Published in final edited form as:
Curr Treat Options Oncol. 2012 December ; 13(4): 437–450. doi:10.1007/s11864-012-0208-2.

The Spectrum of Vaccine Therapies for Patients With Glioblastoma Multiforme

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Opinion statement

Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the central nervous system (CNS) and one of the most lethal cancers in adults and children. Despite aggressive treatment with surgery, radiation, and chemotherapy, median survival is less than 15 months and overall survival is less than 10 % at 5 years. Development of therapeutics for malignant gliomas has been hampered by their natural complexity as well as protective mechanisms unique to the CNS. Better understanding of the pathogenesis of GBM is opening the path to novel, specific-targeted therapies. Recently, multiple immunotherapy approaches have been acquiring substantial indication of therapeutic efficacy with a very safe profile. Examples of the leading clinical approaches for GBM will be discussed in detail in this review.

Keywords

Glioblastoma; Glioblastoma multiforme; Malignant gliomas; Vaccine; Immunotherapy

Introduction

The incidence of brain tumors is increasing and treatment is usually unsuccessful, making this a growing, unmet medical need [1]. In the United States alone, 22,910 new cases and 13,700 deaths from primary nervous system tumors are expected in 2012 (American Cancer Society, 2012). Malignant gliomas represent more than 70 % of these, with a median age at diagnosis of 64 years [2]. Malignant gliomas include WHO Grade III malignant astrocytomas and grade IV tumors, of which GBM are most common [3]. GBM are differentiated histologically by the presence of microvascular proliferation and necrosis. In addition, they are characterized by finger-like infiltration of surrounding brain and even distant diffuse infiltration, making complete surgical resection and curative radiation therapy a challenge (Fig. 1) [4, 5].

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Disclosure

No potential conflicts of interest relevant to this article were reported.

Genetic alterations often contribute to the pathogenesis of GBM and may provide insight for developing targeted therapies and targets for immune-mediated therapies. Approximately 5 % of patients with malignant glioma have a family history associated with syndromes, such as neurofibromatosis types 1 and 2, Li-Fraumeni syndrome (germline p53 mutations), and Turcot's syndrome (intestinal polyposis and brain tumors) [6]. Loss of heterozygosity (LOH) in chromosome 10q is the most common genetic alteration [7]. Epidermal growth factor receptor (EGFR) amplification is found in 40–50 % of primary GBMs [8]. A constitutively active mutant receptor, EGFRvIII, is found in approximately half of EGFR amplified GBMs [8]. This mutant receptor is a potential therapeutic target and is being evaluated in late-stage clinical trials for a peptide-vaccine (described below). GBM have multiple genetic alterations that may serve as targets for recognition by the patients' immune system.

Molecular factors also may predict prognosis or response to specific therapies. For patients receiving temozolomide chemotherapy, hypermethylation of the O6-methylguanine methyltransferase (MGMT) gene has been associated with improved survival [9]. A CpG island methylator phenotype (CIMP), a phenomenon previously reported in colorectal cancer, has been identified recently for gliomas and is associated with improved prognosis and *IDH1* somatic mutations [10]. A gene expression-based molecular classification of GBM was recently reported identifying four subtypes with differing response characteristics: Proneural, Neural, Classical, and Mesenchymal [11]. Aggressive treatment with concurrent chemoradiation was associated with a significant survival advantage only in the Classical and Mesenchymal subtypes. The Mesenchymal class is characterized by necrosis and inflammatory infiltrates, which may have implications for the development of vaccine approaches. It is likely that better genomic characterization of these tumors will lead to better immunotherapeutic selection and targeting, especially for monovalent, single-antigen vaccine approaches.

It is clear that the immune system plays an important role in the development and prognosis of malignant gliomas. Patients with hyperactive immunity, such as atopy and elevated IgE levels, have decreased incidence and mortality from gliomas [6]. Increased levels of $CD8^+$ T-cell infiltration in GBMs has been associated with improved survival [12]. Regulatory T cells (Treg), the "brakes" of an immune response, are found at a higher frequency in GBM than in lower-grade gliomas [13]. In fact, some suggest that chemotherapies, such as temozolomide, may enhance responses to immunotherapy by decreasing the frequency of Treg [14]. Therefore, malignant gliomas are good candidates for immunotherapy approaches.

