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Cellular immunotherapy for high-grade glioma

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Abstract

The outcome for patients with the most common primary brain tumor, glioblastoma multiforme (GBM), remains poor. Several immunotherapeutic approaches are actively being pursued including antibodies and cell-based therapies. While the blood–brain barrier protects brain tumor cells from therapeutic antibodies, immune cells have the ability to traverse the blood–brain barrier and migrate into GBM tumors to exert their therapeutic function. Results of Phase I clinical studies with vaccines to induce GBM-specific T cells are encouraging and Phase II clinical trials are in progress. Nonvaccine-based cell therapy for GBM has been actively explored over the last four decades. Here we will review past clinical experience with adoptive cell therapies for GBM and summarize current strategies on how to improve these approaches.

Keywords

cell therapy; gene therapy; glioma; immunotherapy; NK cell; T cell

Primary brain tumors continue to pose significant clinical challenges. Tumors derived from astrocytes are the most common and among them glioblastoma multiforme (GBM) is the most aggressive. Despite advances in surgical procedures, radiation therapy and chemotherapy, the outcome for GBM is only slowly improving and 5-year overall survival rates remain low [1,2]. Thus, new targeted therapies are needed to improve current treatment strategies. Among them, immunotherapy is an attractive approach since it does not rely on the cytotoxic pathways of conventional therapies [3–5]. Cellular immunotherapies for GBM, which are nonvaccine based, have been explored for the last four decades (Table 1) [6–25]. In this article, we will review these approaches and summarize potential strategies on how to improve them. Vaccine-based approaches have been recently reviewed elsewhere [3,5,26,27].

Glioblastoma multiforme & the immune system

There is convincing evidence that GBMs express antigens that are recognized by the patient's immune system and that the cellular immune response, which is designed to kill virus-infected cells, can also recognize and kill GBMs [3–5]. Nonspecific killer cells, such as NK cells and lymphokine-activated killer (LAK) cells, recognize changes on the cell

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surface, such as carbohydrate abnormalities or low expression of MHC class I. T cells recognize 'foreign' peptides derived from cytosolic proteins presented on the cell surface by MHC molecules [28–30].

T cells recognize peptides derived from tumor-associated antigens (TAAs) expressed by GBMs. TAAs are immunogenic because they:

- Are normally only expressed during fetal development or at immunoprivileged sites;
- Are expressed at higher than normal levels;
- Contain a novel peptide sequence created by gene mutation or rearrangement.

Over the past decades, numerous TAAs have been identified and antigens relevant for GBMs are listed in Table 2 [31–56]. While the expression of TAAs indicate that glioma cells have the potential to be recognized by the immune system, the immune system fails to prevent the development of GBMs. This is probably due to the tumor location as well as the elaborate immune evasion strategies developed by GBMs.

While the CNS is not strictly 'immunologically privileged', it is 'immunologically quiescent' with a low frequency of professional antigen-presenting cells (APCs) and lack of an organized lymphatic system [57–59]. In addition, GBMs are protected by the blood–brain barrier (BBB) [60]. However, the mere fact that GBMs have developed intricate immune evasion strategies strongly argues that the BBB does not present an insurmountable barrier to the cellular arm of the immune system.

Glioblastoma multiforme evade the immune responses by a variety of mechanisms and recent studies have highlighted the prominent role of glioma stem cells in creating the immunosuppressive microenvironment in gliomas [61–64]. Nevertheless, glioma stem cells can be recognized and killed by antigen-specific T cells [40,65]. While several intra-cellular signaling pathways are involved, persistent activation of the signal transducer and activator of transcription (STAT)3 most likely plays a major role, and the advent of STAT3 inhibitors offer the potential to pharmacologically reverse the 'immunosuppressive glioma haven' [41,62,66].

Glioblastoma multiforme:

- Release inhibitory cytokines;
- Interfere with the antigen-presentation pathway or mutate the antigen;
- Downregulate cell adhesion or costimulatory molecules, resulting in failure to activate specific immune responses either directly or indirectly;
- Induce inhibitory cells such as myeloid-derived suppressor cells, immunosuppressive microglia or regulatory T cells [67–71].

At present, the relative contribution of each of the aforementioned immune evasion mechanism is unknown and further studies are needed. Besides GBMs, many malignancies including Hodgkin's and non-Hodgkin lymphoma have developed intricate immune evasion strategies [72,73]. T-cell therapies for these malignancies indicate that this hostile tumor microenvironment can be overcome, best exemplified by long-term remissions achieved with infused T cells, specific for the Epstein–Barr virus (EBV)-associated antigen, LMP2, which is expressed in EBV-positive lymphomas [74]. In addition, as discussed in the section 'Enhancing effector T-cell function' of this article, genetic engineering of T cells enables the generation of T cells that are resistant to the immunosuppressive glioma microenvironment.

Adoptive transfer of immune cells is one strategy to enhance anti-GBM immune responses. In preclinical or clinical studies, the use of leukocytes, NK cells, γδ T cells, activated T cells, tumor-infiltrating lymphocytes (TILs), antigen-specific T cells, or genetically modified T cells has been evaluated and we will review each of these cellular immunotherapy approaches in the following sections.

Leukocytes

Several investigators demonstrated in the 1970s that lymphocytic infiltrates could be detected in a subset of patients with malignant glioma, suggesting that the patients' immune systems are able to recognize the tumors and mount a response, albeit an ineffective one [75,76]. Evidence suggested that the degree of lymphocytic infiltrate, particularly in the perivascular regions of tumors, was positively correlated with survival; however, this correlation was contentious [77,78]. Nevertheless, these findings provided rationale for early trials of adoptive cell therapy in which patients with recurrent GBM were given autologous leukocyte infusions intracranially [6–9]. Although these studies demonstrated the safety of infusing autologous leukocytes into the tumor resection cavity, the efficacy of using leukocytes was limited.

NK cells

The observation that stimulating peripheral blood lymphocytes for 3–4 days with IL-2 was sufficient for obtaining cells with significant antitumor activity prompted a number of groups to evaluate LAK cells as cellular immunotherapy [79]. LAK cells are a mixture of lymphokine-activated $CD3^+$ T lymphocytes and $CD3^-/CD56^+/CD16^+$ NK cells and exhibit *ex vivo* cytolytic activity against a broad range of solid tumors. More than 100 GBM or high-grade glioma patients have been treated with LAK cells by local injection [10–16]. Most of these trials were conducted in the 1980s and early 1990s in the pre-temozolomide era. While some of the clinical studies have shown promising results in prolonging disease-free survival, a randomized Phase II clinical study was never conducted. This has limited the enthusiasm to pursue these cells as immunotherapy for GBM, especially since for other malignancies the use of LAK cells in combination with IL-2 was not superior to the use of IL-2 alone [80]. However, owing to recent advances in the field of NK cell biology, there is renewed interest in NK cell-based immunotherapy for cancer [28].

Several strategies are being pursued to enhance the antitumor activity of NK cells. First, the use of artificial APCs expressing membrane bound IL-15 and 4-1BB ligand has allowed, for the first time, the generation of a highly cytotoxic NK-cell population with enhanced antitumor activity against malignancies [81]. Second, genetic modification of NK cells with chimeric antigen receptors (CARs), as described in the section 'Antigen-specific T cells' of this article, has shown promise in preclinical studies to enhance the effector function of NK cells [82,83]. For example, NK cells expressing CARs specific for CD19 have demonstrated enhanced anti-leukemia activity in preclinical models, and a Phase I clinical study with NK cells expressing CD19-specific CARs is in progress [83]. This approach could be readily adapted to GBMs since CARs specific for GBM-associated tumor antigens such as IL-13 receptor subunit α-2 (IL-13Rα2) and HER2 are available [32,37,40].

