DISCOVERY MEDICINE®
www.discoverymedicine.com
ISSN: 1539-6509; eISSN: 1944-7930

Novel Approaches and Mechanisms of Immunotherapy for Glioblastoma

MEENAKSHI HEGDE, KEVIN J. BIELAMOWICZ, AND NABIL AHMED

Abstract: Glioblastoma (GBM) is the most aggressive primary brain tumor. Combination therapy with surgery, radiation, and chemotherapy is not curative at present and carries a significant risk of toxicity. Advancements in the knowledge of tumor biology and tumor microenvironment have led to the development of novel targeted therapies for glioblastoma. In the past 15 years, a vast amount of pre-clinical data has been generated for glioblastoma immunotherapy. Translating these promising results into the clinic is, however, still an evolving process. Early clinical trials have demonstrated the feasibility and safety of several such approaches in patients with recurrent as well as newly diagnosed glioblastoma. Both passive as well as active immunotherapeutic modalities have also shown potential clinical benefit in at least a subset of these patients. This brief review discusses 'why' and 'how' various types of immunotherapies are being employed to treat glioblastoma. [*Discovery Medicine* 17(93):145-154, March 2014]

Introduction

Glioblastoma (GBM) is the most common primary brain tumor in adults with the annual incidence of over 17,000 in the United States (Grossman *et al.*, 2010; Omuro and DeAngelis, 2013). Despite the considerable improvements made in the conventional therapy for glioblastoma over the recent years, prognosis remains extremely poor with a median survival of 18 to 21 months (Finlay *et al.*, 1995; Grossman *et al.*, 2010; Johnson *et al.*, 2012). Complete tumor resection is difficult owing to the diffusely infiltrative nature of the tumor (Grossman *et al.*, 2010). Concomitant and adjuvant chemotherapy with the alkylating agent temozolomide along with radiation has only been shown to improve median survival by 2.5 months (Stupp *et al.*, 2005). In the past decade, substantial amount of progress has been made in dissecting the glioblastoma biology in relation to its microenvironment as well as the host immune system. This has paved the way for researchers to explore novel targeted immunotherapeutic approaches that have the potential to improve cure rates with minimal toxicities, due to sparing of the surrounding normal brain structures. Here we review the advances made in some of the passive and active immunotherapeutic strategies for glioblastoma.

***Meenakshi Hegde**, M.D., **Kevin J. Bielamowicz**, M.D., and **Nabil Ahmed**, M.D., are at the Center for Cell and Gene Therapy; Texas Children's Cancer Center; Department of Pediatrics, Baylor College of Medicine; Houston, TX 77030, USA.*

Corresponding Author: Meenakshi Hegde, M.D. (mghegde@bcm.edu) or Nabil Ahmed, M.D. (nahmed@bcm.edu).

Targeted Immunotherapy with Monoclonal Antibodies (mAb)

Monoclonal antibodies (mAb) recognize specific antigens present on the tumor cell surface in a Major Histocompatibility Complex (MHC)-unrestricted manner and induce cell death by a host of immune and non-immune mediated mechanisms. Safe and effective anti-tumor therapy with monoclonal antibodies (mAb) requires that the target antigen be confined to the tumor and its microenvironment with absent or very low frequency of expression on normal tissues. Glioblastomas are known to be highly vascular tumors that secrete pro-angiogenic factors like vascular endothelial growth factor (VEGF) (Huang *et al.*, 2005). VEGF is a key regulator of angiogenesis and is known to play a major role in tumor growth (Folkman, 1971; Hicklin and Ellis, 2005). VEGF production is increased under hypoxic conditions, e.g., in rapidly growing tumors with necrotic centers and its over-expression appears to correlate with poor prognosis (Carmeliet, 2005; Huang *et al.*, 2005). VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3) belong to a family of platelet derived growth factor tyrosine kinase receptors (PDGFR) that upon binding with its ligand activates downstream signaling leading to angiogenesis and increased vascular

permeability (Karkkainen and Petrova, 2000; Petrova *et al.*, 1999). Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody that neutralizes the biologic activity of isoform VEGF-A by blocking its binding to VGFR-1 and VGFR-2 on tumor endothelial cells (Ferrara *et al.*, 2005). Bevacizumab, either as a single agent or in combination with irinotecan was shown to be effective for recurrent glioblastoma (Friedman *et al.*, 2009; Kreisl *et al.*, 2009). Multiple ongoing clinical trials are further testing the therapeutic efficacy of bevacizumab as a single agent as well as in combination with other cytotoxic agents and/or radiation (Table 1). Though recent evidence suggests the permeability of blood brain barrier (BBB) to intravenous bevacizumab (Friedman *et al.*, 2009; Kreisl *et al.*, 2009), the use of the intra-arterial route is being investigated as well (Table 1).

Epidermal growth factor receptor (EGFR) gene mutation is a frequent finding in glioblastoma with a deletion mutation EGFR*v*III (epidermal growth factor receptor variant III) being the most common (Aldape *et al.*,

Table 1. Summary of Clinical Trials of Monoclonal Antibodies (mAb) for Glioblastoma.

Monoclonal Antibody (mAb)	Targeted Antigen	Trial Description / Phase	ClinicalTrials.gov Identifier
Bevacizumab	VEGF	+ conventional therapy (XRT, and/or TMZ) / phase II	NCT01149850; NCT01939574; NCT01860638; NCT01987830; NCT01443676; NCT01478321; NCT01740258
		+/- TPI 287 (microtubule-stabilizing agent)	NCT01933815; NCT02047214
		+ BKM120 (oral inhibitor of PI3 kinase) / phase II	NCT01349660
		+ NovoTTF-100A* to stunt tumor cell growth / phase II	NCT01894061; NCT01925573; NCT01954576
		+ Lomustine (CCNU) / phase II, III	NCT01067469; NCT01290939
		+ AMG 386 (trebananib; anti-angiogenic agent) / phase I, II	NCT01290263; NCT01609790
		Intraarterial cerebral infusion of bevacizumab plus carboplatin / phase I, II	NCT01386710
		+ radiosurgery and irinotecan / phase I, II	NCT01086345
		+/- anti-endoglin mAb TRC105 / phase I, II	NCT01648348; NCT01564914
		+ radiation and valproic acid	NCT00879437
		Super-selective Intraarterial Cerebral Infusion / phase I, II	NCT01269853; NCT00968240; NCT01811498
		+ dasatinib (tyrosine kinase inhibitor) / phase II	NCT00892177
		+ TMZ and bortezomib / phase I	NCT01435395
		+/- vorinostat / phase I, II	NCT01266031; NCT01738646
Nivolumab	PD-1	+/- Ipilimumab vs. bevacizumab / randomized phase II	NCT02017717
AMG 102 (Rilotumumab)	HGF	With bevacizumab	NCT01113398
Cetuximab	EGFRvIII	Super-selective intra-arterial cerebral infusion	NCT01238237
		Super-selective intra-arterial cerebral infusion with bevacizumab	NCT01884740
AMG 595	EGFRvIII	An immunoconjugate of anti-EGFRvIII human mAb with a cytotoxic agent maytansinoid DM1	NCT01475006

