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Challenges in Clinical Design of Immunotherapy Trials for Malignant Glioma

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Abstract

Glioblastoma multiforme (GBM) is the most common and lethal primary malignant brain tumor. The traditional treatments for GBM, including surgery, radiation, and chemotherapy, only modestly improve patient survival. Therefore, immunotherapy has emerged as a novel therapeutic modality. Immunotherapeutic strategies exploit the immune system's ability to recognize and mount a specific response against tumor cells, but not normal cells. Current immunotherapeutic approaches for glioma can be divided into three categories: immune priming (active immunotherapy), immunomodulation (passive immunotherapy), and adoptive immunotherapy. Immune priming sensitizes the patient's immune cells to tumor antigens using various vaccination protocols. In the case of immunomodulation, strategies are aimed at reducing suppressive cytokines in the tumor microenvironment or using immune molecules to specifically target tumor cells. Adoptive immunotherapy involves harvesting the patient's immune cells, followed by *ex vivo* activation and expansion prior to re-infusion. This review will provide an overview of the interactions between the central nervous system and the immune system and discuss the challenges facing current immunotherapeutic strategies.

Keywords

glioblastoma multiforme (GBM); immunotherapy; clinical trials; brain tumors; vaccines; dendritic cells; cytokines

Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary malignant brain tumor, with an incidence of 5 – 8/100,000 population and a median survival of 14 months [1]. The current standard of care for newly diagnosed GBM patients is a tripartite regimen of surgery, radiotherapy, and chemotherapy. The most meaningful improvement for the treatment of GBM has been the efficacy of temozolomide (TMZ). According to the study conducted by Stupp et al., the median survival rate with radiotherapy alone was 12.1 months compared to 14.6 months with radiotherapy plus TMZ [1]. In addition to the efficacy of TMZ, improvements in delivery have also greatly enhanced the treatment of GBM, including local delivery of chemotherapeutics to tumor cells and convection enhanced delivery (reviewed by Sampson) [2].

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A major limitation in the treatment of GBM is its location within the brain and the blood brain barrier (BBB). Recently, evidence of immune surveillance within the CNS and a role of T cells within glioma have led to the development of novel immunotherapeutic strategies [3-6]. Immunotherapy seeks to exploit the immune system's ability to specifically recognize and mount a response against the tumor cells, while leaving the normal brain tissue intact. The success of immunotherapy is fueled by the growing understanding of the immune mechanisms in play within the CNS and glioma immunobiology. These immunotherapeutic strategies fall into three categories: immune priming, immunomodulation, and adoptive immune therapy. Additionally, antibodies or immune peptides fused to toxins have also been used to treat GBM.

The emergence of novel immunotherapeutic strategies has cultivated a renewed optimism for the treatment of GBM. Most of these strategies are focused on the induction of specific immune responses against tumor associated antigens (TAA). Currently, targeting two of these TAA, EGFRvIII (NCT00458601) and IL-13R α 2 (NCT00089427), are in clinical trials and we will discuss them in further detail. Another major immunotherapeutic strategy that has gathered a lot of attention is dendritic cell (DC) vaccination, albeit only demonstrating modest success in clinical trials.

Despite the fact that there are a number of immunotherapeutic strategies currently in clinical trials (Table 1), which were successful in animal models of glioma, convincing evidence of their efficacy remains unclear in patients. It has been difficult to study novel immunotherapeutic strategies in clinical trials because of the rarity of GBM in the population. Moreover, the design of clinical trials is often flawed, especially with regard to patient enrollment in targeted treatment studies. The eligibility criteria should include a screening to assess the expression of target molecules prior to enrollment. The emergence of better imaging protocols, end-point analyses, and substantial improvements in protocol design should further aid in the development of clinical trials to assess the efficacy of targeted tumor therapies.

Target identification

EGFRvIII

The epidermal growth factor receptor (EGFR) is frequently over-expressed in solid tumors. Glioma cells often express a mutated form of EGFR, referred to as EGFR variant III, which has an in-frame deletion from the extracellular domain of the EGFR (Fig. 1) [7-15]. This mutation results in increased tumorigenicity and migration, and confers radiation and chemotherapeutic resistance to tumor cells [16-24]. A retrospective analysis of Japanese patients with GBM enrolled in clinical trials determined that EGFR amplification was a negative prognostic factor, and in cases where EGFR amplification occurred with EGFRvIII, the prognosis was even worse [25]. The restriction of EGFRvIII expression to tumors makes it an ideal target for anti-tumor immunotherapy.