Other strategies to improve the efficacy of NK cell-based therapy are based on the observation that NK cells express activating receptors such as NKG2D as well as inhibitory receptors called killer-cell immunoglobulin-like receptors (KIR). Thus, NK cell activation by tumor cells depends on the balance of activating and inhibitory ligands on their cell surface. Several investigators have shown that epigenetic modifiers such as histone deacetylase inhibitors enhance the expression of activating NK cell ligands on tumor cells, resulting in enhanced NK cell-mediated killing [84]. Since inhibitory ligands are encoded by

HLA-C molecules, another strategy to overcome the presence of inhibitory ligands is the use of haploidentical NK cells, which lack the corresponding KIR [85,86]. Indeed, the infusion of haploidentical NK cells is safe and has resulted in promising antitumor effects [87]. Since allogeneic T cells have been injected locally into GBMs with an encouraging safety profile, exploring the use of allogeneic, KIR-mismatched NK cells might also be feasible [88].

γδ T cells

γδ T cells are a subset of T lymphocytes, which express T-cell receptors (TCRs) that consist of one γ-chain and one δ-chain. Unlike conventional αβ T cells that recognize only specific peptide antigens presented in the context of a MHC molecule, γδ T cells recognize a broader range of antigens in a MHC-independent fashion. These antigens include MHC-like stress-induced self-antigens such as the NKG2D ligands, glycolipids presented by CD1c and phosphoantigens produced as a byproduct of bacterial metabolic pathways [89].

γδ T cells have been shown, in a number of preclinical studies, to have potent cytolytic activity against GBM cells [90]. In early studies, it was shown that γδ T cells could be effectively isolated and expanded from the blood of GBM patients by removing the $CD4^+$, $CD8^+$ and $CD16^+$ fractions from peripheral blood mono-nuclear cells (PBMCs) and culturing the negative fraction with OKT3 and IL-2 [91]. These γδ T cells were able to lyse autologous GBM in cytotoxicity assays, and this activity was enhanced by the addition of IL-12 and IL-15 [92,93]. More recently, it has been shown that although the absolute count of γδ T cells decreases and their proliferative capacity is diminished in GBM patients, these γδ T cells can still be activated and expanded *ex vivo* and are cytotoxic against primary GBM tumors, while sparing normal astrocytes [94]. Finally, γδ T cells had antitumor activity in GBM xenograft models [95]. To date, no clinical experience with the adoptive transfer of γδ T cells is available. One of the major limitations in the past has been the inability to generate sufficient numbers of γδ T cells that retain their broad antitumor activity without becoming exhausted or anergic from overstimulation. However, recent studies indicate that these limitations can be overcome [95]. Phase I/II trials have demonstrated the feasibility of stimulating and expanding γδ T cells *in vivo* through the use of aminobisphosphonates in patients with non-Hodgkin's lymphoma, multiple myeloma, and metastatic prostate cancer [96,97]. The efficiency of *in vivo* activation and expansion of γδ T cells correlated in general with antitumor activity. *In vivo* activation of γδ T cells has not been evaluated for high-grade glioma.

Activated T cells

Several clinical studies have been conducted with mitogen-activated T cells (mitogen-activated killer cells or autologous stimulated lymphocytes) [17–19]. As for other malignancies, these cells only had marginal antitumor activity in GBM patients. With the advent of better *ex vivo* stimulation techniques to overcome tumor-induced T-cell anergy using anti-CD3- and anti-CD28-coated beads, there is renewed interest in this approach. While initial studies have shown that the adoptive transfer of these cells is safe and reconstitutes cellular immune responses post-hematopoietic stem cell transplantation, antitumor responses have been limited [98–101]. One strategy to enhance the anti-tumor activity of adoptively transferred CD3/CD28-activated T cells is to vaccinate patients post-T-cell infusion with tumor antigens. In this regard, results of a single Phase I/II clinical study for patients with myeloma are encouraging [102]. At present, no clinical studies have been conducted with GBM patients.

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes represent a source of effector T cells that presumably have already been selected for their ability to recognize and respond to the specific tumor antigens that are present in the tumor. In contrast to LAK cells, TILs recognize tumor cells in a MHC-restricted fashion [103]. While TILs may not possess sufficient antitumor activity in the highly immunosuppressive microenvironment established by tumors, activation and expansion of TILs *ex vivo* can overcome the tumor-induced immunosuppressive effects and allow for the generation of sufficient numbers of TILs for adoptive immunotherapy. Most clinical experience with TILs is available for melanoma. Although TILs by themselves had only limited antitumor activity, clinical studies have shown that high-dose chemotherapy and radiation, in combination with TIL transfer and IL-2, results in significant antimelanoma effects [104,105]. However, for malignancies other than melanoma, it has been very difficult to expand TILs from tumor tissues [106]. For GBM, only one study has been published. TILs were obtained from the resected tumors and expanded *ex vivo* in media containing IL-2. The patients received two injections of the cells 2 weeks apart with doses between 3×10^8 and 1×10^9 along with intratumoral IL-2 injected three-times a week [20]. Of the six patients, one had a complete response, two had partial responses and three died of progressive disease at long-term follow-up. In summary, while TIL therapy continues to be explored for melanoma, enthusiasm to pursue TILs for GBM, as for other malignancies, is limited.

Antigen-specific T cells

The *ex vivo* generation of clinical grade polyclonal tumor antigen-specific T cells for the adoptive immunotherapy of malignancies has proven difficult unless viral-associated tumor antigens are targeted. For example, the infusion of EBV-specific T cells in patients with EBV-positive malignancies has resulted in promising antitumor responses, not only for lymphomas but also for EBV-positive nasopharyngeal carcinoma [74,107–109]. In this regard, the recent demonstration of cytomegalovirus (CMV) antigens in GBM opens the opportunity to evaluate the safety and efficacy of CMV-specific T cells for patients with GBM since robust *ex vivo* expansion protocols are available for the generation of CMV-specific T cells [53,110,111]. Indeed, Phase I clinical studies with CMV-specific T cells for GBM patients have been initiated or are being developed.

While there is no clinical study published with the adoptive transfer of polyclonal, tumor-antigen-specific T cells, the infusion of antigen-specific T-cell clones has been reported. These studies indicate that T-cell clones can induce tumor regressions; however, antigen-loss variants rapidly emerge, highlighting the risk of tumor escape mutants with the use of T-cell clones [112]. A case report indicates that this problem can be potentially overcome if the initial T-cell-mediated tumor cell destruction results in the activation of endogenous immune responses against other tumor antigens (epitope spreading) [113].

Several reports indicate that is feasible to generate polyclonal, GBM-specific T cells by stimulating patients' T cells with autologous GBMs in the presence of IL-2 [24,25]. In general, this approach is not very effective because tumor cells are poor APCs: they do not express costimulatory molecules and even inhibit T-cell responses by the secretion of inhibitory cytokines such as TGF-β. Despite these concerns, investigators have used this approach to generate GBM-specific T cells. The antitumor activity of these T cells was restricted to autologous tumors, suggesting that antigen recognition was HLA dependent; however, the recognized antigens have not yet been identified. Adoptive transfer of these cells resulted in transient antitumor responses, warranting further exploration of this approach [24,25].