Abbreviations: TMZ, temozolomide; XRT, radiation therapy; VEGF, vascular endothelial growth factor; PD-1, programmed death-1; HGF, hepatocyte growth factor; EGFRvIII, epidermal growth factor receptor variant III.
* NovoTTF-100A is a device that delivers low intensity, alternating electrical fields to the brain via the electrodes placed on scalp to disrupt tumor cell division.

2004; Smith *et al.*, 2001; Wong *et al.*, 1992). Mutated EGFR*v*III is a transmembrane glycoprotein that is expressed in 20 to 30% of GBMs (Moscatello *et al.*, 1995) and has constitutive tyrosine kinase activity that plays an important role in tumorigenesis and development of chemoresistance (Nagane *et al.*, 1996; Nishikawa *et al.*, 1994). Recombinant human/mouse chimeric anti-EGFR*v*III mAb cetuximab was found to be well tolerated when given intravenously in patients with recurrent GBM with encouraging results (Hasselbalch *et al.*, 2010). However, combination therapy with cetuximab and bevacizumab/irinotecan was not found to be superior to bevacizumab and irinotecan alone (Hasselbalch *et al.*, 2010). Safety and efficacy of intra-arterial infusion of cetuximab alone and in combination with bevacizumab is now being investigated (Table 1). Another anti-EGFR*v*III antibody nimotuzumab has completed phase III trial and results are expected (NCT00753246). AMG 595, an immunoconjugate of anti-EGFRvIII human mAb with a cytotoxic agent maytansinoid DM1, is currently in a phase I trial (NCT01475006). Binding of the mAb to the EGFRvIII on tumor cell surface leads to internalization and disruption of the microtubule by maytansinoid DM1 resulting in inhibition of tumor cell proliferation. Nivolumab is a fully human mAb that blocks the activation of negative immunoregulatory cell surface receptor PD-1 (programmed death-1) by its ligands, PD-L1 and PD-L2, leading to activation of cytotoxic T-lymphocytes (CTLs) against tumor cells (Robert *et al.*, 2013; Wolchok *et al.*, 2013). Efficacy of nivolumomab is currently being tested in combination with ipilimumab (NCT02017717), a mAb that enhances T-cell activation by binding cytotoxic T-lymphocyte-associated antigen-4 (CTLA4; CD152) thus offsetting its inhibitory effect that is mediated through CD80 (B7-1) and CD86 (B7-2) (Robert *et al.*, 2013; Wolchok *et al.*, 2013).

Induction of *In Vivo* Anti-tumor Response Using Tumor Vaccines

For active specific immunotherapy of glioblastoma, autologous dendritic cells (DCs) are most commonly used as antigen presenting cells (APCs). In addition to being the most powerful activators of innate and adaptive immune system, DCs have been shown to activate Natural Killer (NK) cells and NK T cells (Dhodapkar *et al.*, 2004; Rock *et al.*, 1990; Vidard *et al.*, 1996). Peripheral blood monocyte (PBMC)-derived DCs obtained from GBM patients by leukapheresis are pulsed *ex vivo* with tumor lysates or acid eluted membrane peptides, or by fusing the DCs with tumor cells (De Vleeschouwer *et al.*, 2008; Liau *et al.*, 2005; Wheeler *et al.*, 2008; Yamanaka *et al.*, 2005; Yu *et al.*, 2001). Tumor antigen-loaded DCs are injected into the patient, most often intradermally, though other routes of administration (i.e., subcutaneous and intravenous) have been explored. Injected DCs then migrate to the lymph nodes to activate tumor antigen specific cytotoxic T lymphocytes (CTLs) *in vivo*, and induce sustained anti-tumor response in the host by forming immunological memory (de Vries *et al.*, 2005; Morse *et al.*, 1999).

Researchers have made considerable progress in translating the glioma vaccine therapy from the bench to the bedside. DC vaccines for glioblastoma have been well tolerated in early clinical trials with considerable efficacy (Bregy *et al.*, 2013; Chang *et al.*, 2011; Cho *et al.*, 2012; Phuphanich *et al.*, 2013; Yu *et al.*, 2004; 2001). Most of the current clinical trials are designed to further test their efficacy and answer the key questions, such as ideal time interval between vaccines and the total duration of therapy required to sustain the host anti-tumor response (Table 2). As most immunotherapeutic approaches for cancer are known to be beneficial in the setting of minimal disease burden, post-operative adjuvant therapy with DC vaccines has been investigated. Improved PFS (progression free survival) and OS (overall survival) was reported following vaccination with autologous, mature, tumor lysate-loaded DC as an adjuvant therapy after reoperation (HGG-IMMUNO) in patients with relapsed GBM (n=56; 7 to 77 years of age) (De Vleeschouwer *et al.*, 2008). Total resection and younger age were shown to be the predictors of better outcome with trend to improved PFS with faster DC vaccination schedule (De Vleeschouwer *et al.*, 2008). A phase I study being conducted in collaboration with the HGG-IMMUNO group to investigate the anti-tumor immunity following intradermal injection of autologous DC vaccine with imiquimod (immune response modifier) after surgical resection is currently recruiting patients (NCT01808820). Rindopepimut, a peptide vaccine which evokes humoral and cellular immune response against EGFR*v*III, has demonstrated improved PFS and OS with minimal side effects in adults with glioma (Heimberger *et al.*, 2003; Sampson *et al.*, 2009) and is currently being tested in a randomized phase III trial for adults with newly diagnosed GBM (NCT01480479). Recently, there has been a tremendous amount of interest in targeting cancer stem cells (CSCs) as they are believed to lead to tumorigenesis in the human brain and to play a key role in chemoresistance/radioresistance seen in glioblastoma, and in tumor recurrence (Altaner, 2008; Bao *et al.*, 2006). Safety of autologous DC vaccine against CD133, the stem-like cell marker expressed in the glioblastoma cells, is currently being tested in a first-in-man trial (NCT02049489). Single antigen based vaccines carry

