Published in final edited form as:
Curr Neurol Neurosci Rep. 2010 July ; 10(4): 259–266. doi:10.1007/s11910-010-0111-9.

Immunotherapy Approaches for Malignant Glioma From 2007 to 2009

Laura A. Johnson and
Duke Brain Tumor Immunotherapy Program, Preston Robert Tisch Brain Tumor Center, Division of Neurosurgery, Department of Surgery, Duke University Medical Center, DUMC 3050, Sands Research Building, Durham, NC 27710, USA laura.a.johnson@duke.edu

John H. Sampson
Duke Brain Tumor Immunotherapy Program, Preston Robert Tisch Brain Tumor Center, Division of Neurosurgery, Department of Surgery, Duke University Medical Center, DUMC 3050, Sands Research Building, Durham, NC 27710, USA john.sampson@duke.edu

Abstract

Malignant glioma is a deadly disease for which there have been few therapeutic advances over the past century. Although previous treatments were largely unsuccessful, glioma may be an ideal target for immune-based therapy. Recently, translational research led to several clinical trials based on tumor immunotherapy to treat patients with malignant glioma. Here we review 17 recent glioma immunotherapy clinical trials, published over the past 3 years. Various approaches were used, including passive transfer of naked and radiolabeled antibodies, tumor antigen-specific peptide immunization, and the use of patient tumor cells with or without dendritic cells as vaccines. We compare and discuss the current state of the art of clinical immunotherapy treatment, as well as its limited successes, pitfalls, and future potential.

Keywords

Malignant glioma; Immunotherapy; Review; Clinical trial; Glioblastoma multiforme; Cancer; Treatment

Introduction

Each year, more than 12,000 new cases of malignant glioma (MG) are diagnosed in the Unites States alone. Gliomas are the most common primary brain tumor and are classified in four types: ependymomas, oligodendrogliomas, mixed gliomas, and astrocytomas. Astrocytomas are defined further as grades I through IV, becoming progressively more malignant. Stage IV glioma, glioblastoma multiforme (GBM), is the most malignant and, unfortunately, also the most common type of glial tumor. More common than Hodgkin's disease, multiple myeloma, and testicular cancer, GBM is responsible for more deaths each year than malignant melanoma [1]. Because tumors derive from normal cells, they may be difficult to target without incurring substantial collateral damage, which in the brain, may be debilitating or even deadly. Even with the best available treatment, life expectancy for

Correspondence to: Laura A. Johnson.

Disclosure Dr. Sampson has stock options with Celldex Therapeutics and also has received consulting and license fees from that company. No other potential conflicts of interest relevant to this article were reported.

patients with GBM is less than 15 months from diagnosis [2]. GBM brain tumors invariably recur, and are invariably fatal.

In this review, we identify 17 reports of glioma immunotherapy clinical trials published between late 2007 and 2009. For the purpose of this review, we include only trials that actively involved immune targeting of the tumor or used the patients' own immune system; therefore, we do not include any of the several publications on vascular endothelial growth factor (VEGF)-specific monoclonal antibodies (mAbs).

Standard of Care

Over the years, continued improvement in standard-of-care treatment has increased life expectancy for patients with GBM; however, even with the best available treatment, it remains less than 15 months. Currently, the standard of care includes surgical resection followed by radiation therapy (RT) and chemotherapy with temozolomide (TMZ) for newly diagnosed patients, with the addition of anti-VEGF mAbs for patients with recurrent disease.

Before the advent of what has become today's standard therapy, the average life expectancy for a patient diagnosed with GBM was even more dismal. One of the early advances in GBM patient treatment was published in 1980 by Walker et al. [3], who demonstrated in a randomized clinical trial that patients with newly diagnosed MG treated with RT, or RT combined with BCNU (carmustine), survived longer than similar patients not receiving RT. In 1995, Brem et al. [4] reported a survival benefit with BCNU over placebo in patients with recurrent MG. Then in 2003, Westphal et al. [5] published a phase 3 trial of newly diagnosed MG patients treated with either BCNU or placebo and concluded that BCNU also conferred a survival advantage to newly diagnosed patients. Stupp et al. [2] published a phase 3 clinical trial in 2005, in collaboration with 85 institutions in 15 countries, evaluating a total of 573 patients with newly diagnosed GBM. Patients were randomly assigned to receive the conventional treatment of resection and RT or resection, RT, and the addition of TMZ chemotherapy using a 5-day schedule. Patients from all institutions involved in the study demonstrated markedly similar survival rates. Patients who received resection and RT alone survived a median of 12.1 months, whereas those who received resection, RT, and TMZ survived a median of 14.6 months, setting the "gold standard" by which expected survival in newly diagnosed GBM patients is estimated today. In 2006, Stummer et al. [6] demonstrated that increasing total tumor resection in MG patients improved survival compared with those left with residual disease. Most recently, in 2009, Friedman et al. [7] published results from a clinical trial in which patients with recurrent GBM were treated with the anti-VEGF mAb bevacizumab, increasing survival from approximately 6 months to 9.2 months. However, although 14.6 months for patients with newly diagnosed GBM and 9.2 months for those with recurrent GBM may be an improvement over previous life expectancy, there is still a dire need for further improvement.