Since initial priming and/or activation of polyclonal, tumor-antigen specific T cells is difficult *ex vivo*, one strategy to overcome these limitations is to prime/activate tumor-specific T cells *in vivo* and expand these cells *ex vivo* before reinfusion. This approach has been evaluated in three clinical trials. Activated T cells were either isolated from draining lymph nodes of the vaccine site or from PBMCs. Plautz *et al.* employed the first strategy in two Phase I studies for patients with recurrent or newly diagnosed high-grade gliomas [21,22]. For both studies, resected tumors were cultured short term and irradiated prior to intradermal injection with GM-CSF into the upper thigh. T cells were isolated from the draining inguinal lymph nodes a week after vaccination and expanded *ex vivo* using a combination of CD3 monoclonal antibodies, staphylococcal enterotoxin A and low-dose IL-2. T cells were administered intravenously, given previous experience with animal models demonstrating that T cells could be found infiltrating intracranial tumors following systemic injection. Of the ten patients with recurrent disease, two patients showed radiographic regression of at least 6 months, one patient had stable disease of over 17 months, and the rest had progressive disease. Of the 12 patients in the second study, four patients showed partial regression of residual tumor by MRI. The systemic infusion of these T cells was associated with only minor short-term side effects including fever, chills, nausea and myalgias. In the third study, Peres *et al.* vaccinated three patients with autologous tumor cells [23]. Postvaccination, patients' PBMCs were collected, expanded *ex vivo* and reinfused into patients following a course of high-dose chemotherapy and peripheral blood stem-cell rescue. Two out of three patients had a clinical and radiographic response. Clearly, both of these approaches are promising; however, larger studies are needed to better evaluate the efficacy of this strategy.

Genetically modified T cells

Gene transfer allows the rapid generation of antigen-specific T cells for adoptive immunotherapy or T cells with enhanced effector function (Table 3) [114–135]. This approach can circumvent tolerance to the self-antigens expressed by tumor cells. Successful gene transfer strategies to generate antigen-specific T cells include the forced expression of α/β TCRs or antigen-specific CARs.

α/β T-cell receptors

α/β *TCR* genes have been cloned for several HLA-restricted epitopes encoded by TAAs [115–119]. Genetic modification of T cells with α/β TCRs requires high expression and correct pairing of two different receptor molecules from a single vector, which has proved problematic for transgenic α/β TCRs. However, in the last 5 years, there has been significant progress in overcoming both of these limitations and three Phase I clinical studies with α/β TCR T cells for patients with refractory, metastatic melanoma have been completed [118,119]. A total of 34 patients were infused with T cells expressing a low-affinity MART1-specific α/β TCR T cells and objective clinical responses, including two complete responses, were observed. A total of 36 patients received T cells expressing high-affinity α/β TCR specific for either MART1 or gp100. Infusion of high-affinity α/β TCR T cells was associated with antitumor activity in nine patients including one complete response and eight partial responses. However, T cells also recognized normal tissues, which expressed low levels of the targeted TAAs. At present, no clinical study with α/β TCR T cells has been conducted in GBM patients.

Chimeric antigen receptors

Tumor-specific T cells can be generated by genetically modifying human T cells with tumor-specific CARs. CARs consist of an extracellular binding domain, a transmembrane domain and a cytoplasmic signaling domain [120–123]. The extracellular binding domain is

most commonly derived from a single-chain variable fragment of a monoclonal antibody; however, ligands specific for antigens expressed on the cell surface of tumor cells have also been used. The cytoplasmic signaling domains are derived from the TCR ζ-chain and costimulatory molecules such as CD28, CD134 and CD137. T cells with CARs have numerous advantages over immunotherapies based on monoclonal antibodies or T cells alone. They can be directed toward any antigen that is expressed on the cell surface. Because CARs provide T-cell activation in a non-MHC-restricted manner, they are immune to some of the major mechanisms by which tumors avoid MHC-restricted T-cell recognition, such as downregulation of HLA class I molecules and defects in antigen processing. CAR-expressing T cells are more likely to eradicate tumor cells than antibodies alone, since they can migrate through microvascular walls, extravasate and penetrate the core of solid tumors to exert their cytolytic activity, sequentially kill a multiplicity of target cells and recruit additional components of the immune system, thus amplifying the antitumor or antiviral immune response.

Chimeric antigen receptors have been generated for the glioma-specific antigens, including IL-13Rα2, HER2 and EGFRvIII [32,37,40]. T cells expressing these GBM-specific CARs had potent antitumor activity in preclinical animal models. In addition, the study with T cells expressing HER2-specific CARs showed that these cells had potent antitumor activity against HER2-positive, CD133-positive glioma stem cells, which are chemo- and radiotherapy resistant [40]. A Phase I clinical study with T cells expressing IL-13Rα2-specific CAR is ongoing and a study with T cells expressing HER2-specific CARs is in the development phase [136].

For other diseases, initial clinical studies with T cells expressing a CAR with only a TCR ζ-chain displayed limited clinical benefits [137,138]. The most pertinent issue being that CAR T cells failed to expand and rapidly lost their function *in vivo*. Several approaches have been pursued to overcome the limitation of CAR T cells. Incorporation of additional signaling domains from the costimulatory molecules CD28, CD134 and CD137 into CARs, as well as the coexpression of cytokines or their receptors, have resulted in enhanced effector function of CAR T cells [82,139,140]. A more pragmatic approach to overcome the signaling defect of CARs with a single ζ-signaling domain might be to express the receptors in antigen-specific T cells, which can be activated and expanded through their endogenous TCR. This concept of bispecific T cells was validated in an animal model with alloreactive T cells expressing CARs. Clinical relevant examples of this strategy include the expression of CARs in EBV-, influenza- or varicella zoster virus-specific T cells [123,141–143]. In a Phase I clinical study, EBV-specific T cells, expressing a GD2-ζ CAR, persisted significantly longer than autologous GD2-ζ T cells. In addition, the infusion of GD2-specific T cells resulted in tumor necrosis or regression (including a complete remission) in four out of eight patients with refractory/relapsed neuroblastoma [123]. Since a subset of GBMs is positive for CMV antigens, CMV-specific T cells might be an ideal ‘T-cell platform’ for GBM-specific CARs [144].

Enhancing effector T-cell function

Genetic modification not only allows for the generation of GBM-specific T cells, but also opens the opportunity to improve the effector function of T cells (Table 3) [126–135]. For example, preclinical models have shown that:

- Expression of a dominant negative TGF-β receptor renders T cells resistant to the inhibitory effects of TGF-β [131,132];
- Transgenic expression of cytokines improves T-cell persistence and function [126–128];

- Expression of chemokine receptors enhances T-cell migration [145,146];
- Zinc-finger nuclease-mediated disruption of the glucocorticoid receptor gene locus renders T cells resistant to the inhibitory effects of steroids [134].

Clearly, several of these strategies are directly applicable to cell therapies for GBMs and warrant further exploration.

Combinatorial cell therapy

There is increasing evidence that the efficacy of cell therapies can be enhanced when combined with other therapies. First, the use of lymphodepleting chemotherapy and/or radiation prior to cell transfer has resulted in enhanced *in vivo* expansion and antitumor effects in clinical studies [105]. Other strategies to enhance the *in vivo* expansion of T cells include lymphodepleting monoclonal antibodies as well as vaccines [147–149]. Second, investigators have shown in a preclinical melanoma model that VEGF antibodies enhance trafficking of adoptively transferred T cells to tumor sites resulting in enhanced antitumor effects [150]. This approach is readily applicable to GBMs, since the VEGF antibody Avastin was approved for the treatment of GBM in 2009. Third, agents that either increase the expression of tumor antigens or reverse the inhibitory microenvironment have the potential to enhance cell therapies for GBM patients in the clinic. For example:

- Combining the adoptive transfer of T cells with HDAC inhibitors, which increase MHC and tumor antigen expression in tumor cells, has resulted in enhanced antitumor effects in a preclinical melanoma model [151];
- Blocking STAT3 in combination with the adoptive transfer of T cells resulted in enhanced antitumor effects [41,152].