In experimental animal models, EGFRvIII-expressing cell lines or an EGFRvIII-specific 14-amino acid peptide (PEPvIII) chemically conjugated to keyhole limpet hemocyanin (KLH) (PEPvIII-KLH) have been used for the generation of EGFRvIII-specific antibodies and the induction of cellular immune responses [26-36]. EGFRvIII vaccination in mouse models of established intracerebral glioma showed tumor regression compared to controls [27]. EGFRvIII has also been shown to be immunogenic in humans [37,38]. Purev *et al.*, determined that patients with EGFRvIII-expressing breast adenocarcinomas and malignant gliomas developed EGFRvIII-specific antibodies [37,38]. The authors also observed weak CTL epitopes restricted by major histocompatibility complex (MHC) Class I and Class II motifs which were sufficient to induce EGFRvIII-specific lymphocyte proliferation and cytokine production.

According to Heimberger et al., EGFRvIII peptide vaccination in animal models of intracerebral and subcutaneous glioma demonstrated significant efficacy over controls [27]. In phase II trials, patients were administered the EGFRvIII peptide vaccine along with temozolomide and radiation, following a complete surgical resection. This study demonstrated efficacy over historical controls. The observed time to progression was 12.8 months and the overall median survival was ≥ 18 months. A peptide vaccine directed against EGFRvIII is currently in phase II trials (NCT00458601). Tumor-specific mutation is targeted currently under a Phase I (conducted at Duke University, PI: John H. Sampson) and one multi-institutional Phase II immunotherapy trial (conducted at Duke University, PI: John H. Sampson; and the University of Texas, M.D. Anderson Cancer Center, PI: Amy B. Heimberger) demonstrating that vaccines targeting EGFRvIII are capable of inducing potent T- and B-cell immunity [38]. The authors surmise that the vaccine approach has been highly successful at eliminating tumor cells expressing EGFRvIII, very similar to the experimental animal model studies, without any evidence of toxicity [10,38].

The limitations of clinical studies to evaluate the efficacy of peptide vaccines include patient selection and immune editing. First, expression of EGFRvIII should be confirmed prior to patient selection for efficacy studies. Second, immune editing was observed in 20/23 patients with recurrent tumor, as the tumor biopsies failed to express EGFRvIII (unpublished data from CDX-110 clinical trials). Therefore, on the basis of glioma restricted expression of EGFRvIII and mechanism of action in glioma, future trials should focus on EGFRvIII targeting in primary glioma patients to assess efficacy. Moreover, to circumvent immune editing in recurrent glioma, initial treatments should target multiple tumor associated antigens either using whole tumor lysates or personalized peptide vaccines.

IL-13R α 2

Similar to EGFR, IL-13R α 2 is highly expressed in glioma cells, but not normal brain cells, making it a suitable target for immune cell activation [39]. Despite the over-expression of IL-13R α 2 in glioma cells, its role in glioma cells remains undefined. According to a pre-clinical study conducted by Okano and colleagues, the IL-13R α 2 protein contains an antigenic peptide that activates CD8⁺ T cells to secrete IFN γ and lyse IL-13R α 2⁺ tumor cells [40]. This deserves further analysis to determine the benefits of IL-13R α 2 targeting *in vivo*. Furthermore, a fusion protein composed of human IL-13 and *Pseudomonas* exotoxin A (IL13-PE38QQR) showed limited efficacy in 50 patients that received localized intracerebral administration. Moreover, a phase III study in which the IL-13R α 2 fusion peptide was compared to carmustine wafers was completed and showed no significant benefits. As is the case with EGFRvIII studies, the major challenge facing IL-13R α 2 studies is prospective identification of patients that are likely to respond, based on the expression of IL-13R α 2.