Tumor Immunotherapy

The goal of cancer immunotherapy is to take the patient's own immune system and redirect it to recognize and destroy tumors with a high degree of specificity. The unique specificity and potency of the immune response, directed against tumor targets, has the potential to prolong the quality and duration of life or, potentially, even to cure patients with cancer. In general, tumor immunotherapy uses specific antigenic proteins and peptides displayed by tumor cells as targets. Antitumor antibodies may be used either naked or as a platform to deliver radioisotopes and toxins to tumors, whereas adaptive immunity can be manipulated to generate effector T lymphocytes from the patient's immune system to seek out and destroy tumor cells. For a more comprehensive review of the evolution of glioma tumor immunotherapy, please refer to the recent review by Mitchell et al. [8].

The field of tumor immunotherapy is well established in treating certain types of cancer, with successes in melanoma, renal cell cancer, and hematologic malignancies [9]. Over the past decade or so, extensive work has been done in an attempt to generate an endogenous T cell-mediated antitumor response by "vaccinating" cancer patients with tumor antigen peptides, with generally disappointing results (2–3% response rate) [10]. More recently, however, there has been a resurgence in the use of T lymphocytes as effectors against tumors, generating them either endogenously by delivering adjuvants or dendritic cells (DCs) or exogenously through ex vivo manipulation and adoptive transfer back to the patient [11, 12•, 13].

In comparing immunotherapy of glioma with that used for other types of cancer, there are several differences and challenges to overcome. In using immune therapy to target glioma, one caveat is the "immune privilege" provided by the blood–brain barrier. Serving the purpose of keeping potentially dangerous substances out of the brain, the blood–brain barrier also may hinder the ability of immune-based therapies to cross over into the brain. There also is concern regarding induction of potential autoimmunity as peripheral autoimmunity in tumor immunotherapy trials for melanoma was recently reported [12•]. In the brain, this could be similar to the debilitating experimental autoimmune encephalomyelitis seen in murine models. Although tissues outside the central nervous system (CNS) may undergo collateral damage without causing death, in the brain this toxicity would potentially be fatal. To avoid CNS toxicity, selected antigenic targets must be present only on tumors, and these targets must be recognized with exquisite specificity by the immune system.

Nonetheless, several groups have been moving forward in clinical glioma immunotherapy research, and this review presents results from 17 recently published trials (Table 1). In comparing each of these findings to the benchmarks provided earlier, it becomes apparent that with a few promising exceptions (each with only a few patients), most recent clinical interventions have had minimal impact, demonstrating the need for continued improvement.

Antibody-Specific Tumor Immunotherapy

Unlabeled Antibodies—One way to target tumor antigens in vivo is to use mAbs. mAbs can be generated against almost any antigen and can bind that antigen with high specificity and affinity. They have been used successfully in immunotherapy treatments for patients with cancers such as lymphoma (rituximab) and breast cancer (trastuzumab), as reviewed by Harris [14].

In one recent glioma immunotherapy trial by Neyns et al. [15], the authors evaluated the effects of delivering the mAb recognizing the epidermal growth factor receptor (EGFR), often overexpressed in GBM. Anti-EGFR mAb was administered to patients with recurrent grade IV GBM or grade III MG. Three patients exhibited grade 3 skin rash, and there was some radiographic evidence of objective response; however, patient outcomes did not differ from those expected from standard treatment (Table 1).