In summary, most combinatorial cell therapies have not been evaluated in clinical studies, however, the promising results obtained in pre-clinical models warrant further exploration of these approaches.

Conclusion & future perspective

Cell therapies for GBM have now been evaluated for more than four decades and most published clinical studies have used nonspecific cell products. While antitumor effects of adoptively transferred cells were observed in several studies, these findings were never confirmed in randomized clinical trials. This lack of definitive Phase II studies for cell therapies is not limited to GBM-directed therapies, but is a prevailing problem for the entire field of cell therapy. Currently, several ‘definitive’ clinical cell therapy studies are being developed for other malignancies, and GBM-directed cell therapies will hopefully follow suit. Improved methods to generate GBM-specific T cells, either using APCs or genetic approaches, and the ability to engineer T cells that are resistant to the inhibitory GBM microenvironment, offer exciting new approaches, which need to be tested in carefully designed clinical studies. Finally, combining cell therapies with other therapies holds the promise to enhance their efficacy. Paul Bucy, the former editor of *Surgical Neurology*, put out the following challenge in a commentary in 1973: “The field [brain tumor and immunity] is rapidly developing and we are in need of a better understanding of it” [153]. In the last 37 years, we have made significant progress in understanding the complex relationship between brain tumors and the immune system. The challenge now is how to translate these findings into immunotherapies that will benefit GBM patients. We believe that cell therapies will play an important role in this endeavor.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. DeAngelis LM. Brain tumors. N Engl J Med. 2001; 344(2):114–123. [PubMed: 11150363]
2. Buckner JC. Factors influencing survival in high-grade gliomas. Semin Oncol. 2003; 30(6 Suppl 19):10–14. [PubMed: 14765378]
3▪. Okada H, Kohanbash G, Zhu X, et al. Immunotherapeutic approaches for glioma. Crit Rev Immunol. 2009; 29(1):1–42. Comprehensive review of immunotherapeutic approaches for gliomas. [PubMed: 19348609]
4. Johnson LA, Sampson JH. Immunotherapy approaches for malignant glioma from 2007 to 2009. Curr Neurol Neurosci Rep. 2010; 10(4):259–266. [PubMed: 20424975]
5. Mitchell DA, Sampson JH. Toward effective immunotherapy for the treatment of malignant brain tumors. Neurotherapeutics. 2009; 6(3):527–538. [PubMed: 19560742]
6. Young H, Kaplan A, Regelson W. Immunotherapy with autologous white cell infusions (‘lymphocytes’) in the treatment of recurrrent glioblastoma multiforme: a preliminary report. Cancer. 1977; 40(3):1037–1044. [PubMed: 198084]
7. Steinbok P, Thomas JP, Grossman L, Dolman CL. Intratumoral autologous mononuclear cells in the treatment of recurrent glioblastoma multiforme. A Phase I (toxicity) study. J Neurooncol. 1984; 2(2):147–151. [PubMed: 6090602]
8. Vaquero J, Martinez R, Ramiro J, Salazar FG, Barbolla L, Regidor C. Immunotherapy of glioblastoma with intratumoural administration of autologous lymphocytes and human lymphoblastoid interferon. A further clinical study. Acta Neurochir (Wien). 1991; 109(1–2):42–45. [PubMed: 2068965]
9. Vaquero J, Martinez R, Oya S, et al. Intratumoural injection of autologous lymphocytes plus human lymphoblastoid interferon for the treatment of glioblastoma. Acta Neurochir (Wien). 1989; 98(1–2): 35–41. [PubMed: 2472737]
10. Jacobs SK, Wilson DJ, Kornblith PL, Grimm EA. Interleukin-2 or autologous lymphokine-activated killer cell treatment of malignant glioma: Phase I trial. Cancer Res. 1986; 46(4 Pt 2): 2101–2104. [PubMed: 3512079]
11. Yoshida S, Tanaka R, Takai N, Ono K. Local administration of autologous lymphokine-activated killer cells and recombinant interleukin 2 to patients with malignant brain tumors. Cancer Res. 1988; 48(17):5011–5016. [PubMed: 3261631]
12. Barba D, Saris SC, Holder C, Rosenberg SA, Oldfield EH. Intratumoral LAK cell and interleukin-2 therapy of human gliomas. J Neurosurg. 1989; 70(2):175–182. [PubMed: 2643685]
13. Hayes RL, Koslow M, Hiesiger EM, et al. Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. Cancer. 1995; 76(5):840–852. [PubMed: 8625188]
14. Dillman RO, Duma CM, Ellis RA, et al. Intralesional lymphokine-activated killer cells as adjuvant therapy for primary glioblastoma. J Immunother. 2009; 32(9):914–919. [PubMed: 19816190]
15. Dillman RO, Duma CM, Schiltz PM, et al. Intracavitary placement of autologous lymphokine-activated killer (LAK) cells after resection of recurrent glioblastoma. J Immunother. 2004; 27(5): 398–404. [PubMed: 15314549]
16. Ishikawa E, Tsuboi K, Saijo K, et al. Autologous natural killer cell therapy for human recurrent malignant glioma. Anticancer Res. 2004; 24(3b):1861–1871. [PubMed: 15274367]
17. Ingram M, Buckwalter JG, Jacques DB, et al. Immunotherapy for recurrent malignant glioma: an interim report on survival. Neurol Res. 1990; 12(4):265–273. [PubMed: 1982172]
18. Lillehei KO, Mitchell DH, Johnson SD, McCleary EL, Kruse CA. Long-term follow-up of patients with recurrent malignant gliomas treated with adjuvant adoptive immunotherapy. Neurosurgery. 1991; 28(1):16–23. [PubMed: 1994273]