Enlisting the immune system against cancer has been tried for more than a hundred years with little success. However, the field has evolved drastically in the past decade, and two recent approvals of therapeutic immunotherapy drugs have driven optimism for additional applications: Provenge, an autologous dendritic cell vaccine, for hormone-resistant recurrent prostate cancer [15] and ipilimumab, an antibody that blocks the CTLA4 T-cell regulatory pathway, for metastatic melanoma [16].

Many vaccine approaches are being evaluated for GBM, including 1) dendritic-cell–based vaccines, 2) autologous tumor cells, 3) peptide vaccines, and 4) gene-transfer mediated in situ vaccines. Progress in recent studies with these approaches is described below.

Standard treatment for GBM

Surgery

Surgical resection is the first line of treatment. The extent of resection is an important prognostic factor with a step-wise improvement in survival with more extensive resection [17, 18]. Recently, intraoperative MRI has been used to facilitate more extensive resection while preserving quality of life [19]. However, due to their infiltrative nature, tumor cells remain in the surrounding brain tissue after resection and often lead to tumor recurrence in a matter of months.

Radiation therapy

Due to the remaining tumor cells, the addition of radiation therapy to surgery was shown to increase survival from 3–4 months to 7–12 months [20, 21]. External beam radiation therapy is routinely used with a 2- to 3-cm margin based on studies indicating that most recurrences were within a few centimeters of the enhancing tumor [22]. Median progression-free survival is still less than 6 months [23].

Upon recurrence, GBM also may be treated with radiation, including stereotactic radiosurgery [24] or interstitial brachytherapy. However, both have limited efficacy and the potential for toxicity [25].

Pharmacologic treatment

Temozolomide is the primary chemotherapy used for GBM. It is an oral alkylating agent that was approved for use during (concomitant phase) and after (adjuvant phase) radiation based on a randomized Phase III trial comparing this regimen to radiation alone. The addition of temozolomide led to an improvement in median survival from 12.1 to 14.6 months and an improvement of 2-year survival from 10.9 % to 27.2 % [21]. Tumors with an unmethylated MGMT promoter and consequently high expression of the MGMT protein have a worse prognosis than those with low expression [26]. Efforts to overcome MGMT effects by dose intensification (RTOG 0525) have not been successful [27]. Implantable BCNU (carmustine)-polymer wafers (Gliadel, Eisai, Woodcliff Lake, NJ) were approved as up-front adjuvant treatment based on a modest increase in median survival over placebo (13.8 vs. 11.6 months) [28]. Temozolomide continues to be the most widely used chemotherapy approach for GBM.

For recurrent GBM, there is little that is effective and nothing that is curative. Chemotherapy results in median survivals of less than 6 months [29]. BCNU-wafers also are approved for recurrent malignant glioma patients undergoing surgery [30]. Bevacizumab, an anti-VEGF antibody designed as an anti-angiogenic agent, was approved for recurrent GBM based on an objective response rate of 28 %, decreased corticosteroid requirement, and a 6-month progression-free survival rate of 42.6 % [31]. Two additional Phase 3 trials are evaluating bevacizumab in up-front GBM. Interestingly, bevacizumab appears to change the pattern of recurrence by inhibiting local enhancing tumor recurrence while allowing diffuse infiltrative tumor growth [29].

Emerging immunotherapies for glioblastoma multiforme

Vaccine approaches are an attractive adjuvant therapy for solid tumors due to their potential to generate long-term immune surveillance against cancer cells. The critical components required for stimulating an effective antitumor immune response are target tumor antigens, antigen presenting cells, such as dendritic cells (DCs), and effector lymphocytes, which include T cells and B cells. Effector T cells include $CD4^+$ helper cells, which produce

cytokines that stimulate and regulate other immune components and $CD8^+$ cytotoxic T cells (CTL), which can directly kill tumor cells. Innate immune components, such as natural killer (NK) cells, heat shock proteins, and Toll-like receptors also are important. Different forms of antigen delivery/exposure may be used in vaccine approaches to stimulate potent immune responses. Examples of approaches analyzed in clinical studies for GBM are highlighted below and listed in Table 1.