IL-4R

IL-4R is over-expressed in primary tumor specimens and cell lines in a variety of human malignancies, including glioma [41-46]. According to Joshi et al., IL-4 signals via the heterodimeric IL-4R α and IL-13R α 1 receptor in tumor cells [42]. Therapeutic strategies aimed at specifically targeting tumor cells have utilized IL-4R over-expression using IL-4 fused to *Pseudomonas* exotoxin (IL4(38-37)-PE38KDEL). *In vitro* studies using glioma cell lines found IL4(38-37)-PE38KDEL caused glioma cell death, similar to IL13-PE38QQR.[42] Furthermore, in animal models of glioma using human tumors, IL4(38-37)-PE38KDEL was toxic to glioma cells, but largely spared normal brain parenchyma. Phase I trials revealed that IL4(38-37)-PE38KDEL was well tolerated, with no incipient drug related toxicity. The most notable finding from the related dose-escalation study was a long-term survival of three years in a patient with recurrent malignant glioma treated with a single intratumoral dose of IL4

(38-37)-PE38KDEL. The findings of these earlier trials were promising and as such, IL4 (38-37)-PE38KDEL is still under further consideration.

Dendritic cell vaccination

Dendritic cells (DC) are hematopoietically derived cells that act as antigen presenting cells to activate innate and adaptive immune responses. DC based vaccination strategies seek to exploit the potent APC activity of these cells. The potential to generate large numbers of mature DC *in vitro* from patient blood or bone marrow has resulted in an abundance of DC based vaccination strategies. These studies have utilized DCs pulsed with either tumor peptides eluted from tumor cells or whole tumor lysates [26,47-50]. In short, autologous DC are matured and loaded with tumor specific peptides or tumor lysate and then infused into the patient. A few key issues underlie the use of DC cell vaccines and must be resolved prior to the routine use of DC vaccines to treat GBM. These include the best source of DC, the *in vitro* maturation protocol, the route and dose of DC administration and the source of antigen.

In an early study conducted by Yu and colleagues, four out of seven patients that received DC pulsed with eluted MHC class I peptides had developed cytotoxic responses against the tumor, and at the time of re-operation, two out of those four patients had effector and memory CD8⁺ T cell infiltrates in the tumor [48]. In this study, DC vaccination was not associated with any adverse side effects. In a phase I trial, 16 patients with malignant glioma were immunized intradermally with autologous DC pulsed with KLH conjugated to EGFRvIII peptides [38]. This study showed promising results based on the increased time to progression and median survival time. Stable disease was observed in two out of three grade III patients. The mean time to progression was 46.9 weeks and the median survival was 110.8 weeks. A similar study conducted by Liau et al., in 12 GBM patients, showed that intradermal infusion of peptide-pulsed DC improved survival compared to historical controls. The median time to progression was 15.5 months and the median survival was 23.4 months [49]. Additionally, 100% survival was observed at six months, 75% at one year, and 50% at two years, with two patients surviving long-term (≥ 4 year). Presumably, the administration of DC intradermally allowed the DC to traffic to the lymph nodes, where they are able to activate tumor antigen-specific T cells.

One of the largest DC vaccine studies to date, the HGG-Immuno study, conducted by De Vleeschouwer and colleagues, assessed 56 patients with recurrent GBM [51]. The patients were separated into three groups and treated with autologous DC pulsed with autologous tumor lysate, followed by tumor lysate boosts every four weeks. The clinical response was minimal, with a median progression free survival of 3 months and overall median survival of 24 – 36 months. Overall, this treatment strategy was not significantly better than historical controls. Despite disappointing results from these clinical trials, multiple other clinical trials are under way.

A large multi-institutional randomized placebo control study is currently being sponsored by Northwest Biotherapeutics (DCVax-Brain, Phase II, NCT00045968). DCVax-Brain is a personalized (autologous) dendritic cell based vaccine. The vaccine is prepared from PBMC obtained from the patient and are then loaded with tumor lysate from surgically resected tumor tissue. According to the sponsors, in phase I trials, 8 out of 19 GBM patients treated with DCVax-Brain, in addition to the standard of care for GBM, were still alive with stable disease. The median overall survival was 33.6 months. The median time to progression was 18.1 months. In this study, 90% of the patients surpassed the standard of care median time to disease progression of 8.1 months and median overall survival time of 17.0 months. The two year survival rate is 68% and 42% of the patients have survived longer than 4 years (reviewed by Wheeler et al, 2009) [52].