Radiolabeled Antibodies—To increase the impact of antitumor mAbs, they can be used as a delivery system, bringing a dose of toxin or radiation directly to tumor cells. In the past year, two groups published results from clinical trials using intratumoral (IT) or intracavitary (IC) delivery of radiolabeled antibodies to treat patients with recurrent grade III/IV MG/GBM. One study, published by Casaco et al. [16], used the anti-EGFR mAb coupled to the beta and gamma radio-particle emitter ^{188}Re. Overall survival was comparable with that predicted by conventional (non-TMZ-inclusive) treatments; however, severe adverse events (AEs) were reported. With increasing dosage, two of four patients experienced severe neurologic symptoms and developed hemorrhagic brain necrosis, halting the trial.

A separate radiolabeled mAb trial conducted at Duke University used ^{211}At, an alpha-emitting radionuclide, coupled to the tumor-associated chimeric antitenascin mAb (ch81C6) [17•]. Unlike gamma-emitters, alpha-emitters confer high-intensity radiation over only very short (1–2 mm) distances. The authors treated patients after maximal surgical resection, to target any remaining residual disease. Among 14 patients with recurrent GBM treated, the authors observed an overall survival of 12 months, a potential increase over the 9 to 10 months observed previously in patients with recurrent GBM treated with conventional therapy plus TMZ or anti-VEGF mAb [18, 19]. Additionally, there were no adverse effects above grade II attributed to the treatment. Although these results show promise, further study in a larger randomized trial is needed.

Cytokine-Based Immunotherapy

In the immune system, cytokines are signals that induce different functions in tumor target cells and in effector T lymphocytes. Some cytokines, including the interferons (IFNs), promote immunity and tumor destruction, whereas others, such as tumor growth factor (TGF)-β, inhibit immune function and promote tumor growth. Both cytokine actions have been targeted for tumor immunotherapy. Cytokines may be administered to patients in several different ways. In three recently published clinical trials, IFNs were administered to patients with recurrent glioma [20–22]. One IFN trial examined the effects of subcutaneously (SC) introducing either long-acting (L) IFN-α coupled with polyethylene glycol (PEG; n=29) or a shorter-acting version (S) without PEG (n=34) [21]. L or S IFN-α, along with TMZ, was given to patients with recurrent GBM; some patients experienced grade 3 or 4 fatigue, but there was no improvement in survival. In a separate trial, IFN-α-2B was given SC along with BCNU to nine patients with recurrent GBM, two of whom experienced grade 3 fatigue [22]. Neither of these trials demonstrated any improvement in patient outcome compared with conventional therapies.

One trial used adenovirus-mediated IFN-α gene therapy delivered directly IT [20]; however, this trial was suspended early because of a case of grade 4 hemorrhagic brain necrosis; thus, no follow-up results are available.

In a different cytokine modulation approach, AP12009 peptide inhibitors were delivered to patients IT in attempts to neutralize the immune-suppressive effects of TGF-β [23]. However, the overall results were not substantially different from standard therapy outcomes.

Influencing the Immune System: Adjuvants, Antigens, and Tumor Vaccines

The immune system has several requirements that must be met before it can induce an adaptive antitumor cellular immune response. First, immune-surveillant DCs in the tissues must encounter the tumor and take up tumor-associated antigens. Although DCs process the antigen intracellularly, only upon activation by danger signals can they prime a T-cell response. DCs must present tumor-derived peptides in the context of major histocompatibility (MHC) molecules on the cell surface, then migrate into the lymphoid organs, where they encounter naïve or memory CD4 and CD8 T cells. Upon interacting with the appropriate T cell in the presence of the appropriate costimulatory molecules, DCs prime T cells to become effectors. The effector T cells then circulate through the body, whereupon they can encounter and destroy the tumor.

Adjuvants—Different aspects of immune priming are being explored for tumor immunotherapy. Butowski et al. [24, 25] recently reported on two separate clinical trials using polyinosinic–polycytidylic acid with polylysine and carboxymethylcellu-lose, an immune adjuvant given to patients intramuscularly. The first trial treated patients with

recurrent grade III MG; one patient had a radiographic response, although there was also a case of grade 3 fatigue [25]. The second trial added standard RT to the treatment for patients with newly diagnosed GBM, although this trial had no notable responses or AEs above grade 2 [24]. The patient outcomes of both studies were similar to those observed with the standard treatment of resection, RT, and TMZ [2].

Cancer Vaccines—Eight recent reports, including our own, were published on cancer vaccine treatments consisting of different combinations of DCs, adjuvants, autologous tumors, and/or peptides administered to patients with grade III or IV glioma in efforts to prime an endogenous antitumor immune response [26–31, 32•, 33•].