Using dendritic cells loaded with autologous tumor or peptides

The concept is to preload antigen-presenting cells with tumor associated antigens and return them to the patient so that they can stimulate T cells that recognize those antigens. Multiple groups have evaluated DC approaches for gliomas [5]. Large numbers of peripheral blood mononuclear cells must be collected from the patient, usually via leukapheresis, cultured with cytokines, and then loaded with antigen. The antigen source may be DNA, RNA, or proteins, single antigens or autologous tumor cells or tumor cell proteins. For the latter two, surgery is required to obtain sufficient tumor cells, but, unlike single peptides, these approaches have the potential to elicit a polyvalent immune response against many of the patient's own tumor associated antigens.

Results of studies using dendritic cells loaded with autologous tumor

Prins et al. (2011) at University of California, Los Angeles (UCLA) [32•]

Vaccine type: DCs loaded with autologous tumor lysate. Immunoadjuvant: either imiquimod or poly-ICLC, administered with the DC vaccine during the booster phase.

Study design: Phase I dose-escalation, adult newly diagnosed or recurrent GBM. Three dose levels were compared: 1, 5, and 10×10^6 DC/dose injected intradermally. Initial phase: three biweekly vaccinations. Booster phase: every 3 months up to 10 times or until tumor progression.

Patient population: N=23; 15 newly diagnosed and 8 recurrent. The mean age was 51 years (range, 26–74) and Karnofsky Performance Score (KPS) was 60–100.

Immune response: Serum IL-6 and TNF-α levels increased after DC vaccination and to greater extent after booster vaccines with adjuvant. $CD3^+$ and $CD8^+$ T-cell infiltration was increased after vaccination in tumors resected or biopsied at recurrence. This increase was associated with the *mesenchymal* gene expression signature but not with the dose of DC.

Clinical Outcomes: There were no grade 3 or 4 adverse events. The most common adverse events associated with the vaccinations were injection-site reactions and flu-like symptoms. Interestingly, patients with the *mesenchymal* gene expression signature (n=9) had significantly better survival than a randomly selected control *mesenchymal* group (n=82), whereas no difference was seen in patients with the *proneural* signature. These data suggest a potential differential responsiveness to vaccine therapy that may be related to gene expression, such as in the *mesenchymal* gene expression signature.

Wheeler et al. (2008) Cedars-Sinai Medical Center, Los Angeles [33]

Vaccine type: DCs loaded with autologous tumor lysate.

Study design: Phase II, adult newly diagnosed or recurrent GBM. The dose was 1 to 4×10^7 DCs injected subcutaneously. DC vaccination started approximately 15 weeks after surgery with 3 biweekly vaccinations and a fourth 6 weeks after the third.

Patient population: N=34; 11 newly diagnosed and 23 recurrent. Ages ranged from 22–74 years.

Immune response: Immunologic responders (17/32 patients tested) were defined as patients whose levels of interferon (IFN)-γ RNA expression from peripheral blood cells increased ≥1.5-fold compared with baseline.

Clinical outcomes: There were no grade 3 or 4 toxicities associated with the vaccinations. Time to progression (TTP) and overall survival (OS) was significantly longer in patients who had a positive immunological response (n=17) than in nonresponders (n =15). The immunologic responders and nonresponders had no significant differences in age, KPS, or extent of resection, but the responders did have a significantly higher number of newly diagnosed (47 %) compared with the nonresponders (20 %). In the recurrent patient population, 2-year survival was significantly higher for the immunologic responders (5/9, 56 %) than for the nonresponders (1/12, 8 %). In responders, there was a loose correlation between the level of IFN-γ expression and survival. For TTP, this correlation also existed for the interval of postvaccination recurrence to subsequent progression during which chemotherapy was administered, suggesting that immune responders may have an improved response to subsequent chemotherapy.

De Vleeschouwer et al. (2008) Catholic University of Leuven, Belgium [34]

Vaccine type: DCs loaded with autologous tumor lysate and matured with TNF-α, IL-1β, and PGE2.