Parajuli and colleagues investigated the best protocol for antigen preparation for DC vaccination strategies [53]. DC were isolated and matured from patient derived PBMC. The four conditions evaluated were: DC fused with glioma cells; DC pulsed with apoptotic tumor cells; DC pulsed with total tumor RNA; and DC pulsed with tumor lysate. All four conditions produced similar amounts of mature DC, however, DC pulsed with apoptotic tumor cells or total tumor RNA were the best at inducing CTL. Furthermore, DC pulsed with apoptotic tumor cells were also able to induce NKT cell activation. Collectively, these data suggest that DC pulsed with apoptotic cells are the best preparation for autologous DC vaccination strategies.

Heat Shock Proteins-tumor peptide vaccination

Heat shock proteins (HSP) are chaperone proteins which are localized to the ER and aid in nascent protein folding and also play a role in antigen presentation via MHC class I (reviewed by Srivastava et al) [54]. Recent studies have shown that at least two HSP, Gp96 and HSP70, have antigenic properties and are able to generate immune responses directed against the proteins to which they are associated [55,56]. The benefits of using HSP-peptide complexes for vaccination is the potential to limit immune editing since HSP are associated with a broad range of the tumor peptide repertoire. Furthermore, HSP have been identified as potent activators of APCs, making them ideal candidates for tumor immunotherapy [57].

Gp96 has been shown to induce immunity specifically against antigens found in the cells from which it has been isolated. This has been exploited in the case of tumor cells to generate antitumor immune responses. According to Binder et al., one potential mechanism by which this may occur is through cross-presentation by DC via Gp96 binding CD91 expressed on DC [58]. In a study of 12 patients with recurrent high grade glioma, patients received 4 injections over 2-4 weeks. Seven out of 8 patients had a survival time of 10.5 months compared to the historical survival time of 6.5 months [59]. More importantly, Gp96 vaccination has garnered success in the treatment of malignant melanoma and renal cell carcinoma, and hopefully similar success will be obtained in malignant glioma [15,60-62]. Currently, the Gp96-tumor peptide vaccination strategy is in phase I/II clinical trials (NCT00293423).

Immunomodulation

Cytokines

The cytokine milieu of the CNS ensures that primarily humoral immune responses are generated in order to prevent damage due to inflammation. The normal humoral response is further skewed in glioblastoma patients [63,64]. Additionally, immunosuppressive cytokines, such as TGF- β 2 and IL-10, are highly expressed in glioma cell lines and patient specimens [65-68]. These cytokines suppress T cell proliferation and IL-2 production, and also support glioma cell growth. To alter the cytokine milieu of glioma, studies have focused on supplementing the immuno-activating cytokine IL-2, or conversely, inhibiting the immunosuppressive cytokine TGF β .

IL-2 is the cytokine most often associated with T cell activation and expansion. Recent studies have shown that IL-2 is required for differentiation of naïve T cells into cytokine producing effector cells. According to a study conducted by Colombo and colleagues, IL-2 was administered as a transgene in combination with herpes simplex virus tyrosine kinase in a retroviral vector to 12 patients with recurrent GBM [69]. Two out of the 12 patients had a partial response, four had a minor response, four had stable disease, and two had progressive disease. In another study, five patients with recurrent glioma were infused with IL-2 in combination with cytotoxic T cells [70]. Although, two patients with GBM died, the other patients showed no evidence of tumor at least 28 weeks post-treatment. These studies suggest that IL-2, either in combination with effectors cells or alone, may be beneficial in the treatment

of glioma. However, it is important to note that these studies were small, and lacked adequate randomization or controls.

TGF- β 2 was originally named for its ability to suppress T cell growth and IL-2 production, and was isolated and cloned from glioblastoma cell lines [67,71]. The expression of TGF- β 1 and - β 2 in two glioblastoma cell lines and newly isolated patient samples was confirmed at the mRNA level [65]. However, only TGF β -2 was detected in the supernatant of glioma cell lines and in the cerebral spinal fluid of patients with malignant glioma [66]. Primary glioma cells treated with antisense TGF β -2 (Antisense Pharma, AP 12009) showed a significant reduction in TGF β -2 expression from 73% positive cells to 49% positive cells, and glioma cell proliferation [72]. According to a phase I/II trial (NCT00844064), Hau and colleagues showed promising results in 24 patients with malignant glioma treated with antisense oligonucleotides (AP 12009) [72]. A complete remission was observed in two patients with anaplastic astrocytoma (AA), and the overall survival in AA (146.6 weeks) and GBM (44 weeks) patients was increased relative to historical controls. The two year survival for the treatment group was 80%. This immunotherapeutic strategy is designed to improve the immune system's ability to mount antitumor immune responses and is currently in phase III trials (NCT00761280).