Tumor-based vaccines: Ishikawa et al. [27] treated patients with newly diagnosed GBM, as well as those with recurrent GBM, with intradermal (ID) injections of formalin-fixed autologous tumor; however, no improvement over standard therapies was observed. Okada et al. [28] published the results of two trials, both using irradiated patient tumors in combination with autologous fibroblasts transfected to produce interleukin-4, delivered ID with the intent to polarize endogenous DCs to a type 1 antitumor phenotype. The first trial treated patients with recurrent disease (grade III/IV), and the authors observed some radiographic response, as well as T-lymphocyte infiltration into tumor. The second trial used the same treatment with the addition of DCs pulsed with tumor lysate to treat patients with newly diagnosed GBM. No objective responses were observed, and neither trial improved survival compared with that reported for standard therapy.

DC vaccines: Walker et al. [29], Wheeler et al. [30], De Vleeschouwer et al. [26], and Prins et al. [32•] all reported on glioma clinical trials using patient tumors alone or DCs pulsed with irradiated tumor as a vaccine. The trials by Walker [29] and De Vleeschouwer [26], which included both patients with newly diagnosed and those with recurrent GBM, showed no improvement in response. Wheeler et al. [30] detected an immune response in peripheral blood but did not report on patient survival. However, there was one notable severe AE: in one patient, irradiated GBM injected peripherally to test for delayed-type hypersensitivity (DTH) skin response resulted in the growth of GBM at the injection site and metastasis to one nearby lymph node. This DTH testing was immediately discontinued.

Prins et al. [32•] recently published an interesting observation in a GBM patient being treated with tumor lysate-pulsed DCs. The authors detected an increase in circulating cytomegalovirus (CMV)-reactive T lymphocytes after vaccination, suggesting that CMV antigens present in tumor expanded endogenous CMV-reactive T cells. This finding complements recent published work, including our own, demonstrating high expression of CMV in glioma tumors [34, 35] and supporting the potential use of CMV antigen as a target for glioma immunotherapy.

Peptide vaccines with or without DCs: Izumoto et al. [31] published a clinical trial using the tumor associated WT-1 peptide to vaccinate patients with recurrent GBM, with no improvement in either WT-1 immunity or patient outcome. Although there has been little efficacy attributed to clinical trials using peptides alone to therapeutically vaccinate patients with cancer [10], those mediated by ex vivo DCs have shown more promise. Recently, our group conducted a clinical trial at Duke University treating newly diagnosed GBM patients with DCs that had been pulsed with tumor-specific EGFRvIII antigen coupled to KLH adjuvant [33•]. Only 12 patients were treated in this trial, and no AE above grade 2 was seen; however, radiographic responses were observed, and patient overall survival was 18 months, a potential increase over the 15-month survival observed with standard therapy [2]. However, this study was performed in a small group of patients, and a larger-scale randomized trial is needed.

Adverse Events

In general, AEs in these immunotherapy trials were similar to those expected with the current standard treatment of resection plus RT and/or chemotherapy, suggesting that immune therapy itself generally does not result in severe toxicities. Some of the AEs observed in the trials discussed here included instances of transient hematologic depression, pulmonary and thromboembolytic events, seizures, headaches, nausea, fatigue, cerebral hemorrhage, diarrhea, and several reports of bowel perforation. However, only three trials reported severe AEs that were possibly or likely attributable to the immune therapy. Two of these included the beta- and gamma-radiation–emitting ^{188}Re-labeled anti-EGFR mAb [16], and IT delivery of adenoviral-vector IFN-α gene therapy [20]. Each of these treatments resulted in severe neurologic symptoms and evidence of brain necrosis, halting the studies. In a third trial, Wheeler et al. [30] reported on the use of DTH testing by injecting irradiated GBM, which resulted in new GBM growth at the injection site and metastasis to the draining lymph node.

Outcomes and Immune Monitoring

Potentially confounding factors exist within and among patient populations that affect survival outcome, such as patient age, Karnofsky performance status, and medical history. Even when these factors are controlled for, many groups report results differently [36]. Often, groups evaluate the overall survival or the progression-free survival of patients over time, or they may assess survival as a percentage of patients remaining alive at a given time point, perhaps at 6 months or 1 or 2 years after treatment. Although these numbers may suggest a treatment impact, determining true efficacy is not possible in the absence of a large-scale randomized trial.