Study design: Phase II; pediatric and adult patients with recurrent GBM. DCs were injected intradermally. Three cohorts with different vaccine schedules were compared: cohort A, 2 DC vaccines given at weeks 1 and 3 after surgery and then every 4 weeks until progression; cohort B, 5 DC vaccines every 2 weeks and then additional DC vaccines every 4 weeks until progression; cohort C, 4 weekly DC vaccinations and then additional boosts with tumor lysate without DCs every 4 weeks.

Patient population: N=56. Median age was 45 years (range, 7–77) years, and there was a trend toward a larger pediatric population on cohort A. Median KPS was 80 (range, 50–100).

Immune response: Delayed type hypersensitivity (DTH) skin tests using tumor lysate were positive in 9 of 17 patients after vaccination compared with 9 of 21 patients at diagnosis. There was no correlation reported between DTH results and PFS or OS.

Clinical outcomes: Adverse events were reported as mild except for one patient who developed grade 4 neurotoxicity associated with perilesional edema necessitating corticosteroid treatment. Median OS from time of reoperation for all cohorts (n=56) was 9.6 months; at 12, 24, and 36 months, OS was 37.4 %, 14 %, and 11.1 %, respectively. Comparison of outcomes for adult patients of the three cohorts revealed a significantly improved PFS and a trend toward better survival in cohort C.

Yamanaka et al. (2005) Niigata University School of Medicine, Japan [35]

Vaccine type: DCs loaded with autologous tumor lysate and the immunoadjuvant Keyhole Limpet Hemocyanin (KLH). In addition, in seven patients, half of the DCs were matured with penicillin-killed *Streptococcus pyogenes* (OK-432), a vaccine adjuvant approved in Japan that induces DC maturation via the toll-like receptor (TLR) pathway [36].

Study design: Phase I/II; adult recurrent malignant glioma. DCs were injected intradermally, close to a cervical lymph node, every 3 weeks for up to 10 vaccinations. In 11 patients, DCs with KLH also were injected intratumorally via an Ommaya reservoir.

Patient population: N=24, 18 with GBM and 6 with WHO grade III malignant gliomas. Mean age was 48.9 years (range, 20–80). The mean number of vaccinations was 7.4 (range, 1–22) intradermally and 4.6 (range, 1–18) intratumorally.

Immune response: DTH with tumor lysate was positive in 8 of 17 patients tested. Tumor lysate reactive IFN-γ producing T cells in peripheral blood (ELISPOT assay) were increased after vaccination in 6 of 16 patients tested.

Clinical outcomes: There were no significant adverse events related to the vaccinations. GBM patients who received DCs matured with OK-432 (n=7) had longer survival (p=0.027) than GBM patients who received DCs without OK-432 (n=11). GBM patients in the intratumoral/intradermal group (n=7) had longer survival (p=0.042) than GBM patients with only intradermal injection (n=11). In addition, GBM patients with a positive immunologic response to autologous tumor (DTH or ELISPOT assay) had significantly increased survival compared with those without a response. The GBM patients (n=18) were compared with a matched control group (n=27) and found to have increased median overall survival (480 vs. 400 days) and increased 2-year survival (23.5 % vs. 3.7 %), respectively.

Results of study using dendritic cells loaded with peptides—Peptides are an alternative source of antigens for DC-based vaccines. The peptides are synthesized and thus do not require autologous tumor collection and processing. However, the number of antigen targets for the immune response is limited, making tumor escape a potential problem. In addition, trials using peptide antigens usually require restriction to one or a few HLA types.

Okada et al. (2011) University of Pittsburgh [37••]

Vaccine type: α-type 1 polarized DCs (αDC1) loaded with synthetic HLA-A2 restricted peptides for 4 glioma-associated antigens (GAA). αDC1 are DCs matured with cytokines, in this case IL-1β, TNF-α, IFN-α, and IFN-γ, and polyinosinic:polycytidylic acid (poly I:C). αDC1 are able to produce high levels of interleukin-12 and induce type-1T-cell responses. An immunoadjuvant, polyI:C stabilized by lysine and carboxymethylcellulose (poly-ICLC) also was administered. Poly-ICLC is a TLR agonist that enhances vaccine efficacy in mouse glioma tumor models [38].

