compared to Temozolomide, Given With Radiation Therapy, for Newly-diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer)	Nivolumab + Temozolomide + Radiotherapy	Phase 3	Overall survival (OS) | Progression free survival (PFS) | Overall survival
NCT02431572	A Pilot Study to Evaluate PBR PET in Brain Tumor Patients Treated With Chemoradiation or Immunotherapy	PBR PET + Cancer Immunotherapy + Radiation and chemotherapy		Change in PBR uptake (changes in PBR uptake by PET)
NCT02502708	Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients With Progressive Primary Malignant Brain Tumors	Indoximod + Temozolomide + Conformal Radiation	Phase 1	Incidence of regimen limiting toxicities (RLTs) | Objective Response Rate | Pharmacokinetics: Serum concentrations (Cmax/Steady State) | Safety and Tolerability of Indoximod combined with Temozolomide as assessed by incidence and severity of adverse events, dose interruptions and dose reductions. | Progression Free Survival (PFS) | Time to Progression | Overall Survival | Safety and Feasibility of Indoximod combined with conformal radiation as assessed by incidence and severity of adverse events, dose interruptions and dose reductions.

Table 2: Checkpoint blockade-based clinical trials for GBM. Source: clinicaltrials.gov

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 $CD4^+$ Th helper cells and Tregs appear to play the dominant physiological role. Moreover, CTLA-4 serves to dampen $CD4^+$ Th 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 *Ctla-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 enhance endogenous 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 (NCT02311920) 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 70% (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, NCT02311920, NCT02337491, NCT02337686, NCT02658279, NCT02336165).

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.

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