For immunotherapy trials, immune function should also be evaluated. Immune monitoring may be accomplished in many ways, from measuring specific antibodies to detecting the presence of antigen-reactive T lymphocytes or cytokines. Commonly, immune-related cytokines are measured in blood or using ex vivo T-cell assays against peptides, tumors, or by triggering T-cell activation using other means. Some issues regarding comparison of results arise with the different methods used: enzyme-linked immunosorbent assay (ELISA) or ELISpot to measure cell cytokine secretions by concentration produced per number of cells, or by intracellular cytokine capture, staining, and flow cytometry. Although each assay may be a valid evaluation of immune induction, it makes it challenging to compare among different groups using different assays. Indeed, some groups do not evaluate patient clinical outcomes at all, but instead use only a measure of immune monitoring to determine whether a trial was successful, making evaluation of therapeutic efficacy impossible.

In assessing whether a clinical tumor immunotherapy trial has established the intended goals, we believe immune function must be established in the context of patient clinical response. Both of these factors need to be assessed to determine whether the treatment actually induced the intended immunologic response in patients, and whether that response was sufficient to impact their clinical disease. Ideally, these elements should be thoroughly validated in a randomized phase 3 clinical trial.

Future Directions

Although some of the early results in glioma immunotherapy are promising, they are far from a cure for brain cancer. However, several types of immunotherapy have demonstrated success in treating patients with cancer, one of which is adoptive cell transfer (ACT).

Adoptive Cell Transfer

ACT of expanded tumor-infiltrating T cells uses the cellular immune system to target discrete tumor antigens and has been used successfully to treat patients with advanced cancer [11]. Unfortunately, ACT therapy has been limited mostly to small numbers of melanoma patients because of the challenges involved in obtaining and preparing endogenous tumor-reactive cells from each patient. Although T lymphocytes may be potent effectors in the antitumor immune response, endogenous circulating T cells generally are of insufficient affinity and avidity to eliminate tumors in vivo [37, 38]. This limitation recently was overcome by using high-avidity T-cell receptor (TCR) gene therapy to redirect patient T cells to tumors. In clinical trials, this method was shown to cause tumor regression in patients with advanced metastatic melanoma [12•, 13]. However, use of antigen-specific TCR remains limited by patient MHC restriction and the need to identify immunodominant tumor-specific epitopes and a corresponding TCR of sufficient avidity and affinity [12•, 13, 37]. ACT could be applied to treating glioma patients, as GBM expresses known specific tumor-restricted antigens, such as EGFRvIII [39, 40], or viral-derived CMV antigens [35], which are not found in normal tissues.

Chimeric Antigen Receptors

It is possible to engineer extremely potent T cells ex vivo. It also is possible to synthesize antibodies against glioma tumors in vitro [41]. With recent technologic advances in genetic engineering, it is now possible to utilize the exquisite specificity and high affinity of mAbs for tumor antigens, combined with the potency of effector T cells, by recombining mAbs and TCRs. This combination creates a chimeric antigen receptor (CAR) [42], which can redirect T cells to specific tumor targets based on the mAb chosen. Because the CAR targets antigens using mAbs, it is not restricted to a specific MHC haplotype, as TCR gene therapy is, and might be used to treat a broader patient population.

Early reports of clinical trials using CAR-expressing T cells demonstrated antigen specificity [43, 44], and a recent clinical trial treating pediatric neuroblastoma patients with GD2-specific CAR T cells produced promising results, with reduction of tumor burden in some patients [43]. We believe this to be a promising immune therapy to pursue in the treatment of patients with brain tumors.

Conclusions

Over the past year or so, there have been several immunotherapy clinical trials for patients with MG. Some have treated patients with tumor-specific mAbs, either naked or tied to radioactive isotopes. Others have delivered immunostimulatory cytokines or have inhibited immunosuppressive cytokines. Several groups have delivered tumors directly, or pulsed onto DCs, as vaccines. Immune adjuvants have been incorporated to improve the endogenous antitumor immune response, and immune cells have been isolated ex vivo and engineered to be more potent. Although these recent clinical trials in glioma immunotherapy have had only limited successes, they have established the methodology and safety profiles required for future clinical success. Several trials, although limited to small numbers, have provided encouraging reports of individual patients who continue to survive long term after treatment, in some cases longer than 5 years. These individual successes are what fuel the future of cancer immunotherapy.