Study design: Phase I/II; adult recurrent malignant gliomas restricted to HLA-$A2^+$ patients. Two dose levels: 1×10^7 and 3×10^7 αDC1/dose administered by intranodal injection every 2 weeks×4 and additional booster vaccinations until progression. Poly-ICLC (20 μg/kg) was administered by intramuscular (IM) injection twice per week for 8 weeks.

Patient population: N=22, 11 on each of the two dose levels, 13 with GBM, and 9 WHO grade III. All received at least one vaccination, 19 completed the initial four-vaccine schedule, 9 completed five additional booster vaccinations. Median age was 48 years (range, 28–71).

Immune response: T-cell responses to the peptides used in the vaccine were evaluated by IFN-γ ELISPOT and/or tetramer assay comparing the frequency of reactive T cells in peripheral blood before and after vaccination. Positive responses to at least one peptide were found after four vaccinations in six of ten and five of nine patients in dose levels 1 and 2, respectively. Three additional patients developed T-cell responses after booster vaccines.

Upregulation of mRNA expression for type 1 cytokines and chemokines, specifically IFN-α1, CXCL10, and TLR3, was found in peripheral blood cells after the first and fourth DC vaccinations. IFN-γ increased only after the fourth vaccination. Serum protein levels of IFN-α, CXCL10, IL-15, MCP-1, and MIP-1β increased after vaccination. Three of five tumors evaluated after progression expressed mRNA for CXCL10, a chemokine important in trafficking of $CD8^+$ T cells.

Clinical outcomes: There were no significant toxicities. Common side effects were injection site reactions and transient flu-like symptoms. Two patients with GBM had response on MRI. One had complete response at week 17 that was durable for at least 13 months. The other had partial response at week 9 and pseudo-progression after 2 booster vaccines when biopsy revealed intense infiltration of $CD8^+$ T cells and $CD68^+$ macrophages without mitotically active tumor; the patient subsequently developed recurrence. Both of these patients also had positive T-cell responses. Median time to progression was 4 months for GBM and 13 months for grade III gliomas. At the time of publication, 9 patients (4 GBM and 5 grade III) were progression-free for at least 12 months.

In summary, DCs mixed ex vivo with autologous tumors or peptides have consistently given encouraging results. Although there is high variability and conflicting data from immune response analyses, this approach is promising for future advances.

Using direct tumor cell or peptide immunization

Another approach to stimulate a vaccine effect is to use killed autologous tumor cells or peptides mixed with an adjuvant to stimulate the immune response. In this case, antigen presentation is done by antigen presenting cells at the injection site, putatively stimulated by the co-delivered adjuvant. This approach could avoid the extra logistics and variables of DC isolation and culture but may not be as reliable or potent.

Results of study using autologous tumor cells

Muragaki et al. (2011) Tokyo Women's Medical University, Japan [39•]

Vaccine type: Autologous formalin-fixed tumor vaccine (AFTV) admixed with Bacillus-Calmette Guérin (BCG) derived adjuvant.

Study design: Phase I/IIa; newly diagnosed GBM in combination with radiation therapy. Standard fractionated radiotherapy (2 Gy per fraction to total dose of 60 Gy) was started 2–3 weeks after resection. When radiation reached 32–36 Gy, AFTV injections were given intradermally once per week for 3 weeks.

Patient population: N=24, 22 evaluable. Median age was 58 years (range, 18–70), median pre-operative KPS was 90, and 73 % had complete surgical resection.

Immune response: DTH tests were performed to AFTV without the BCG adjuvant at baseline and 2 weeks after the third vaccination.

Clinical outcomes: The most common vaccine-related adverse events were mild injection site reactions, but no adverse events greater than grade 1 were reported. Median OS was 21.4 months and actuarial 2-year survival rate was 40 %. Median PFS was 7.6 months. Patients with a positive DTH response (55 %) had significantly longer PFS: 13.9 months vs. 4.3 months. OS trended longer but did not meet statistical significance.