19. Jeffes EW III, Beamer YB, Jacques S, et al. Therapy of recurrent high grade gliomas with surgery, and autologous mitogen activated IL-2 stimulated killer (MAK) lymphocytes: I. Enhancement of MAK lytic activity and cytokine production by PHA and clinical use of PHA. J Neurooncol. 1993; 15(2):141–155. [PubMed: 8509819]
20. Quattrocchi KB, Miller CH, Cush S, et al. Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. J Neurooncol. 1999; 45(2):141–157. [PubMed: 10778730]
21▪. Plautz GE, Miller DW, Barnett GH, et al. T cell adoptive immunotherapy of newly diagnosed gliomas. Clin Cancer Res. 2000; 6(6):2209–2218. Demonstrates that it is feasible to expand in vivo-primed T cells ex vivo and administer to glioma patients. [PubMed: 10873070]
22. Plautz GE, Barnett GH, Miller DW, et al. Systemic T cell adoptive immunotherapy of malignant gliomas. J Neurosurg. 1998; 89(1):42–51. [PubMed: 9647171]
23. Peres E, Wood GW, Poulik J, et al. High-dose chemotherapy and adoptive immunotherapy in the treatment of recurrent pediatric brain tumors. Neuropediatrics. 2008; 39(3):151–156. [PubMed: 18991194]
24. Kitahara T, Watanabe O, Yamaura A, et al. Establishment of interleukin 2 dependent cytotoxic T lymphocyte cell line specific for autologous brain tumor and its intracranial administration for therapy of the tumor. J Neurooncol. 1987; 4(4):329–336. [PubMed: 3494820]
25▪. Tsuboi K, Saijo K, Ishikawa E, et al. Effects of local injection of *ex vivo* expanded autologous tumor-specific T lymphocytes in cases with recurrent malignant gliomas. Clin Cancer Res. 2003; 9(9):3294–3302. Clinical study demonstrating feasibility of generating autologous tumor-specific T cells ex vivo and their subsequent transfer into glioma patients. [PubMed: 12960115]
26. Wheeler CJ, Black KL. DCVax-Brain and DC vaccines in the treatment of GBM. Expert Opin Investig Drugs. 2009; 18(4):509–519.
27. Van Gool S, Maes W, Ardon H, Verschuere T, Van Cauter S, De Vleeschouwer S. Dendritic cell therapy of high-grade gliomas. Brain Pathol. 2009; 19(4):694–712. [PubMed: 19744041]
28. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol. 2007; 7(5):329–339. [PubMed: 17438573]
29. Leen AM, Rooney CM, Foster AE. Improving T cell therapy for cancer. Ann Rev Immunol. 2007; 25:243–265. [PubMed: 17129181]
30. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. Science. 2004; 305(5681):200–205. [PubMed: 15247469]
31. Humphrey PA, Wong AJ, Vogelstein B, et al. Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma. Proc Natl Acad Sci USA. 1990; 87(11):4207–4211. [PubMed: 1693434]
32. Bullain SS, Sahin A, Szentirmai O, et al. Genetically engineered T cells to target EGFRvIII expressing glioblastoma. J Neurooncol. 2009
33. 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. 2004; 64(14): 4980–4986. [PubMed: 15256472]
34. Sahin U, Koslowski M, Tureci O, et al. Expression of cancer testis genes in human brain tumors. Clin Cancer Res. 2000; 6(10):3916–3922. [PubMed: 11051238]
35. Scarcella DL, Chow CW, Gonzales MF, Economou C, Brasseur F, Ashley DM. Expression of MAGE and GAGE in high-grade brain tumors: a potential target for specific immunotherapy and diagnostic markers. Clin Cancer Res. 1999; 5(2):335–341. [PubMed: 10037183]
36. Debinski W, Slagle B, Gibo DM, Powers SK, Gillespie GY. Expression of a restrictive receptor for interleukin 13 is associated with glial transformation. J Neurooncol. 2000; 48(2):103–111. [PubMed: 11083073]
37▪. Kahlon KS, Brown C, Cooper LJ, Raubitschek A, Forman SJ, Jensen MC. Specific recognition and killing of glioblastoma multiforme by interleukin 13-ζ kine redirected cytolytic T cells. Cancer Res. 2004; 64(24):9160–9166. Generation of IL-13 receptor α2-specific T cells using a genetic approach for the adoptive immunotherapy of gliomas. [PubMed: 15604287]

38▪. Zhang JG, Eguchi J, Kruse CA, et al. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. Clin Cancer Res. 2007; 13(2 Pt 1):566–575. Comprehensive antigen profiling of glioma cells. [PubMed: 17255279]

39. Zhang JG, Kruse CA, Driggers L, et al. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. J Neurooncol. 2008; 88(1): 65–76. [PubMed: 18259692]

40▪. Ahmed N, Salsman VS, Kew Y, et al. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res. 2010; 16(2): 474–485. Demonstrates that glioma stem cells are sensitive to autologous antigen-specific T cells. [PubMed: 20068073]

41. Fujita M, Zhu X, Sasaki K, et al. Inhibition of STAT3 promotes the efficacy of adoptive transfer therapy using type-1 CTLs by modulation of the immunological microenvironment in a murine intracranial glioma. J Immunol. 2008; 180(4):2089–2098. [PubMed: 18250414]

42. Liu G, Yu JS, Zeng G, et al. AIM-2: a novel tumor antigen is expressed and presented by human glioma cells. J Immunother. 2004; 27(3):220–226. [PubMed: 15076139]

43. Ueda R, Yoshida K, Kawase T, Kawakami Y, Toda M. Preferential expression and frequent IgG responses of a tumor antigen, SOX5, in glioma patients. Int J Cancer. 2007; 120(8):1704–1711. [PubMed: 17230535]

44. Ueda R, Yoshida K, Kawakami Y, Kawase T, Toda M. Expression of a transcriptional factor, SOX6, in human gliomas. Brain Tumor Pathol. 2004; 21(1):35–38. [PubMed: 15696967]

45. Jin M, Komohara Y, Shichijo S, et al. Identification of EphB6 variant-derived epitope peptides recognized by cytotoxic T-lymphocytes from HLA-A24^{+} malignant glioma patients. Oncol Rep. 2008; 19(5):1277–1283. [PubMed: 18425388]

46. Harada M, Ishihara Y, Itoh K, Yamanaka R. Kinesin superfamily protein-derived peptides with the ability to induce glioma-reactive cytotoxic T lymphocytes in human leukocyte antigen-A24^{+} glioma patients. Oncol Rep. 2007; 17(3):629–636. [PubMed: 17273744]

47. Friese MA, Platten M, Lutz SZ, et al. MICA/NKG2D-mediated immunogene therapy of experimental gliomas. Cancer Res. 2003; 63(24):8996–9006. [PubMed: 14695218]

48. Wu A, Wiesner S, Xiao J, et al. Expression of MHC I and NK ligands on human CD133^{+} glioma cells: possible targets of immunotherapy. J Neurooncol. 2007; 83(2):121–131. [PubMed: 17077937]

49. Castriconi R, Daga A, Dondero A, et al. NK cells recognize and kill human glioblastoma cells with stem cell-like properties. J Immunol. 2009; 182(6):3530–3539. [PubMed: 19265131]

50. Tsuda N, Nonaka Y, Shichijo S, et al. UDP-Gal: β GlcNAc β1, 3-galactosyltransferase, polypeptide 3 (GALT3) is a tumour antigen recognised by HLA-A2-restricted cytotoxic T lymphocytes from patients with brain tumour. Br J Cancer. 2002; 87(9):1006–1012. [PubMed: 12434293]

51. Hatano M, Eguchi J, Tatsumi T, et al. EphA2 as a glioma-associated antigen: a novel target for glioma vaccines. Neoplasia. 2005; 7(8):717–722. [PubMed: 16207473]

52. Okada H, Low KL, Kohanbash G, McDonald HA, Hamilton RL, Pollack IF. Expression of glioma-associated antigens in pediatric brain stem and non-brain stem gliomas. J Neurooncol. 2008; 88(3): 245–250. [PubMed: 18324354]

53▪. Cobbs CS, Harkins L, Samanta M, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 2002; 62(12):3347–3350. Establishes cytomegalovirus as a potential tumor antigen in glioma. [PubMed: 12067971]

54. Scheurer ME, Bondy ML, Aldape KD, Albrecht T, El Zein R. Detection of human cytomegalovirus in different histological types of gliomas. Acta Neuropathol. 2008; 116(1):79–86. [PubMed: 18351367]

55. Mitchell DA, Xie W, Schmittling R, et al. Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. Neuro Oncol. 2008; 10(1):10–18. [PubMed: 17951512]

56. Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C. The detection of CMV pp65 and IE1 in glioblastoma multiforme. J Neurooncol. 2010 (Epub ahead of print). 10.1007/s11060-010-0383-6