the risk of creating target-antigen negative tumor cell variants (Sampson *et al.*, 2010), whole tumor cell derived multi-peptide vaccines consisting of a panel of tumor-associated antigens (TAAs) along with some non-specific antigens are hence preferred (Van Gool *et al.*, 2009). While the risk of inducing immune response against normal host tissues is a consideration with this strategy, none has been reported in GBM trials so far.

Passive Immunotherapy with Adoptive Cell Transfer

Adoptive cell transfer involves directly transferring effector immune cells to a host in order to induce anti-tumor activity. These *ex vivo*-generated effector cells may be innate immune cells or cells capable of more specific cell recognition. Nonspecific effector cells such as NK cells and lymphokine-activated killer (LAK) cells react innately as they recognize cell surface abnormalities, such as low expression of MHC class I molecules or carbohydrate abnormalities. T cells recognize foreign peptides presented on the cell surface by MHC molecules. While T cells specific for tumor antigens can be identified within the tumor tissues or elsewhere, most are present at a low frequency and

Table 2. Tumor Vaccine Trials for Glioblastoma.

Vaccine / Phase	Targeted Antigen / Description	ClinicalTrials.gov Identifier	Trial Site or Sponsor
Autologous DC vaccine / phase I	CD-133*	NCT02049489	Cedars-Sinai Medical Center
Autologous DC vaccine / phase I	Pulsed with lysate derived from an allogeneic glioblastoma stem-like cell line	NCT02010606	Cedars-Sinai Medical Center
Autologous DC vaccine / phase I	Tumor-lysate pulsed	NCT01957956	Mayo Clinic
DC vaccine / phase I	Tumor-lysate pulsed / with imiquimod (immune response modifier)	NCT01808820; NCT01902771	University of Miami Sylvester Comprehensive Cancer Center
Autologous DC vaccine	Tumor-lysate pulsed / +/- resiquimod (immunostimulator) or Poly-ICLC	NCT01204684	Jonsson Comprehensive Cancer Center
BTSC mRNA-loaded DC vaccine / phase I	Pulsed with BTSC-specific mRNA.	NCT00890032	National Cancer Institute
ERC1671/GM-CSF / phase I	Mixture of the autologous and allogeneic cells and lysates/ with bevacizumab and oral cyclophosphamide	NCT01903330	University of California, Irvine
Rindopepimut (CDX-110) / phase II, III	EGFRvIII With GM-CSF and bevacizumab	NCT01498328; NCT01480479	Celldex Therapeutics
ADU-623 (Live-attenuated *Listeria monocytogenes* strain expressing the EGFRvIII-NY-ESO-1) / phase I	EGFRvIII NY-ESO-1**	NCT01967758	Providence Health & Services
DEC-205-NY-ESO-1 fusion protein vaccine / phase I	NY-ESO-1**	NCT01522820	Roswell Park Cancer Institute
Heat Shock Protein-Peptide Complex-96 (HSPPC-96) vaccine/ phase II	+/- bevacizumab	NCT01814813	Alliance for Clinical Trials in Oncology
IMA 950 multi-tumor associated peptide vaccine / phase I, II	With Poly-ICLC*** (adjuvant), TMZ and radiation therapy	NCT01920191	University Hospital, Geneva
Montanide ISA-51/ phase I	Survivin peptide vaccine	NCT01250470	Roswell Park Cancer Institute

Abbreviations: DC, dendritic cell; GM-CSF, granulocyte macrophage colony stimulating factor; EGFRvIII, epidermal growth factor receptor variant III; BTSC, brain tumor stem cell; TMZ, temozolamide.
* CD-133 is a stem cell marker in glioblastoma.
** NY-ESO-1 is a cancer/testis antigen.
*** Ply-ICLC is a synthetic complex of carboxymethylcellulose, polyinosinic-polycytidylic acid, and poly-L-lysine double-stranded RNA.

many have receptors with low avidity for the tumor antigens, and are commonly anergic. An alternative strategy is to activate T cells *ex vivo* to circumvent these limitations and to overcome suppressive factors present *in vivo*, thus augmenting the anti-tumor activity (June, 2007). It is necessary to enrich for anti-tumor cells with the appropriate properties or to redirect the specificity of a non-tumor-specific population that can then be expanded to large numbers *ex vivo* for subsequent adoptive transfer. In addition, the host can be manipulated before adoptive cell transfer to provide an optimal environment for the transferred cells. In general, the transfer of *ex vivo* generated effectors could potentially overcome some of the current limitations of other targeted immunotherapies since T cells can expand, actively migrate through microvascular walls and penetrate the core of solid tumors to exert their antitumor activity (Marras *et al.*, 2003; Plautz *et al.*, 2000; Tsuboi *et al.*, 2003).

Early work introduced NK cells, lymphokine activated killer cells (LAKs), and γδ T cells as ways to expand and activate the immune system and tip the balance towards an antitumor effector function in the face of a substantially immunosuppressive tumor microenvironment. LAK cells have been safely administered within the CNS resulting in improved long term survival in adult patients with recurrent glioma (Hayes *et al.*, 1995). In a phase II trial of adult patients with GBM (n=33) treated with intralesional autologous LAK cells after initial primary treatment, those with higher doses of LAK cells had longer survival, and overall survival was encouraging compared to controls (Dillman *et al.*, 2009). *Ex vivo* activation of tumor-draining lymph node cells induces potent effector function (Porter *et al.*, 2011). This strategy has been used in patients with recurrent and newly diagnosed malignant glioma after surgery and radiation therapy. Several objective clinical responses were noted in both adult and pediatric patients with no significant toxicity (Plautz *et al.*, 2000; Peres *et al.*, 2008). Intracavitary administration of allogeneic mixed reactive T cells for recurrent gliomas (n=5) has shown promising results and the benefits of intra-tumoral administration is being studied (Kruse *et al.*, 1997; Wang *et al.*, 2008).