Results of studies using peptide vaccines

Sampson et al. (2010 and 2011) Duke University and MD Anderson Cancer Center [40••, 41••]

Vaccine type: EGFRvIII antigen peptide conjugated to KLH (PEPvIII-KLH) administered with GM-CSF.

Study design: Two Phase II studies; newly diagnosed adult GBM. Restricted to *EGFR-vIII*–expressing tumors that had gross total resection, without radiographic evidence of progression on MRI 2–4 weeks after completion of chemo-radiation and KPS ≥80.

First study—ACTIVATE: three vaccinations at 2-week intervals starting 4 weeks after completion of radiation and continued monthly until tumor progression without adjuvant temozolomide [40••].

Second study—ACT II: three vaccinations at 2-week intervals starting after chemoradiation completion and before adjuvant temozolomide. Subsequent vaccinations were given on day 21 of each 28-day cycle of temozolomide until tumor progression [41••]. Two cohorts comparing varying temozolomide doses: standard dose (STD, 200 mg/m^2 per day for the first 5 days of each 28-day cycle) and dose-intensified (DI, 100 mg/m^2 per day for 21 days of each 28-day cycle).

Patient population: ACTIVATE: N=21 of which 3 patients were not included in the analysis due to <95 % tumor resection. Median age was 52 years (range, 29–67).ACT II: N=22, 12 on the STD cohort and 10 on the DI cohort. All patients received at least one vaccination. Mean age was 57 years (range, 41–83).

Immune response: ACTIVATE: Of 14 patients tested for antibody response to the EGFRvIII peptide, 6 (43 %) were positive. Of 17 patients tested for DTH, 3 (18 %) were positive. A trend was observed in longer survival for those with positive antibody response and longer PFS and survival for those with positive DTH.

ACT II: All 22 patients developed positive antibody responses that increased over time to a higher titer in the DI cohort. At the time of the eighth vaccination, DTH responses were positive in seven of eight (87.5 %) of the DI cohort and zero of five in the STD cohort.

Clinical outcomes: ACTIVATE: One patient had a presumed severe allergic reaction, with perioral numbness and tingling. No other grade 3 or 4 adverse events related to vaccination were reported. Median PFS and OS were 14.2 and 26 months, respectively. The PFS and OS were not significantly different between patients with unmethylated and methylated MGMT.

ACT II: Four patients in the DI cohort had possible allergic reactions to the vaccinations. Sustained lymphopenia of grade 2 in the STD cohort and grade 3 in the DI cohort occurred. There was no significant difference in PFS or OS between the STD and DI cohorts. Median PFS and OS were 15.2 and 23.6 months, respectively.

In tumors recurring after vaccination and available for analysis of *EGFRvIII*-expression, 20 of 23 (87 %) had lost *EGFRvIII*-expression. This suggests that the vaccine successfully lead to elimination of *EGFRvIII*-expressing tumor cells but also demonstrates that a tumor vaccine with a single antigen can lead to selection of nonexpressing tumor cells.

Direct immunization with autologous cell or peptides with adjuvants have consistently given immune responses, but the translation to survival impact remains to be shown. The

EGFRvIII study clearly demonstrated the efficacy of immunization as well as the risk of tumor escape if the vaccine is monovalent.

Using gene-mediated immunization

Gene transfer can be used to create a vaccine in-situ by direct transfer of a specific antigenic molecule, transfer of immune-modulating molecules, such as cytokines, or by the creation of conditions to generate a local immune response. The latter can generate a polyvalent immune response to autologous tumor without requiring ex vivo processing of patient's cells [42••]. Gene-mediated cytotoxic immunotherapy (GMCI) is a specific example of generating the local conditions for a response (Fig. 1). It uses an adenoviral vector (AdV-tk) expressing the herpes simplex virus thymidine kinase gene (HSV-tk) followed by an antiherpetic prodrug, such as valacyclovir, in combination with standard of care debulking therapies, such as radiation or surgery [42••]. The HSV-tk protein participates in killing tumor cells and in the broad-spectrum activation of effector T cells through induction of cytokine expression. This generates a local immunostimulatory milieu that stimulates a systemic antitumor immune response to the released autologous tumor associated antigens. The approach has shown synergy with radiation, surgery, and chemotherapy in animal models. A Phase 3 trial was recently initiated in prostate cancer based on a threefold decrease in recurrence in a Phase 2 study. Studies in other tumor types, including malignant glioma, are ongoing.