Interferons are normally expressed in response to altered cells. In animal models, IFN- α and - β inhibit glioma growth. On this basis, IFN has been investigated in multiple clinical trials for the treatment of malignant glioma. A phase I trial using IFN- α in combination with BCNU, as an initial treatment modality for high-grade glioma, found that five of nine patients had a partial response and a median survival of four years [73]. In a phase II trial of 21 patients with recurrent high-grade glioma, seven patients had partial response and six patients maintained stable disease following treatment with IFN- α and BCNU [74]. In contrast to these earlier trials, a phase III trial of 214 eligible patients with high-grade glioma, in which patients received BCNU in combination with IFN α , the response was no better than in patients that received BCNU alone, with regard to time to disease progression or overall survival [75]. A few caveats of the early studies involved patient selection and inconsistent endpoint analysis. It is important to note that in addition to being ineffective for the treatment of glioma, systemic IFN administration also causes severe adverse reactions, including neurocortical effects, fever, chills, and myalgias.

Depletion of regulatory T cells

Regulatory T cells (Tregs; CD4⁺CD25⁺FOXP3⁺) Tregs are a fraction of the T cell population that suppress immune activation and thereby maintain homeostasis and tolerance to self-antigens. Functional deletion of Tregs induce autoimmunity, facilitates transplantation tolerance and also increases immunity to tumors [3,76,77]. A lack of immune rejection of neoplastic cells is believed to be maintained by Tregs in many malignancies including colorectal, esophageal, pancreatic, breast, lung, ovarian and brain tumors [3,78-81]. An increased fraction of regulatory T cells has been reported to infiltrate glioma contributing to the immunosuppressive status associated with glioma [3-5,82,83]. It is therefore very important to understand the biology and function of Tregs for its potential therapeutic potentials.

The precise mechanism(s) by which Tregs suppress effector T cell-mediated immune response have not been definitively characterized. Some studies highlight the importance of cytokines in the regulation, and others, cell-to-cell contact with effector T cells in which case membrane bound TGF- β and cytotoxic T-lymphocyte protein (CTLA-4) plays an important role.[84-86] Heme oxygenase-1 (HO-1), a rate limiting enzyme in heme metabolism also plays a role in Treg mediated immune suppression. HO-1 is constitutively expressed in human Tregs and is induced by FoxP3 expression [87,88]. It is suggested that HO-1 suppresses effector T cell by carbon-monoxide production [89,90].

In 2006, our group demonstrated tumor infiltration of Tregs in glioblastoma multiforme (GBM) patients [4]. The expression of FoxP3⁺ Tregs was significantly higher in patients with GBM than in controls, whereas these cells were absent from control brain specimens. Higher levels of FoxP3 expression were observed in regulatory T cells isolated from the tumor tissue in comparison to autologous patient blood and blood from control individuals. In an *in vitro* suppression assay, Tregs inhibited T cell proliferation in a dose-dependent manner. Among various markers analyzed, the expression of CD62L and CTLA-4 was elevated in the glioma infiltrating Tregs in comparison to that of the controls. We showed improved survival of mice with experimental brain tumors, following the depletion of Tregs with anti-CD25 monoclonal antibody (PC61) [3].

A prominent population of Tregs and a corresponding lack of effector/activated T cells was demonstrated in GBM patient specimens [82]. Absolute counts of both CD4⁺ T cells and FoxP3⁺CD45RO⁺ Tregs were greatly diminished in the peripheral pool of patients with malignant glioma, but the Tregs fraction was increased in the remaining CD4 compartment in 5 out of the 8 patients evaluated [5]. The proportion of Tregs in the peripheral blood of patients with GBM was 2.63 times higher than that found in the blood of normal volunteers. The patients with an elevated Tregs fraction showed significant CD4⁺ T cell lymphopenia, whereas the patients without Tregs elevation possessed normally proliferating CD4⁺ T cell levels. T cells from the patients bearing malignant gliomas regained their function after Tregs depletion *in vitro*, and proliferated to levels equivalent to those of normal controls.