To be effective in the future, clinical tumor immunotherapy treatments likely will need to incorporate multiple modalities addressing both the innate immune system (DCs) and the adaptive response (effector T cells and mAbs). Highly avid antitumor T cells will be required to recognize tumors with high specificity, and they must persist in vivo long

enough to eliminate all of the tumor. These cells may need to be further activated in vivo, either with vaccines or cytokine support, because of inherent immune deficits such as anergy, or to combat the effects of immunosuppressive tumor environments. By incorporating these combined treatments, it may be possible to eliminate patients' glioma tumors. To combine therapies effectively, it will be necessary for clinical researchers to evaluate results between different trials, which will require a standardized method for reporting results.

Only recently has the field of biomedical research been able to provide the advanced tools needed to implement an effective and potentially curative antitumor immune therapy. Individually, there has been progress on each of the immunologic fronts we discuss here; indeed, it now may only be a matter of finding the right combination of immune therapies to successfully treat patients with brain cancer.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

1. United States Cancer Statistics: National Program of Cancer Registries. Available at http://apps.nccd.cdc.gov/uscs/
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005; 352:987–996. [PubMed: 15758009]
3. Walker MD, Green SB, Byar DP, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med. 1980; 303:1323–1329. [PubMed: 7001230]
4. Brem H, Piantadosi S, Burger PC, et al. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. Lancet. 1995; 345:1008–1012. [PubMed: 7723496]
5. Westphal M, Hilt DC, Bortey E, et al. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro Oncol. 2003; 5:79–88. [PubMed: 12672279]
6. Stummer W, Pichlmeier U, Meinel T, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol. 2006; 7:392–401. [PubMed: 16648043]
7. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009; 27:4733–4740. [PubMed: 19720927]
8. Mitchell DA, Fecci PE, Sampson JH. Immunotherapy of malignant brain tumors. Immunol Rev. 2008; 222:70–100. [PubMed: 18363995]
9. Rosenberg SA. Progress in human tumour immunology and immunotherapy. Nature. 2001; 411:380–384. [PubMed: 11357146]
10. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004; 10:909–915. [PubMed: 15340416]
11. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002; 298:850–854. [PubMed: 12242449]
12•. 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; 114:535–546. [PubMed: 19451549] [This study demonstrated that ex vivo genetically engineered T cells can eliminate tumors in patients with advanced metastatic disease.]
13. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006; 314:126–129. [PubMed: 16946036]

14. Harris M. Monoclonal antibodies as therapeutic agents for cancer. Lancet Oncol. 2004; 5:292–302. [PubMed: 15120666]
15. Neyns B, Sadones J, Joosens E, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. Ann Oncol. 2009; 20:1596–1603. [PubMed: 19491283]
16. Casaco A, Lopez G, Garcia I, et al. Phase I single-dose study of intracavitary-administered Nimotuzumab labeled with 188 Re in adult recurrent high-grade glioma. Cancer Biol Ther. 2008; 7:333–339. [PubMed: 18094616]
17•. Zalutsky MR, Reardon DA, Akabani G, et al. Clinical experience with alpha-particle emitting 211At: treatment of recurrent brain tumor patients with 211At-labeled chimeric antitenascin monoclonal antibody 81C6. J Nucl Med. 2008; 49:30–38. [PubMed: 18077533] [This preliminary clinical trial suggested improved patient survival compared with historical controls.]
18. Yung WK, Albright RE, Olson J, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. Br J Cancer. 2000; 83:588–593. [PubMed: 10944597]
19. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clin Cancer Res. 2007; 13:1253–1259. [PubMed: 17317837]
20. Chiocca EA, Smith KM, McKinney B, et al. A phase I trial of Ad. hIFN-beta gene therapy for glioma. Mol Ther. 2008; 16:618–626. [PubMed: 18180770]
21. Groves MD, Puduvalli VK, Gilbert MR, et al. Two phase II trials of temozolomide with interferon-alpha2b (pegylated and non-pegylated) in patients with recurrent glioblastoma multiforme. Br J Cancer. 2009; 101:615–620. [PubMed: 19672263]
22. Olson JJ, McKenzie E, Skurski-Martin M, et al. Phase I analysis of BCNU-impregnated biodegradable polymer wafers followed by systemic interferon alfa-2b in adults with recurrent glioblastoma multiforme. J Neurooncol. 2008; 90:293–299. [PubMed: 18679580]
23. Hau P, Jachimczak P, Schlingensiepen R, et al. Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. Oligonucleotides. 2007; 17:201–212. [PubMed: 17638524]
24. Butowski N, Chang SM, Junck L, et al. A phase II clinical trial of poly-ICLC with radiation for adult patients with newly diagnosed supratentorial glioblastoma: a North American Brain Tumor Consortium (NABTC01-05). J Neurooncol. 2009; 91:175–182. [PubMed: 18797818]
25. Butowski N, Lamborn KR, Lee BL, et al. A North American brain tumor consortium phase II study of poly-ICLC for adult patients with recurrent anaplastic gliomas. J Neurooncol. 2009; 91:183–189. [PubMed: 18850068]
26. De Vleeschouwer S, Fieuws S, Rutkowski S, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. Clin Cancer Res. 2008; 14:3098–3104. [PubMed: 18483377]
27. Ishikawa E, Tsuboi K, Yamamoto T, et al. Clinical trial of autologous formalin-fixed tumor vaccine for glioblastoma multiforme patients. Cancer Sci. 2007; 98:1226–1233. [PubMed: 17517052]
28. Okada H, Lieberman FS, Walter KA, et al. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas. J Transl Med. 2007; 5:67. [PubMed: 18093335]
29. Walker DG, Laherty R, Tomlinson FH, et al. Results of a phase I dendritic cell vaccine trial for malignant astrocytoma: potential interaction with adjuvant chemotherapy. J Clin Neurosci. 2008; 15:114–121. [PubMed: 18083572]
30. Wheeler CJ, Black KL, Liu G, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res. 2008; 68:5955–5964. [PubMed: 18632651]
31. Izumoto S, Tsuboi A, Oka Y, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. J Neurosurg. 2008; 108:963–971. [PubMed: 18447714]
32•. Prins RM, Cloughesy TF, Liau LM. Cytomegalovirus immunity after vaccination with autologous glioblastoma lysate. N Engl J Med. 2008; 359:539–541. [PubMed: 18669440] [The authors present a clinical observation of CMV-reactive T cells induced in a patient by vaccination with glioma tumor.]