Results of studies using gene transfer technology

Chiocca et al. (2011) OSU, Columbus, OH and The Methodist Hospital, Houston, TX [43••]

Vaccine type: Replication-defective adenoviral vector (AdV-tk) delivered by injection into the tumor bed at the time of surgery, followed by oral antiherpetic prodrug, valacyclovir.

Study design: Phase Ib, dose-escalation; adult newly diagnosed malignant gliomas. Three dose levels of AdV-tk were evaluated, 3×10^{10}, 1×10^{11}, and 3×10^{11} vector particles, followed by 14 days of valacyclovir. Radiation was started 1 week after AdV-tk injection and temozolomide was administered after completing valacyclovir.

Patient population: Thirteen patients were enrolled; 12 were evaluable, 10 with GBM and 2 WHO grade III. Three patients each on dose levels 1 and 2 and six on dose level 3. Median age was 59.5 years (range, 41–72), and median KPS was 90 (range, 70–100).

Immune response: In four of four cases analyzed, significant $CD3^+$ T cell $CD68^+$ macrophages infiltration was found after AdV-tk injection. These were predominantly $CD8^+$ in one case analyzed for T-cell subsets.

Clinical outcomes: There were no dose-limiting toxicities and no complications related to starting radiation within 7 days of surgery. Survival was 33 % at 2 years and 25 % at 3 years. There did not appear to be a correlation between MGMT methylation and survival; the longest surviving GBM patient had unmethylated MGMT and survived for 46.4 months. Three patients developed pseudoprogression that gradually resolved. Quality of life assessed by FACT-Br questionnaire was stable or improved after treatment.

Gene transfer technology creates the potential for generating an in situ patient specific, polyvalent vaccine. If successful, this could greatly simplify the logistics of vaccine production compared with autologous DC or killed-tumor vaccines and make it more difficult for the tumor to escape immune surveillance.

Other approaches in development

Additional clinical trials are ongoing with the approaches described as well as some for which data are not yet published (Table 1). Two pediatric studies are in progress: one at Children's Hospital of Pittsburgh, using dendritic cells loaded with peptides similar to the Okada et al. adult study; the other is at Dana Farber Cancer Institute/Boston Children's Hospital using the described GMCI approach, similar to the Chiocca et al. adult study. At Duke University, a Phase I study is evaluating DCs loaded with autologous brain tumor stem cell mRNA and ImmunoCellular Therapeutics has a Phase II study underway with DCs loaded with a set of synthetic peptides. Cancer Research UK along with Immatics Biotechnologies are testing a multipeptide vaccine, IMA950, in two Phase I studies. Additional approaches for gliomas not considered vaccines but using similar mechanisms or components are adoptive T-cell therapy and replication conditional viruses used to kill tumor cells (virotherapy).

Future success in therapy for GBM will likely require a combination approach adding to current multimodality therapies. Immunotherapy has the potential to harness the immune system as an additional weapon but is unlikely to be successful as a monotherapy. Its application is most likely to succeed as complimentary to standard of care, especially since its toxicity is minimal. The studies described above provide encouraging data on immune responses and clinical outcomes. Randomized, controlled trials are required to assess efficacy.

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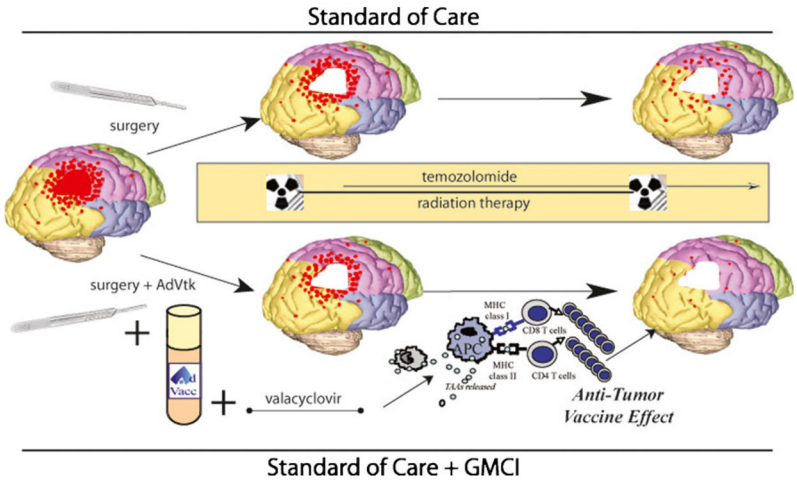