The depletion of Tregs is normally achieved using anti-CD25 antibodies, which may also deplete activated T cells which express CD25. Curtin et al demonstrated the efficacy of immunotherapy using anti-CD25 depleting antibodies (PC61) in experimental animal of glioma. Interestingly, the efficacy of Tregs depletion was time dependent and greatly influenced by tumor burden [91]. Systemic depletion of Tregs 15 days after tumor implantation improved long-term survival, but Tregs depleted 24 days after tumor implantation showed no improvement in survival. It is very important to note this observation suggests that immunotherapy alone may not be the fail-safe therapeutic strategy. Moreover, Tregs depletion should be performed prior to immunotherapy to limit depletion of effector cells along with Tregs following the administration of anti-CD25 antibodies.

Small Molecule Inhibitors of STAT-3

Signal transducer and activator of transcription-3 (STAT-3) is a convergence point of several signaling pathways in quite a multiple malignancies including glioblastoma, breast, lung, ovarian, pancreatic, skin and prostate cancer [92,93]. It has recently emerged as a potential target for glioma immunotherapy. The binding of STAT3 to its target genes affects proliferation, survival, differentiation and development. Receptor engagement by members of IL-6 cytokine family like IL-6, oncostatin M and Leukemia inhibitory factor, or growth factors like platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and epithelial growth factor (EGF) activate STAT-3. The activation of STAT-3 requires the activation of receptor-associated kinases like Janus kinase (JAK) family members, FHFR, EGFR, PDGFR or nonreceptor-associated kinases like Ret, Src or Bcl-Abl. STAT-3 activity is attenuated by suppressors of cytokine signaling (SOCS) by down-regulating its upstream kinase activity, while protein inhibitors of activated STAT (PIAS) and protein tyrosine phosphatases target STAT-3 directly [94-96]. Other than promoting oncogenesis, active STAT-3 also enables tumor growth by suppressing tumor recognition by the immune system.[97] STAT-3 promotes tumor immune evasion by inhibiting pro-inflammatory cytokine signaling and amplifying Tregs. STAT-3 activity in cancers other than glioblastoma has been targeted in several different therapeutic strategies. STAT-3 inhibition has been approached from two fronts either through

RNA interference or chemical inhibitors, or through modulation of endogenous regulators such as PIAS3 and SOCS-3 (Fig. 2).

Several compounds block STAT-3 signaling by directly targeting the STAT-3 protein. Platinum compounds like CPA-1 and CPA-7 have been successfully used to block STAT-3 activity and induce apoptosis in breast, lung, and prostate cancer cell lines [98]. More recently, Zhang et al (2009) used CPA-7 to successfully block STAT-3 activation in glioma-associated microglia [99]. Decoy oligonucleotides like G-quartets or Transcription factor decoy (TFD) oligodeoxynucleotides, and inhibitors like S31-201 have been used by researchers for directly blocking STAT-3 signal transduction in human cancer cells [100-103]. Furthermore, the knock down of STAT-3 in human glioma cell lines by STAT-3 siRNA induced apoptosis and inhibited survival [104,105]. STAT3 decoy ODN treatment in U251 and A172 glioma cell lines blocked STAT3 signaling and inhibited glioma proliferation by inducing apoptosis and cell-cycle arrest [103].

The pharmacological inhibitors of growth factor receptors and upstream tyrosine kinases have also been very successful at blocking STAT-3 activity. Inhibitors of JAK and Src showed potential STAT-3 inhibition and are in early stages of experimental testing [106,107]. Preliminary *in vivo* studies showed that WP1066, a JAK inhibitor, has the potential to cross the blood-brain barrier, which is very important for glioma patients. WP1066 abrogated immune tolerance in glioblastoma patients and stimulated T cell proliferation by up-regulating secretion of costimulatory molecules and T-cell effector cytokines and improved immunogenic responses [108]. In an independent experiment, growth of glioma xenografts was restricted by decreased STAT-3-mediated expression of Bcl-xL, Mcl-1, and c-Myc when STAT-3 was inhibited with WP1066 [109]. The effects were also very tumor-specific as normal astrocytoma cells were not affected. Attenuation of upstream FGF signaling pathway by dobesilate, a vasoactive drug, in C6 glioma cells triggered apoptosis and growth arrest by inhibiting STAT-3 activation [110]. These observations illustrate a possible relationship between STAT-3 and glioblastoma. Pharmacologic inhibitors of individual kinases that are in command upstream of STAT-3 inhibitors therefore might be an ideal candidate for potential therapeutic intervention of glioma progression.