57. Lampson LA. Brain tumor immunotherapy: an immunologist's perspective. J Neurooncol. 2003; 64(1–2):3–11. [PubMed: 12952281]
58. Tambur AR. Transplantation immunology and the central nervous system. Neurol Res. 2004; 26(3):243–255. [PubMed: 15142316]
59. Khan-Farooqi HR, Prins RM, Liau LM. Tumor immunology, immunomics and targeted immunotherapy for central nervous system malignancies. Neurol Res. 2005; 27(7):692–702. [PubMed: 16197806]
60. de Vries NA, Beijnen JH, Boogerd W, van Tellingen O. Blood–brain barrier and chemotherapeutic treatment of brain tumors. Expert Rev Neurother. 2006; 6(8):1199–1209. [PubMed: 16893347]
61. Wu A, Wei J, Kong LY, et al. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro Oncol. 2010; 12(11):1113–1125. [PubMed: 20667896]
62. Wei J, Barr J, Kong LY, et al. Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. Mol Cancer Ther. 2010; 9(1):67–78. [PubMed: 20053772]
63▪. Di Tomaso T, Mazzoleni S, Wang E, et al. Immunobiological characterization of cancer stem cells isolated from glioblastoma patients. Clin Cancer Res. 2010; 16(3):800–813. Characterizes immunosuppressive effects of glioma stem cells. [PubMed: 20103663]
64. Wei J, Barr J, Kong LY, et al. Glioma-associated cancer-initiating cells induce immunosuppression. Clin Cancer Res. 2010; 16(2):461–473. [PubMed: 20068105]
65. Brown CE, Starr R, Martinez C, et al. Recognition and killing of brain tumor stem-like initiating cells by $CD8^+$ cytolytic T cells. Cancer Res. 2009; 69(23):8886–8893. [PubMed: 19903840]
66▪. Hussain SF, Kong LY, Jordan J, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer Res. 2007; 67(20):9630–9636. Highlights role of signal transducer and activator of transcription 3 in glioma-mediated immunosuppression. [PubMed: 17942891]
67. Albesiano E, Han JE, Lim M. Mechanisms of local immunoresistance in glioma. Neurosurg Clin N Am. 2010; 21(1):17–29. [PubMed: 19944963]
68. Avril T, Saikali S, Vauleon E, et al. Distinct effects of human glioblastoma immunoregulatory molecules programmed cell death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO) on tumour-specific T cell functions. J Neuroimmunol. 2010; 225(1–2):22–33. [PubMed: 20493562]
69. Jia W, Jackson-Cook C, Graf MR. Tumor-infiltrating, myeloid-derived suppressor cells inhibit T cell activity by nitric oxide production in an intracranial rat glioma + vaccination model. J Neuroimmunol. 2010; 223(1–2):20–30. [PubMed: 20452681]
70. Han SJ, Kaur G, Yang I, Lim M. Biologic principles of immunotherapy for malignant gliomas. Neurosurg Clin N Am. 2010; 21(1):1–16. [PubMed: 19944962]
71. Humphries W, Wei J, Sampson JH, Heimberger AB. The role of Tregs in glioma-mediated immunosuppression: potential target for intervention. Neurosurg Clin N Am. 2010; 21(1):125–137. [PubMed: 19944972]
72. Poppema S. Immunobiology and pathophysiology of Hodgkin lymphomas. Hematology (Am Soc Hematol Educ Program). 2005:231–238. [PubMed: 16304386]
73. Alvaro T, Lejeune M, Escriva P, et al. Appraisal of immune response in lymphoproliferative syndromes: a systematic review. Crit Rev Oncol Hematol. 2009; 70(2):103–113. [PubMed: 18996025]
74. Bollard CM, Gottschalk S, Leen AM, et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. Blood. 2007; 110(8):2838–2845. [PubMed: 17609424]
75. Ridley A, Cavanagh JB. Lymphocytic infiltration in gliomas: evidence of possible host resistance. Brain. 1971; 94(1):117–124. [PubMed: 5552158]
76. Palma L, Di Lorenzo N, Guidetti B. Lymphocytic infiltrates in primary glioblastomas and recidivous gliomas. Incidence, fate, and relevance to prognosis in 228 operated cases. J Neurosurg. 1978; 49(6):854–861. [PubMed: 731302]
77. Brooks WH, Markesbery WR, Gupta GD, Roszman TL. Relationship of lymphocyte invasion and survival of brain tumor patients. Ann Neurol. 1978; 4(3):219–224. [PubMed: 718133]

78. Safdari H, Hochberg FH, Richardson EP Jr. Prognostic value of round cell (lymphocyte) infiltration in malignant gliomas. Surg Neurol. 1985; 23(3):221–226. [PubMed: 2983448]
79. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155(6):1823–1841. [PubMed: 6176669]
80. Law TM, Motzer RJ, Mazumdar M, et al. Phase III randomized trial of interleukin-2 with or without lymphokine-activated killer cells in the treatment of patients with advanced renal cell carcinoma. Cancer. 1995; 76(5):824–832. [PubMed: 8625186]
81. Fujisaki H, Kakuda H, Shimasaki N, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res. 2009; 69(9):4010–4017. [PubMed: 19383914]
82. Milone MC, Fish JD, Carpenito C, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy *in vivo*. Mol Ther. 2009; 17(8):1453–1464. [PubMed: 19384291]
83. Imai C, Mihara K, Andreansky M, et al. Chimeric receptors with 4–1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. Leukemia. 2004; 18(4):676–684. [PubMed: 14961035]
84. Diermayr S, Himmelreich H, Durovic B, et al. NKG2D ligand expression in AML increases in response to HDAC inhibitor valproic acid and contributes to allorecognition by NK-cell lines with single KIR-HLA class I specificities. Blood. 2008; 111(3):1428–1436. [PubMed: 17993609]
85. Ruggeri L, Mancusi A, Burchielli E, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis. 2008; 40(1):84–90. [PubMed: 17964828]
86. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295(5562):2097–2100. [PubMed: 11896281]
87. Miller JS, Cooley S, Parham P, et al. Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. Blood. 2007; 109(11):5058–5061. [PubMed: 17317850]
88. 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. 1997; 45(2):77–87. [PubMed: 9390198]
89. Bonneville M, O'Brien RL, Born WK. γδ T cell effector functions: a blend of innate programming and acquired plasticity. Nat Rev Immunol. 2010; 10(7):467–478. [PubMed: 20539306]
90. Lamb LS Jr. γδ T cells as immune effectors against high-grade gliomas. Immunol Res. 2009; 45(1):85–95. [PubMed: 19711198]
91. Yamaguchi T, Fujimiya Y, Suzuki Y, Katakura R, Ebina T. A simple method for the propagation and purification of γ δ T cells from the peripheral blood of glioblastoma patients using solid-phase anti-CD3 antibody and soluble IL-2. J Immunol Methods. 1997; 205(1):19–28. [PubMed: 9236911]
92. Yamaguchi T, Suzuki Y, Katakura R, Ebina T, Yokoyama J, Fujimiya Y. Interleukin-15 effectively potentiates the *in vitro* tumor-specific activity and proliferation of peripheral blood γδ T cells isolated from glioblastoma patients. Cancer Immunol Immunother. 1998; 47(2):97–103. [PubMed: 9769118]
93. Fujimiya Y, Suzuki Y, Katakura R, et al. *In vitro* interleukin 12 activation of peripheral blood $CD3^+CD56^+$ and $CD3^+CD56^-$ γδ T cells from glioblastoma patients. Clin Cancer Res. 1997; 3(4):633–643. [PubMed: 9815731]
94. Bryant NL, Suarez-Cuervo C, Gillespie GY, et al. Characterization and immunotherapeutic potential of γδ T-cells in patients with glioblastoma. Neuro Oncol. 2009; 11(4):357–367. [PubMed: 19211933]
95. Bryant NL, Gillespie GY, Lopez RD, et al. Preclinical evaluation of *ex vivo* expanded/activated γδ T cells for immunotherapy of glioblastoma multiforme. J Neurooncol. 2010; 101(2):179–188. [PubMed: 20532954]
96. Wilhelm M, Kunzmann V, Eckstein S, et al. γδ T cells for immune therapy of patients with lymphoid malignancies. Blood. 2003; 102(1):200–206. [PubMed: 12623838]