Over time, adoptive cell therapies for the treatment of glioblastoma have evolved from being relatively non-specific to tumor-specific, and most current clinical trials utilize targeted approaches such as virus specific cytotoxic T lymphocytes (CTLs) and chimeric antigen receptor (CAR)-modified T cells (Table 3). T cells recognize targets through an antigen-specific T-cell receptor (TCR) and engage with their target when presented in the context of a matching MHC molecule. Developing successful CTL therapies depends on the availability of tumor-associated antigens (TAAs) as targets, their successful processing and presentation by professional APCs, and efficient methods for T-cell activation and expansion. Both viral antigens and TAAs can be used as such targets. Several TAAs have been validated as therapeutic targets in GBM and are actively being studied for potential clinical application (Saikali *et al.*, 2007; Wykosky *et al.*, 2008; Zhang *et al.*, 2008). CD133 is one such TAA of interest in CTL therapy for glioblastoma; CD133+ CTLs have been shown to be cytotoxic to glioma stem cells (Hua *et al.*, 2011). A large percentage of GBM have been shown to express the cytomegalovirus (CMV) immunodominant proteins pp65 and IE1-72 as well as CMV nucleic acid has been detected in GBM cells by *in situ* hybridization (Cobbs *et al.*, 2002; Mitchell *et al.*, 2008; Scheurer *et al.*, 2008). CMV-specific CTLs expanded *ex vivo* from CMV seropositive GBM patients have been shown to recognize and kill CMV-expressing autologous tumor cells (Louis and Brenner, 2009). This has prompted the

Table 3. Current Adoptive Cell Therapy for Glioblastoma.

Immunotherapy Approach / Phase	Target	ClinicalTrials.gov Identifier	Trial Site or Sponsor
Intratumoral injection of Allogeneic CTLs expressing genetically modifies α/β T cells / phase I	IL13Rα2	NCT01082926	City of Hope Medical Center
Allogeneic T-cell infusion / phase I	Not TAA-specific	NCT01144247	Jonsson Comprehensive Cancer Center
Vaccine/CMV-specific cytotoxic lymphocytes / phase I	CMV-specific antigen	NCT00693095	Duke University Medical Center
CAR modified CMV-specific cytotoxic lymphocytes / phase I	HER2	NCT01109095	Baylor College of Medicine
CAR modified T cells / phase I, II	EGFRvIII	NCT01454596	National Cancer Institute (NCI)

Abbreviations: CTL, cytotoxic T lymphocyte; TAA, tumor-associated antigen; CMV, cytomegalovirus; CAR, chimeric antigen receptor.

use of CMV specific CTLs as a therapeutic modality in phase I trials (NCT00693095; NCT01205334; NCT01109095).

Genetic modification of T cells with chimeric antigen receptors (CARs) can also be employed as highly targeted therapies using adoptive cell transfer (Figure 1). CARs are artificial molecules custom made by fusing an extracellular variable domain usually derived from a high-affinity monoclonal antibody specific for a TAA of interest to an intracellular signaling domain derived from the ζ-signaling chain of the TCR (Eshhar *et al.*, 1993). The intracellular signaling domain is responsible for activating the T cell upon encountering the specific antigen. CARs recognize antigens in an HLA-independent manner and hence are able to circumvent some mechanisms by which tumors evade immune-recognition, such as down regulation of MHC molecules (Sadelain *et al.*, 2003). CAR T cells have been shown to be effective even when target antigens are modestly expressed on tumor cells likely because they can multiply in response to antigen encounter and can recruit other effectors as well as additional components of the immune system amplifying the antitumor immune response (Ahmed *et al.*, 2009). In addition, they broaden the range of antigens recognizable by T cells to include carbohydrate and glycolipid antigens. T cells expressing CARs can be reliably generated in a relatively short time for clinical usage, typically 10-15 days (Pule *et al.*, 2003). CAR-modified T cells have been effectively generated against some of the glioma-associated antigens including IL-13 receptor alpha 2 (IL13Rα2), human epidermal growth factor receptor 2 (HER2), ephrin type A receptor 2 (EphA2), and epidermal growth factor receptor variant III (EGFRvIII) (Choi *et al.*, 2014; Liu *et al.*, 2004; Jarboe *et al.*, 2007; Morgan *et al.*, 2012; Wang *et al.*, 2008). HER2-specific CAR T cells generated from GBM patients recognized autologous HER2-positive tumor cells, including their CD133-positive stem cells *in vitro*, and had potent anti-tumor activity against autologous xenografts in orthotopic models of human glioblastoma (Ahmed *et al.*, 2010). A clinical trial of CMV-specific CTLs modified to express HER2-specific CARs is currently underway (NCT01109095). T cells engineered to target IL13Rα2 have also shown tumor recognition and anti-tumor