Figure 1. Residual infiltrating tumor cells after standard of care treatments may be eliminated by tumor-specific T cells stimulated by immunotherapies, such as gene-mediated cytotoxic immunotherapy (GMCI)

Standard of care treatment for newly diagnosed GBM includes surgical resection followed by radiation and temozolomide chemotherapy. However, progression occurs in more than two thirds of patients within 1 year due to growth of residual, infiltrating tumor cells. Immunotherapies may increase the efficacy of the immune system to fight against this minimal residual disease. GMCI involves injection of AdV-tk to the tumor bed at the time of surgery followed by oral valacyclovir prodrug administration to induce immunogenic tumor cell death. Surgery, radiation, and the viral vector stimulate infiltration of antigen-presenting cells (APC). Tumor-associated antigens (TAA) are presented by MHC class I and II molecules on APCs stimulating tumor-specific CD4 and CD8 T cells. The HSV-tk protein expressed from the AdV-tk vector has a superantigen-like effect that further stimulates proliferation of T cells. Thus, the approach generates an antitumor vaccine effect that may compliment standard of care to improve outcomes for patients with GBM.

Table 1

Clinical development of novel vaccines for glioblastoma multiforme

Institution/Sponsor	Indication*	Restrictions	Phase	Status	Source of Information
Dendritic cells with autologous tumor					
University of California, Los Angeles	Primary malignant glioma	Resection	II	Recruiting	Clinicaltrials.gov
Northwest Biotherapeutics	Primary GBM	Resection	III	Recruiting	Clinicaltrials.gov
Cedars-Sinai Medical Center, Los Angeles	Primary and recurrent GBM	Resection	II	Completed	Wheeler et al, 2008
Catholic University of Leuven, Belgium	Recurrent GBM	Resection	II	Completed	De Vleeschouwer et al, 2008
Niigata University, Japan	Recurrent malignant gliomas	Resection	I/II	Completed	Yamanaka et al, 2005
Duke University	Recurrent GBM	Resection	I	Recruiting	Clinicaltrials.gov
Dendritic Cells with Peptides					
University of Pittsburgh, Pennsylvania	Recurrent GBM	HLA-A2	I/II	Completed	Okada et al, 2011
Children's Hospital of Pittsburgh	Pediatric primary or recurrent glioma	HLA-A2	I	Recruiting	Clinicaltrials.gov
ImmunoCellular Therapeutics	Primary GBM	HLA-A1 or A2	IIb	Recruiting	Clinicaltrials.gov
Autologous tumor cells					
Tokyo Women's Medical University, Japan	Primary GBM	Resection	I/IIa	Completed	Muragaki et al, 2011
Peptide					
Celldex Therapeutics	Primary GBM	EGFRvIII^{+} tumor	III	Recruiting	Clinicaltrials.gov
Celldex Therapeutics	Recurrent GBM	EGFRvIII $^{+}$ tumor	II	Recruiting	Clinicaltrials.gov
Cancer Research UK/immatics Biotechnologies GmbH	Primary GBM	HLA-A*02	I	Recruiting	Clinicaltrials.gov
GMCI					
Advantagene Inc.	Primary malignant glioma	Accessible tumor	II	Active, not recruiting	Clinicaltrials.gov
Dana Farber Cancer Institute, Boston	Pediatric malignant glioma	Accessible tumor	I	Recruiting	Clinicaltrials.gov

GBM glioblastoma multiforme; *EGFRvIII* endothelial growth factor receptor variant III; HLA-Human leukocyte antigen

* Primary refers to first diagnosis prior to recurrence