97. Dieli F, Vermijlen D, Fulfaro F, et al. Targeting human γδ T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. Cancer Res. 2007; 67(15): 7450–7457. [PubMed: 17671215]
98. Laport GG, Levine BL, Stadtmauer EA, et al. Adoptive transfer of costimulated T cells induces lymphocytosis in patients with relapsed/refractory non-Hodgkin lymphoma following $CD34^+$-selected hematopoietic cell transplantation. Blood. 2003; 102(6):2004–2013. [PubMed: 12763934]
99. Rapoport AP, Stadtmauer EA, Aqui N, et al. Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. Nat Med. 2005; 11(11):1230–1237. [PubMed: 16227990]
100. Rapoport AP, Stadtmauer EA, Aqui N, et al. Rapid immune recovery and graft-versus-host disease-like engraftment syndrome following adoptive transfer of costimulated autologous T cells. Clin Cancer Res. 2009; 15(13):4499–4507. [PubMed: 19509133]
101. Porter DL, Levine BL, Bunin N, et al. A Phase I trial of donor lymphocyte infusions expanded and activated *ex vivo* via CD3/CD28 costimulation. Blood. 2006; 107(4):1325–1331. [PubMed: 16269610]
102. Rapoport AP, Aqui NA, Stadtmauer EA, et al. Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination based on hTERT and survivin following ASCT for myeloma. Blood. 2011; 117(3):788–797. [PubMed: 21030558]
103. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008; 8(4):299–308. [PubMed: 18354418]
104. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002; 298(5594):850–854. [PubMed: 12242449]
105. Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008; 26(32):5233–5239. [PubMed: 18809613]
106. Balch CM, Riley LB, Bae YJ, et al. Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. Arch Surg. 1990; 125(2):200–205. [PubMed: 1689143]
107. Straathof KC, Bollard CM, Popat U, et al. Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes. Blood. 2005; 105:1898–1904. [PubMed: 15542583]
108. Bollard CM, Aguilar L, Straathof KC, et al. Cytotoxic T lymphocyte therapy for Epstein–Barr virus + Hodgkin's disease. J Exp Med. 2004; 200(12):1623–1633. [PubMed: 15611290]
109. Comoli P, Pedrazzoli P, Maccario R, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein–Barr virus-targeted cytotoxic T lymphocytes. J Clin Oncol. 2005; 23(35):8942–8949. [PubMed: 16204009]
110. Leen AM, Myers GD, Sili U, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. Nat Med. 2006; 12(10):1160–1166. [PubMed: 16998485]
111. Peggs KS, Verfuerth S, Pizzey A, et al. Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. Lancet. 2003; 362(9393):1375–1377. [PubMed: 14585640]
112. Yee C, Thompson JA, Byrd D, et al. Adoptive T cell therapy using antigen-specific $CD8^+$ T cell clones for the treatment of patients with metastatic melanoma: *in vivo* persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci USA. 2002; 99(25):16168–16173. [PubMed: 12427970]
113. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous $CD4^+$ T cells against NY-ESO-1. N Engl J Med. 2008; 358(25):2698–2703. [PubMed: 18565862]
114. Morgan RA, Dudley ME, Rosenberg SA. Adoptive cell therapy: genetic modification to redirect effector cell specificity. Cancer J. 2010; 16(4):336–341. [PubMed: 20693844]
115. Xue SA, Gao L, Thomas S, et al. Development of a WT1-TCR for clinical trials: engineered patient T cells can eliminate autologous leukemia blasts in NOD/SCID mice. Haematologica. 2009; 95(1):126–134. [PubMed: 19679884]

116. Stanislawski T, Voss RH, Lotz C, et al. Circumventing tolerance to a human MDM2-derived tumor antigen by *TCR* gene transfer. Nat Immunol. 2001; 2(10):962–970. [PubMed: 11577350]

117. Engels B, Uckert W. Redirecting T lymphocyte specificity by T cell receptor gene transfer – a new era for immunotherapy. Mol Aspects Med. 2007; 28(1):115–142. [PubMed: 17307249]

118. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009; 114(3):535–546. [PubMed: 19451549]

119▪. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006; 314(5796):126–129. First clinical study using T cells genetically modified with tumor antigen-specific αβ T-cell receptors. [PubMed: 16946036]

120. 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 γ or ζ subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA. 1993; 90(2): 720–724. [PubMed: 8421711]

121. Pule M, Finney H, Lawson A. Artificial T-cell receptors. Cytotherapy. 2003; 5(3):211–226. [PubMed: 12850789]

122. Sadelain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer. 2003; 3(1):35–45. [PubMed: 12509765]

123. Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008; 14(11):1264–1270. [PubMed: 18978797]

124. Till BG, Jensen MC, Wang J, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008; 112(6):2261–2271. [PubMed: 18509084]

125. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010; 116(7):1035–1044. [PubMed: 20439624]

126. Vera JF, Hoyos V, Savoldo B, et al. Genetic manipulation of tumor-specific cytotoxic T lymphocytes to restore responsiveness to IL-7. Mol Ther. 2009; 17(5):880–888. [PubMed: 19259067]

127. Quintarelli C, Vera JF, Savoldo B, et al. Co-expression of cytokine and suicide genes to enhance the activity and safety of tumor-specific cytotoxic T lymphocytes. Blood. 2007; 110(8):2793–2802. [PubMed: 17638856]

128. Kerkar SP, Muranski P, Kaiser A, et al. Tumor-specific $CD8^+$ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts. Cancer Res. 2010; 70(17):6725–6734. [PubMed: 20647327]

129. Stephan MT, Ponomarev V, Brentjens RJ, et al. T cell-encoded CD80 and 4–1BBL induce auto- and transcostimulation, resulting in potent tumor rejection. Nat Med. 2007; 13(12):1440–1449. [PubMed: 18026115]

130. Kalbasi A, Shrimali RK, Chinnasamy D, Rosenberg SA. Prevention of interleukin-2 withdrawal-induced apoptosis in lymphocytes retrovirally cotransduced with genes encoding an antitumor T-cell receptor and an antiapoptotic protein. J Immunother. 2010; 33(7):672–683. [PubMed: 20664359]

131. Foster AE, Dotti G, Lu A, et al. Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF-β receptor. J Immunother. 2008; 31(5):500–505. [PubMed: 18463534]

132. Bollard CM, Rossig C, Calonge MJ, et al. Adapting a transforming growth factor β-related tumor protection strategy to enhance antitumor immunity. Blood. 2002; 99(9):3179–3187. [PubMed: 11964281]

133. Dotti G, Savoldo B, Pule M, et al. Human cytotoxic T lymphocytes with reduced sensitivity to Fas-induced apoptosis. Blood. 2005; 105(12):4677–4684. [PubMed: 15713795]

134. Gardner RA, Reik A, Berger C, et al. Disruption of glucocorticoid regulation in therapeutic human T cells with zinc finger nucleases. Mol Ther. 2010; 18(Suppl 1):S240.