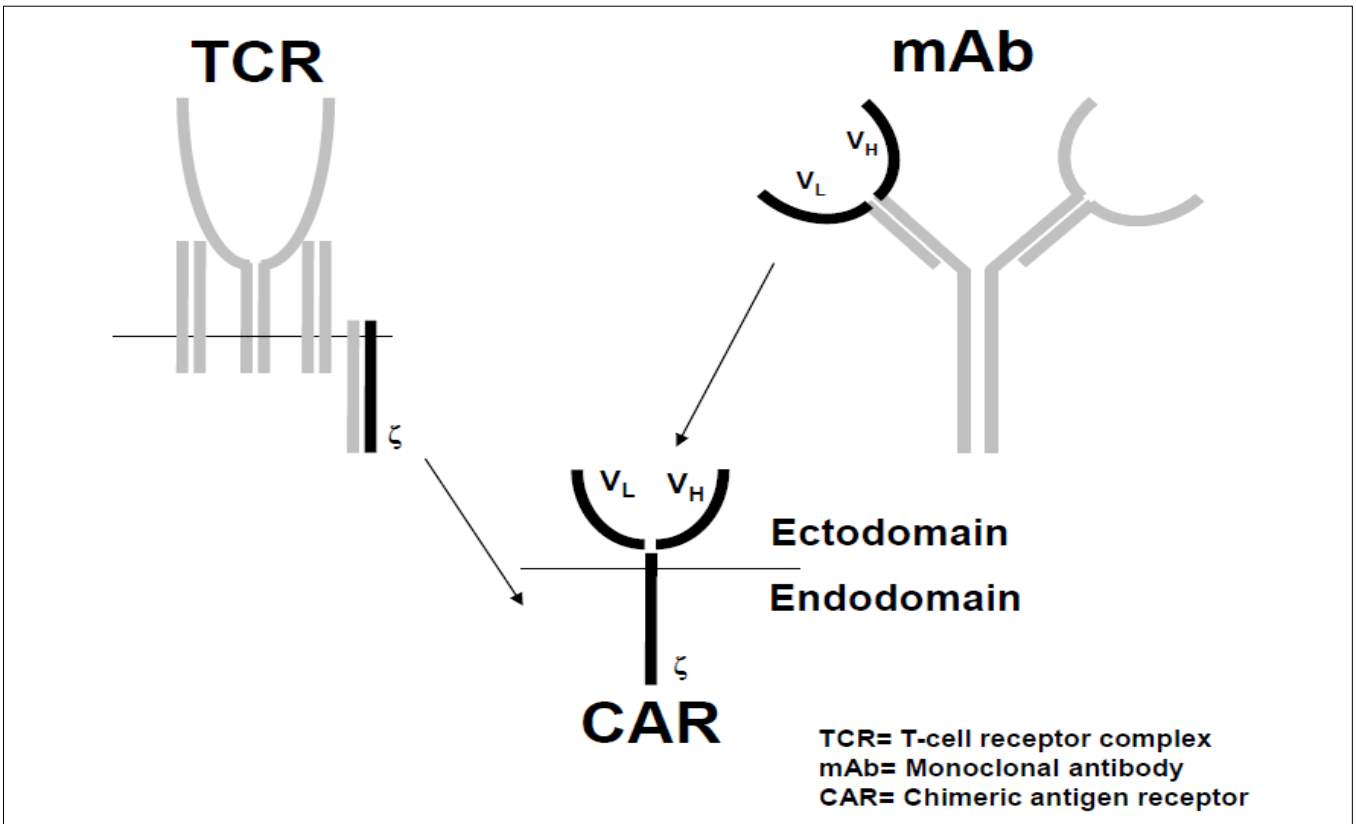

Figure 1. Chimeric antigen receptor (CAR) consists of an extracellular antigen recognition domain (ECTODOMAIN) usually derived from the antigen recognition domain of a tumor antigen specific monoclonal antibody and an intracellular domain (ENDODOMAIN) with a kinase activity derived from the T-cell receptor complex ζ-chain. Upon the engagement of the target molecule by the ectodomain, the endodomain delivers a downstream signal initiating T-cell activation.

effector function (Kahlonn *et al.*, 2004; NCT00730613). IL13Rα2 is currently being explored in a clinical trial by infusion of autologous CAR T cell clones into resection cavities of adult GBM (NCT01082926). Further, a phase I/II trial is investigating the safety and effectiveness of autologous CARs targeting EGFRvIII in adults with glioblastoma (NCT01454596).

Developing an Effective Immunotherapy for Glioblastoma: Challenges Involved

Though the pre-clinical data for adoptive glioblastoma immunotherapy has been largely promising, several obstacles, some posed by glioma cells or their microenvironment and others intrinsic to the immunotherapy products, limit their clinical efficacy. Glioblastoma cells are considered to be poor APCs. They have inadequate phosphorylation and cytoskeletal rearrangements which are required for appropriate APC to T-cell contacts and stimulation of an immune response. T cells obtained from patients with gliomas do not make sufficient contact with APCs and consequently are not appropriately stimulated (Dix *et al*., 1999). Tumor cells can, directly or by influencing the tumor microenvironment to play a protumoral role, manipulate the host's immune response for tumor-protective effects. T cells in the microenvironment and their impact on tumor growth may depend heavily upon the particular tumor infiltrating lymphocyte (TIL) subset. It is fairly agreed upon that the majority of CD4+ T cells favor tumor progression, while CD8+ T cells favor tumor rejection in GBM (Byrd *et al.*, 2012). Regulatory T cells inhibit the effect of T cells against tumor antigens (Heimberger *et al.*, 2003). Cytokines produced by tumors stimulate increased helper T-cell and decreased regulatory T-cell function that decreases natural tumor immunity (Sonabend *et al.*, 2012). Secretion of inhibitory factors such as transforming growth factor beta (TGFβ) by the tumor microenvironment can have inhibitory effects on the immune system and can allow cancers to proliferate and become more invasive (Grauer *et al.*, 2007; Heimberger *et al.*, 2008; Kuppner *et al.*, 1989; Nakano *et al.*, 2006; Platten *et al.*, 2001; Siepl *et al.*, 1988). The *in vivo* induction of antigen-specific T cells using antigen loaded DC is often not reproducible, because tumor-specific T cells are either present at very low frequency due to relatively weak immunogenicity of TAAs or are anergized (Marras *et al.*, 2003; Plautz *et al.*, 2000; Tsuboi *et al.*, 2003). Down-regulation of MHC molecules may limit the effectiveness of tumor vaccine induced cellular immune response as well (Dunn *et al.*, 2004). Problems such as limited *in vivo* expansion following infusion of cellular products are being resolved by optimizing the cellular product (for example including enhanced signaling domains in CAR T cells or infusion of more naïve phenotypes of effectors) and/or optimizing the host by strategies such as lymphodepletion or co-administration of immuno-stimulatory cytokines.