33•. Sampson JH, Archer GE, Mitchell DA, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Mol Cancer Ther. 2009; 8:2773–2779. [PubMed: 19825799] [This preliminary clinical trial suggested improved patient survival compared with historical controls.]

34. Cobbs CS, Harkins L, Samanta M, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 2002; 62:3347–3350. [PubMed: 12067971]

35. 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; 10:10–18. [PubMed: 17951512]

36. Lamborn KR, Yung WK, Chang SM, et al. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. Neuro Oncol. 2008; 10:162–170. [PubMed: 18356283]

37. Johnson LA, Heemskerk B, Powell DJ Jr, et al. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. J Immunol. 2006; 177:6548–6559. [PubMed: 17056587]

38. Lyman MA, Nugent CT, Marquardt KL, et al. The fate of low affinity tumor-specific CD8+ T cells in tumor-bearing mice. J Immunol. 2005; 174:2563–2572. [PubMed: 15728462]

39. 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 U S A. 1990; 87:4207–4211. [PubMed: 1693434]

40. Sugawa N, Ekstrand AJ, James CD, Collins VP. Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. Proc Natl Acad Sci U S A. 1990; 87:8602–8606. [PubMed: 2236070]

41. Wikstrand CJ, Hale LP, Batra SK, et al. Monoclonal antibodies against EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. Cancer Res. 1995; 55:3140–3148. [PubMed: 7606735]

42. 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 receptors. Proc Natl Acad Sci U S A. 1993; 90:720–724. [PubMed: 8421711]

43. 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; 14:1264–1270. [PubMed: 18978797]

44. Savoldo B, Rooney CM, Di Stasi A, et al. Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood. 2007; 110:2620–2630. [PubMed: 17507664]