135. Sawai N, Zhou S, Vanin EF, Houghton P, Brent TP, Sorrentino BP. Protection and *in vivo* selection of hematopoietic stem cells using temozolomide, O6-benzylguanine, and an alkyltransferase-expressing retroviral vector. Mol Ther. 2001; 3(1):78–87. [PubMed: 11162314]

136▪. Yaghoubi SS, Jensen MC, Satyamurthy N, et al. Noninvasive detection of therapeutic cytolytic T cells with 18F-FHBG PET in a patient with glioma. Nat Clin Pract Oncol. 2009; 6(1):53–58. Demonstrates that it is feasible to image adoptively transferred T cells in glioma patients. [PubMed: 19015650]

137. Mitsuyasu RT, Anton PA, Deeks SG, et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4ζ gene-modified autologous $CD4^+$ and $CD8^+$ T cells in human immunodeficiency virus-infected subjects. Blood. 2000; 96(3):785–793. [PubMed: 10910888]

138. Walker RE, Bechtel CM, Natarajan V, et al. Long-term *in vivo* survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. Blood. 2000; 96(2): 467–474. [PubMed: 10887107]

139. Maher J, Brentjens RJ, Gunset G, Riviere I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. Nat Biotechnol. 2002; 20(1): 70–75. [PubMed: 11753365]

140. Yvon E, Del Vecchio M, Savoldo B, et al. Immunotherapy of metastatic melanoma using genetically engineered GD2-specific T cells. Clin Cancer Res. 2009; 15(18):5852–5860. [PubMed: 19737958]

141. Landmeier S, Altvater B, Pscherer S, et al. Gene-engineered varicella-zoster virus reactive $CD4^+$ cytotoxic T cells exert tumor-specific effector function. Cancer Res. 2007; 67(17):8335–8343. [PubMed: 17804749]

142. Savoldo B, Rooney CM, Di Stasi A, et al. Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30ζ artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood. 2007; 110(7):2620–2630. [PubMed: 17507664]

143. Cooper LJ, Al Kadhimi Z, Serrano LM, et al. Enhanced antilymphoma efficacy of CD19-redirected influenza MP1-specific CTLs by cotransfer of T cells modified to present influenza MP1. Blood. 2005; 105(4):1622–1631. [PubMed: 15507526]

144. Ghazi A, Salsman VS, Kew Y, et al. CMV-specific HER2-Redirected T cells for the Adoptive Immunotherapy of Glioblatstoma. Mol Ther. 2010; 18(Suppl 1):S182.

145. Kershaw MH, Wang G, Westwood JA, et al. Redirecting migration of T cells to chemokine secreted from tumors by genetic modification with CXCR2. Hum Gene Ther. 2002; 13(16): 1971–1980. [PubMed: 12427307]

146. Craddock JA, Lu A, Bear A, et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J Immunother. 2010; 33(8):780–788. [PubMed: 20842059]

147. Louis CU, Straathof K, Bollard CM, et al. Enhancing the *in vivo* expansion of adoptively transferred EBV-specific CTL with lymphodepleting CD45 monoclonal antibodies in NPC patients. Blood. 2009; 113(11):2442–2450. [PubMed: 18971421]

148. Park MY, Kim CH, Sohn HJ, Oh ST, Kim SG, Kim TG. The optimal interval for dendritic cell vaccination following adoptive T cell transfer is important for boosting potent anti-tumor immunity. Vaccine. 2007; 25(42):7322–7330. [PubMed: 17889413]

149. Song XT, Turnis M, Zhou X, et al. A Th1-inducing adenoviral vaccine for boosting adoptively transferred T cells. Mol Ther. 2010; 19(1):211–217. [PubMed: 20959814]

150. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res. 2010; 70(15):6171–6180. [PubMed: 20631075]

151. Vo DD, Prins RM, Begley JL, et al. Enhanced antitumor activity induced by adoptive T-cell transfer and adjunctive use of the histone deacetylase inhibitor LAQ824. Cancer Res. 2009; 69(22):8693–8699. [PubMed: 19861533]

152. Herrmann A, Kortylewski M, Kujawski M, et al. Targeting Stat3 in the myeloid compartment drastically improves the *in vivo* antitumor functions of adoptively transferred T cells. Cancer Res. 2010; 70(19):7455–7464. [PubMed: 20841481]

153. Bucy P. Commentary on: Primary brain tumors: tumor immunity and immunocompetence. Surg Neurol. 1973; 1(5):263.

Executive summary

- The outcome for patients with glioblastoma multiforme (GBM) remains poor.
- Immunotherapy with nonspecific cell products has been evaluated in a series of small clinical studies with some clinical benefit; however, these findings were never confirmed in a randomized clinical study.
- Initial clinical studies with the infusion of *in vivo*- or *ex vivo*-activated GBM-specific T cells has shown encouraging results; confirmatory studies are needed.
- Recent advances in the *ex vivo* generation of NK cells, γδ T cells and GBM-specific T cells make clinical studies of these cell products in GBM patients feasible.
- Genetic modification holds the promise to generate T cells that are not only GBM-specific but also have enhanced effector function.
- Combining cell therapies with other therapies has the potential to improve their antitumor activity.
- Phase II clinical studies are needed to define the role of cell therapies.

Table 1

Cell therapies for glioblastoma multiforme

Cell product	Clinical glioblastoma multiforme study (number of published studies)	Ref.
Autologous		
Leukocytes (unmanipulated)	Yes (4)	[6–9]
NK cells		
LAK cells	Yes (7)	[10–16]
NK cells (generated with APCs)	No	
T cells		
γδ T cells	No	
Mitogen-activated T cells	Yes (3)	[17–19]
Antigen-specific T cells		
TIL	Yes (1)	[20]
In vivo activated, *ex vivo* expanded	Yes (3)	[21–23]
Ex vivo activated and expanded with tumor cells	Yes (2)	[24,25]
Ex vivo activated and expanded with APCs	No	
Genetically modified T cells		
αβ TCR	No	
Chimeric antigen receptor	Yes (1)	[136]
Modifications that enhance T-cell function	No	
Allogeneic		
T cells	Yes (1)	[88]

APC: Antigen-presenting cell; LAK: Lymphokine-activated killer; TIL: Tumor-infiltrating lymphocyte; TCR: T-cell receptor.

Table 2

Tumor associated antigens expressed in glioblastoma multiforme[†]

Brain tumor antigens	T-cell therapy glioblastoma multiforme studies		Ref.
	Animal	*Clinical*	
Mutations/novel epitopes in oncogenic proteins			
EGFRvIII	Yes	–	[31,32]
Cancer testis antigens			
MAGE, GAGE, SSX family	–	–	[33–35]
Overexpressed antigens			
IL-13Rα2	Yes	Yes	[36,37]
HER2, gp100	Yes	–	[38–41]
Aim-2, Art-1, EphA2, EphB6, Ezh2, Fos11, GALT-3, GnT-V, HNRPL, hTert, B-cyclin KIF1C, KIF3C, MRP-3, NKG2D ligands, PTH-rP, Sart family, SOX5, SOX6, survivin, tyrosinase, Trp-1&2 Mart-1, Whsc2, YKL-40	–	–	[38,39,42–52]
Viral antigens			
CMV	–	–	[53–56]

[†]Determined either by RT-PCR, western blot or immunohistochemistry.

CMV: Cytomegalovirus; EGFR: EGF receptor; GAGE: G melanoma antigen; GALT: Galactosyltransferase; HNRPL: Heterogeneous nuclear ribonucleoprotein L; IL-13Rα2: IL-13 receptor subunit α-2; MAGE: Melanoma-associated antigen; MRP: Multidrug-resistance protein; NKG2D: NK group 2, member D; RT-PCR: Reverse transcriptase PCR; SOX: SRY-related HMG-box; SSX: Synovial sarcoma, X breakpoint; YKL: Tyr–Lys–Leu.

Table 3

Genetic modifications to improve cell therapy

Goal	Genetic modification	Ref.
Tumor specificity	αβ TCR	[11–119]
	Chimeric antigen receptors	[120–125]
Cell survival and persistence	Cytokines or cytokine receptors	[126–128]
	Costimulatory molecules	[129]
	Antiapoptosis genes	[130]
Resist tumor-mediated inhibition	Dominant negative TGF-β receptor	[131,132]
	shRNA to knock down FAS	[133]
Integrating cell therapy with conventional therapies	Zinc-finger nuclease for glucocorticoid receptor	[134]
	Expression of chemoresistance genes	[135]

TCR: T-cell receptor.