In addition to having variable antigen expression between patients, glioblastoma cells in any individual patient exhibit great heterogeneity and targeted therapies can become ineffective over time as tumors develop antigen escape variants. This could develop because of the high mutation rate in glioblastoma, but might be intrinsic to the tumor cell or induced by selective survival of target negative tumor cells after therapy. Overcoming this mechanism of resistance will be necessary to improve response in patients. There are strategies in the development that could offset antigen escape by co-targeting multiple TAAs, such as use of whole tumor cell derived DC vaccines and using cellular products grafted with multiple CARs (Hegde *et al.*, 2013) or TanCAR, a bispecific CAR molecule that can simultaneously target two TAAs (Grada *et al.*, 2013). The presence of BBB provides challenges in using systemically administered immunotherapy strategy for the treatment of glioblastoma. This barrier also provides immune privilege that makes utilizing host immune responses in treatment of these malignancies challenging (Doolittle *et al.*, 2005). While many studies utilize direct injection of cells to the tumor site to bypass BBB, intravenous, intra-arterial, intranodal, intradermal, and intranasal injections are other options. Investigative comparison of these delivery strategies should be performed to reach the optimal delivery route for effective GBM immunotherapy modalities.

Summary

Developing an effective targeted immunotherapy for glioblastoma has been a considerable challenge due to disease heterogeneity and hostile environment the tumor creates for the immune system. Despite the obstacles, remarkable progress has been made in the field in the past decade. While early clinical trials of these targeted approaches have shown encouraging results in terms of efficacy and safety, substantial testing needs to be undertaken before these novel treatment modalities can be made available as standard therapies across the centers that treat patients with glioblastoma.

Acknowledgment

Authors have funding support from the Alliance for Cancer Gene Therapy (ACGT, Inc.), Alex's Lemonade Stand Pediatric Cancer Foundation (ALSF), CureSearch for Children's Cancer, Children's Cancer

Research Fund, and the Stand Up to Cancer (SU2C)-St. Baldrick's Pediatric Cancer Dream Team Grant.

Disclosure

The Center for Cell and Gene Therapy (CAGT) has research collaboration with Celgene Inc., to develop chimeric antigen receptor (CAR)-based therapeutics that is administered by Baylor College of Medicine. M.H. and N.A. have patent applications in the field of T-cell and gene-modified T-cell therapy for cancer.

References

Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, Grossman RG, Heslop HE, Gottschalk S. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. *Clin Cancer Res* 16(2):474-485, 2010.

Ahmed N, Salsman VS, Yvon E, Louis CU, Perlaky L, Wels WS, Dishop MK, Kleinerman EE, Pule M, Rooney CM, Heslop HE, Gottschalk S. Immunotherapy for osteosarcoma: genetic modification of T cells overcomes low levels of tumor antigen expression. *Mol Ther* 17(10):1779-1787, 2009.

Aldape KD, Ballman K, Furth A, Buckner JC, Giannini C, Burger PC, Scheithauer BW, Jenkins RB, James CD. Immunohistochemical detection of EGFRvIII in high malignancy grade astrocytomas and evaluation of prognostic significance. *J Neuropathol Exp Neurol* 63(7):700-707, 2004.

Altaner C. Glioblastoma and stem cells. *Neoplasma* 55(5):369-374, 2008.

Bao S, Wu Q, Mclendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444(7120):756-760, 2006.

Bregy A, Wong TM, Shah AH, Goldberg JM, Komotar RJ. Active immunotherapy using dendritic cells in the treatment of glioblastoma multiforme. *Cancer Treat Rev* 39(8):891-907, 2013.

Byrd T, Grossman RG, Ahmed N. Medulloblastoma — biology and microenvironment: a review. *Pediat Hematol Oncol* 6:495-506, 2012.

Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology* 69(Suppl 3):4-10, 2005.

Chang CN, Huang YC, Yang DM, Kikuta K, Wei KJ, Kubota T, Yang WK. A phase I/II clinical trial investigating the adverse and therapeutic effects of a postoperative autologous dendritic cell tumor vaccine in patients with malignant glioma. *J Clin Neurosci* 18(8):1048-1054, 2011.

Cho DY, Yang WK, Lee HC, Hsu DM, Lin HL, Lin SZ, Chen CC, Harn HJ, Liu CL, Lee WY, Ho LH. Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. *World Neurosurg* 77(5-6):736-744, 2012.

Choi BD, Suryadevara CM, Gedeon PC, Herndon Ii JE, Sanchez-Perez L, Bigner DD, Sampson JH. Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. *J Clin Neurosci* 21(1):189-190, 2014.

Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG, Britt WJ. Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* 62:3347-3350, 2002.

De Vleeschouwer S, Fieuws S, Rutkowski S, Van Calenbergh F, Van Loon J, Goffin J, Sciot R, Wilms G, Demaerel P, Warmuth-Metz M, Soerensen N, Wolff JE, Wagner S, Kaempgen E, Van Gool SW. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. *Clin Cancer Res* 14(10):3098-3104, 2008.

De Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, Van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nat Biotechnol* 23(11):1407-1413, 2005.

Dhodapkar KM, Cirignano B, Chamian F, Zagzag D, Miller DC, Finlay JL, Steinman RM. Invariant natural killer T cells are preserved in patients with glioma and exhibit antitumor lytic activity following dendritic cell-mediated expansion. *Int J Cancer* 109(6):893-899, 2004.

Dillman RO, Duma CM, Ellis RA, Cornforth AN, Schiltz PM, Sharp SL, Depriest MC. Intralesional lymphokine-activated killer cells as adjuvant therapy for primary glioblastoma. *J Immunother* 32(9):914-919, 2009.

Dix AR, Brooks WH, Roszman TL, Morford LA. Immune defects observed in patients with primary malignant brain tumors. *J Neuroimmunol* 100:216-232, 1999.

Doolittle ND, Abrey LE, Bleyer WA, Brem S, Davis TP, Dore-Duffy P, Drewes LR, Hall WA, Hoffman JM, Korfel A, Martuza R, Peereboom D, Peterson DR, Rabkin SD, Smith Q, Stevens GHJ, Neuwelt EA. New frontiers in translational research in neuro-oncology and the blood-brain barrier: report of the tenth annual blood-brain barrier disruption consortium meeting. *Clin Cancer Res* 11:421-428, 2005.

Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 22:329-360, 2004.

Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell *Proc Natl Acad Sci U S A* 90:720-724, 1993.

Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochem Biophys Res Commun* 333(2):328-335, 2005.

Finlay JL, Boyett JM, Yates AJ, Wisoff JH, Milstein JM, Geyer JR, Bertolone SJ, Mcguire P, Cherlow JM, Tefft M, Et Al. Randomized phase III trial in childhood high-grade astrocytoma comparing vincristine, lomustine, and prednisone with the eight-drugs-in-1-day regimen. Childrens Cancer Group. *J Clin Oncol* 13(1):112-123, 1995.

Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285(21):1182-1186, 1971.

Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WK, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol* 27(28):4733-4740, 2009.

Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS, Corder A, Schonfeld K, Koch J, Dotti G, Heslop HE, Gottschalk S, Wels WS, Baker ML, Ahmed N. TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy. *Mol Ther Nucleic Acids* 2:e105, 2013.

Grauer OM, Nierkens S, Bennink E, Toonen LW, Boon L, Wesseling P, Sutmuller RP, Adema GJ. CD4+FoxP3+ regulatory T cells gradually accumulate in gliomas during tumor growth and efficiently sup-

press antiglioma immune responses in vivo. *Int J Cancer* 121(1):95-105, 2007.

Grossman SA, Ye X, Piantadosi S, Desideri S, Nabors LB, Rosenfeld M, Fisher J, Consortium NC. Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clin Cancer Res* 16(8):2443-2449, 2010.

Hasselbalch B, Lassen U, Hansen S, Holmberg M, Sorensen M, Kosteljanetz M, Broholm H, Stockhausen MT, Poulsen HS. Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: a phase II trial. *Neuro Oncol* 12(5):508-516, 2010.

Hayes RL, Koslow M, Hiesiger EM, Hymes KB, Hochster HS, Moore EJ, Pierz DM, Chen DK, Budzilovich GB, Ransohof J. Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. *Cancer* 76(5):840-852, 1995.

Hegde M, Corder A, Chow KKH, Mukherjee M, Ashoori A, Kew Y, Zhang J, Baskin DS, Merchant FA, Brawley VS, Byrd TT, Krebs S, Wu J, Liu H, Heslop HE, Gottschalk S, Yvon E, Ahmed N. Combinational targeting offsets antigen escape and enhances effector functions of adoptively transferred T cells in Glioblastoma. *Mol Ther* (Previewed online), 2013.

Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, Yang DS, Sun W, Qiao W, Hiraoka N, Fuller GN. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. *Clin Cancer Res* 14(16):5166-5172, 2008.

Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, Friedman HS, Bigner DD, Sampson JH. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. *Clin Cancer Res* 9(11):4247-4254, 2003.

Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 23(5):1011-1027, 2005.

Hua W, Yao Y, Chu Y, Zhong P, Sheng X, Xiao B, Wu J, Yang B, Mao Y, Zhou L. The CD133+ tumor stem-like cell-associated antigen may elicit highly intense immune responses against human malignant glioma. *J Neurooncol* 105(2):149-157, 2011.

Huang H, Held-Feindt J, Buhl R, Mehdorn HM, Mentlein R. Expression of VEGF and its receptors in different brain tumors. *Neurol Res* 27(4):371-377, 2005.

Jarboe JS, Johnson KR, Choi Y, Lonser RR, Park JK. Expression of interleukin-13 receptor alpha2 in glioblastoma multiforme: implications for targeted therapies. *Cancer Res* 67(17):7983-7986, 2007.

Johnson DR, Ma DJ, Buckner JC, Hammack JE. Conditional probability of long-term survival in glioblastoma: a population-based analysis. *Cancer* 118(22):5608-5613, 2012.

June CH. Adoptive T cell therapy for cancer in the clinic. *J Clin Invest* 117(6):1466-1476, 2007.

Kahlonn KS, Brown C, Cooper LJN, Raubitschek A, Forman SJ, Jensen MC. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res* 64:9160-9166, 2004.

Karkkainen MJ, Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. *Oncogene* 19(49):5598-5605, 2000.

Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, Garren N, Mackey M, Butman JA, Camphausen K, Park J, Albert PS, Fine HA. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol* 27(5):740-745, 2009.

Kruse CA, Cepeda L, Owens B, Johnson SD, Stears J, Lillehei KO. Treatment of recurrent glioma with intracavitary alloreactive cytotoxic T lymphocytes and interleukin 2. *Cancer Immunol Immunother* 45:77-87, 1997.

Kuppner MC, Hamou MF, Sawamura Y, Bodmer S, De Tribolet N. Inhibition of lymphocyte function by glioblastoma-derived transforming growth factor beta 2. *J Neurosurg* 71(2):211-217, 1989.

Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, Lin JW, Chute DJ, Mischel PS, Cloughesy TF, Roth MD. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clin Cancer Res* 11(15):5515-5525, 2005.

Liu G, Ying H, Zeng G, Wheeler CJ, Black KL, Yu JS. HER-2, gp100, and MAGE-1 are expressed in human glioblastoma and recognized by cytotoxic T cells. *Cancer Res* 64:4980-4986, 2004.

Louis CU, Brenner MK. Cellular immunotherapy for neuroblastoma: a review of current vaccine and adoptive T cell therapeutics. *Curr Pharm Des* 15(4):424-429, 2009.

Marras C, Mendola C, Legnani FG, Dimeco F. Immunotherapy and biologic modifiers for the treatment of malignant brain tumors. *Curr Opin Oncol* 15:204-208, 2003.

Mitchell DA, Xie W, Schmittling R, Learn C, Friedman A, Mclendon RE, Sampson JH. Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. *Neuro Oncol* 10(1):10-18, 2008.

Morgan RA, Johnson LA, Davis JL, Zheng Z, Woolard KD, Reap EA, Feldman SA, Chinnasamy N, Kuan CT, Song H, Zhang W, Fine HA, Rosenberg SA. Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. *Hum Gene Ther* 23(10):1043-1053, 2012.

Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res* 59(1):56-58, 1999.

Moscatello DK, Holgado-Madruga M, Godwin AK, Ramirez G, Gunn G, Zoltick PW, Biegel JA, Hayes RL, Wong AJ. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer Res* 55(23):5536-5539, 1995.

Nagane M, Coufal F, Lin H, Bogler O, Cavenee WK, Huang HJ. A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer Res* 56(21):5079-5086, 1996.

Nakano Y, Kuroda E, Kito T, Yokota A, Yamashita U. Induction of macrophagic prostaglandin E2 synthesis by glioma cells. *J Neurosurg* 104(4):574-582, 2006.

Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, Huang HJ. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. *Proc Natl Acad Sci U S A* 91(16):7727-7731, 1994.

Omuro A, Deangelis LM. Glioblastoma and other malignant gliomas: a clinical review. *JAMA* 310(17):1842-1850, 2013.

Peres E, Wood GW, Poulik J, Baynes R, Sood S, Abidi MH, Klein J, Bhambhani K, Dansey R, Abella E. High-dose chemotherapy and adoptive immunotherapy in the treatment of recurrent pediatric brain

tumors. *Neuropediatrics* 39(3):151-156, 2008.

Petrova TV, Makinen T, Alitalo K. Signaling via vascular endothelial growth factor receptors. *Exp Cell Res* 253(1):117-130, 1999.

Phuphanich S, Wheeler CJ, Rudnick JD, Mazer M, Wang H, Nuno MA, Richardson JE, Fan X, Ji J, Chu RM, Bender JG, Hawkins ES, Patil CG, Black KL, Yu JS. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. *Cancer Immunol Immunother* 62(1):125-135, 2013.

Platten M, Wick W, Weller M. Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. *Microsc Res Tech* 52(4):401-410, 2001.

Plautz GE, Miller DW, Barnett GH, Stevens GHJ, Maffett S, Kim J, Cohen PA, Shu S. T Cell Adoptive Immunotherapy of Newly Diagnosed Gliomas. *Clin Cancer Res* 6:2209-2218, 2000.

Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 365(8):725-733, 2011.

Pule M, Finney H, Lawson A. Artificial T-cell receptors. *Cytotherapy* 5(3):211-226, 2003.

Robert C, Soria JC, Eggermont AM. Drug of the year: programmed death-1 receptor/programmed death-1 ligand-1 receptor monoclonal antibodies. *Eur J Cancer* 49(14):2968-2971, 2013.

Rock KL, Gamble S, Rothstein L. Presentation of exogenous antigen with class I major histocompatibility complex molecules. *Science* 249(4971):918-921, 1990.

Sadelain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. *Nat Rev Cancer* 3(1):35-45, 2003.

Saikali S, Avril T, Collet B, Hamlat A, Bansard JY, Drenou B, Guegan Y, Quillien V. Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy. *J Neurooncol* 81(2):139-148, 2007.

Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE, 2nd, Lally-Goss D, Mcgehee-Norman S, Paolino A, Reardon DA, Friedman AH, Friedman HS, Bigner DD. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. *Mol Cancer Ther* 8(10):2773-2779, 2009.

Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, Gilbert MR, Herndon JE, 2nd, Mclendon RE, Mitchell DA, Reardon DA, Sawaya R, Schmittling RJ, Shi W, Vredenburgh JJ, Bigner DD. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol* 28(31):4722-4729, 2010.

Scheurer ME, Bondy ML, Aldape KD, Albrecht T, El-Zein R. Detection of human cytomegalovirus in different histological types of gliomas. *Acta Neuropathol* 116(1):79-86, 2008.

Siepl C, Bodmer S, Frei K, Macdonald HR, De Martin R, Hofer E, Fontana A. The glioblastoma-derived T cell suppressor factor/transforming growth factor-beta 2 inhibits T cell growth without affecting the interaction of interleukin 2 with its receptor. *Eur J Immunol* 18(4):593-600, 1988.

Smith JS, Tachibana I, Passe SM, Huntley BK, Borell TJ, Iturria N, O'fallon JR, Schaefer PL, Scheithauer BW, James CD, Buckner JC, Jenkins RB. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. *J Natl Cancer Inst* 93(16):1246-1256, 2001.

Sonabend AM, Ogden AT, Maier LM, Anderson DE, Canoll P, Bruce JN, Anderson RC. Medulloblasoma: challenges for effective immunotherapy. *J Neurooncol* 108(1):1-10, 2012.

Stupp R, Mason WP, Van Den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation For R, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352(10):987-996, 2005.

Tsuboi K, Saijom K, Ishikawa E, Tsurushima H, Takano S, Morishita Y, Ohno T. Effects of local injection of ex vivo expanded autologous tumor-specific T lymphocytes in cases with recurrent malignant gliomas. *Clin Cancer Res* 9:3294-3302, 2003.

Van Gool S, Maes W, Ardon H, Verschuere T, Van Cauter S, De Vleeschouwer S. Dendritic cell therapy of high-grade gliomas. *Brain Pathol* 19(4):694-712, 2009.

Vidard L, Kovacsovics-Bankowski M, Kraeft SK, Chen LB, Benacerraf B, Rock KL. Analysis of MHC class II presentation of particulate antigens of B lymphocytes. *J Immunol* 156(8):2809-2818, 1996.

Wang L-F, Fokas E, Bieker M, Rose F, Rexin P, Zhu Y, Pagenstecher A, Engenhart-Cabillic R, An H-X. Increased expression of EphA2 correlates with adverse outcome in primary and recurrent glioblastoma multiforme patients. *Oncol Rep* 19:151-156, 2008.

Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, Goldfinger D, Ng H, Irvin D, Yu JS. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res* 68(14):5955-5964, 2008.

Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 369(2):122-133, 2013.

Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DS, Vogelstein B. Structural alterations of the epidermal growth factor receptor gene in human gliomas. *Proc Natl Acad Sci U S A* 89(7):2965-2969, 1992.

Wykosky J, Gibo DM, Stanton C, Debinski W. Interleukin-13 receptor alpha 2, EphA2, and Fos-related antigen 1 as molecular denominators of high-grade astrocytomas and specific targets for combinatorial therapy. *Clin Cancer Res* 14(1):199-208, 2008.

Yamanaka R, Homma J, Yajima N, Tsuchiya N, Sano M, Kobayashi T, Yoshida S, Abe T, Narita M, Takahashi M, Tanaka R. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. *Clin Cancer Res* 11(11):4160-4167, 2005.

Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 64(14):4973-4979, 2004.

Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, Yong WH, Incardona F, Thompson RC, Riedinger MS, Zhang W, Prins RM, Black KL. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61(3):842-847, 2001.

Zhang JG, Kruse CA, Driggers L, Hoa N, Wisoff J, Allen JC, Zagzag D, Newcomb EW, Jadus MR. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. *J Neurooncol* 88(1):65-76, 2008.