Table 1

Recent malignant glioma immunotherapy clinical trials

Study	Treatment	Method of delivery	Type of cancer (grade)	N or R	Patients, *n*	Median PFS, *mo*	Median OS, *mo*	6-Mo PFS, %	6-Mo OS, %
Neyns et al., 2009 [15]	Cetuximab (anti-EGFR mAb)	IV	GBM (IV)	R	55	1.9	5.0	7.3	38
Casaco et al., 2008 [16]	^{188}Re-labeled nimotuzumab (anti-EGFR mAb)	IC	AA (III) GBM (IV)	R	3 (grade III) 7 (grade IV)	NA	5.9 (grade IV)	NA	100 (grade III) 43 (grade IV)
Zalutsky et al., 2008 [17•]	^{211}At-labeled antitenascin mAb	IC	MG (III) GBM (IV)	R	4 (grade III) 14 (grade IV)	NA	22.0 (grade III) 12.0 (grade IV)	NA	100 (grade III) 79 (grade IV)
Chiocca et al., 2008 [20]	Adeno-IFN-β	IT	MG (III) GBM (IV)	R	11	NA	NA	NA	NA
Groves et al., 2009 [21]	IFN-α L or S plus TMZ	SC	GBM (IV)	R	29 (L) 34 (S)	4.4 (L) 3.6 (S)	10.0 (L) 7.2 (S)	38 (L) 31 (S)	NA
Olson et al., 2008 [22]	IFN-α-2B + BCNU	SC	GBM (IV)	R	9	NA	5.9	22	NA
Hau et al., 2007 [23]	TGF-β2 peptide inhibitor	IT	AA (III) GBM (IV)	R	5 (grade III) 19 (grade IV)	NA	33.1(grade III) 10.2(grade IV)	NA	100 (grade III) 74 (grade IV)
Butowski et al., 2009 [25]	Poly-ICLC	IM	MG (AA, AO, AMO) (HI)	R	45	NA	9.9	24	NA
Butowski et al., 2009 [24]	Poly-ICLC + RT	IM	GBM (IV)	N	30	4.2	15.0	30	NA
Ishikawa et al., 2007 [27]	Formalin-fixed tumor	ID	GBM (IV)	N + R	4(N) 8(R)	NA NA	12.5 (N) 4.75 (R)	50 (N) 25 (R)	100 (N) 50 (R)
Okada et al., 2007 [28]	Trial 1: irrad. tumor + IL-4-TFD fibroblasts	ID	Both trials:	Trial 1: R	Trial 1: 1 (grade IV), 1 (grade III)	Trial 1: 6.0	NA	Trial 1: 0	NA
	Trial 2: as above + tumor lysate on DCs		AA (III) GBM (IV)	Trial 2: N	Trial 2: 5 (grade IV)	Trial 2: 5.0		Trial 2: 20	
Walker et al., 2008 [29]	Irrad. tumor-pulsed DCs	ID	AA (III) GBM (IV)	N + R	4 (AA grade III): 3 N, 1 R 9 (GBM grade IV): 7 N, 2 R	NA	18.0 (grade III N + R) 11.0 (grade IV N) 5.0 (grade IV R)	NA	100 (N + R grade III AA) 71 (N grade IV)
Wheeler et al., 2008 [30]	Tumor lysate-pulsed DCs	SC	GBM (IV)	N + R	11 (N) 23 (R)	NA	NA	NA	NA
Izumoto et al., 2008 [31]	WT-1 peptide vaccine	ID	GBM (IV)	R	21	4.5	8.3	33	90

Study	Treatment	Method of delivery	Type of cancer (grade)	N or R	Patients, *n*	Median PFS, *mo*	Median OS, *mo*	6-Mo PFS, %	6-Mo OS, %
De Vleeschouwer et al., 2008 [26]	Tumor lysate-pulsed DCs	ID	GBM (IV)	R	56	3.0	9.6	30[a]	75[a]
Prins et al., 2008 [32•]	Tumor lysate-pulsed DCs	NA	GBM (IV)	N	14 (ongoing)	NA	NA	NA	NA
Sampson et al., 2009 [33•]	EGFRvIII + KLH-pulsed DCs	ID	GBM (IV)	N	12	6.8	18.7	58	92

AA—anaplastic astrocytoma; AMO—anaplastic mixed oligoastrocytoma; AO—anaplastic oligodendroglioma; BCNU—carmustine; CMV—cytomegalovirus; DC—dendritic cell; EGFR—epidermal growth factor receptor; GBM—glioblastoma multiforme; IC—intracavitary; ID—intradermal; IFN—interferon; IL—interleukin; IM—intramuscular; irrad.—irradiated; IT—intratumoral; IV—intravenous; L—long acting; mAb—monoclonal antibody; MG—malignant glioma; N—newly diagnosed; NA—not available; OS—overall survival; PFS—progression-free survival; poly-ICLC—polylysine and carboxymethylcellulose; R—recurrent; RT—radiation therapy; S—short acting; SC—subcutaneous; TFD—transfected; TGF—tumor growth factor; TMZ—temozolomide

[a]Estimated from published data