Active Immunotherapy

LAK cells

In vitro studies using tumor cells from a variety of malignancies, including glioma, showed lymphokine activated killer (LAK) cell lysis [111,112]. Human studies conducted by Rosenberg et al., showed therapeutic benefits of LAK cells in multiple types of tumor cells and they were largely inefficient at lysing normal tissues [113]. A phase I study evaluated 10 patients with recurrent GBM following surgical resection and intratumoral injection of LAK and IL-2 [114]. In this study, steroids were restricted during treatment, unless required for the treatment of acute symptoms of IL-2 toxicity (edema and confusion). The therapeutic efficacy of LAK cells was characterized by a median survival of 53 weeks, with 53% of the patients still being alive after one year, compared to a median survival of 25.5 weeks for the chemotherapy alone group [115]. This study highlighted the potential benefits of LAK cell infusion for the treatment of glioma. To date, the mechanism of action of LAK cells remains unclear, thereby limiting their use in immunotherapy. Furthermore, LAK cells must be administered locally at the tumor site since they fail to effectively home to tumor lesions. In light of these factors, immunotherapeutic strategies have moved from LAK cells toward T cells. Moreover, T cells have been to be more lytic than LAK cells, on a per cell basis.

Effector T cells

Adoptive immunotherapy has emerged as a novel treatment modality for multiple cancers. The use of tumor specific T cells was based on the belief that tumor antigen specific T cells could traffic to tumor lesions and preferentially target tumor cells, over non-tumor cells. In many of these studies, autologous T cells are primed against tumor antigens and expanded *in vitro* prior to re-infusion. Using an animal model of glioma, adoptively transferred CTL were shown to effectively home to and reject tumors following intravenous administration [116]. According to Yamasaki et al., the mean survival time following intravenous administration of *in vitro* expanded CTLs was over 15 weeks (except for one animal that died at 10 weeks) compared to approximately 3.3 weeks in vehicle only or *in vivo* primed CTLs isolated from the draining lymph nodes (3.6 weeks) and spleen (2.0 weeks). Further analysis revealed that the CTL activity of the adoptively transferred cells was specific for tumor cells and not non-glia tumor cells. The ability to generate and maintain tumor specific T cells was a major advantage compared to LAK cells, and propelled it to prominence in the field of adoptive immunotherapy.

In one such study, Kitahara et al., generated CTL *in vitro* from the blood of five malignant glioma patients [117]. Briefly, the peripheral blood lymphocytes were cultured with autologous tumor cells plus IL-2, in order to generate CTL, which were later administered intracranially. The results from this study were largely poor. One patient showed a transient regression for 20 weeks prior to recurrence and one patient had a complete regression to at least 104 weeks. Three other patients progressed quickly and died from recurrent tumor. This study underscored the potential benefits of this treatment modality and served as a building block for future trials.

The use of autologous tumor cells to sensitize CTL *in vitro* requires the isolation and maintenance of tumor cells. Furthermore, in order to increase the amount of T cells harvested from peripheral blood, recent studies used BCG vaccination in combination with GM-CSF and IL-2 infusion. In an attempt to circumvent these issues, a more recent study of nine high-grade glioma patients utilized anti-CD3 for polyclonal T cell activation in combination with IL-2 [118]. Two patients with grade III disease had complete tumor regression to at least five years, and one patient had a partial regression. This treatment strategy was not effective in the GBM patients. Plautz et al obtained encouraging results using autologous CTL [119]. Patients were infused with GM-CSF and T cells were isolated from the draining lymph nodes. Two patients showed tumor regression, one patient did not observe tumor growth out to 17 months, while the remaining 7 patients had progressive disease. Overall, all patients with GBM survived at least a year. Although polyclonal stimulation with anti-CD3 stimulates a large pool of T cells, which may include tumor specific T cells, the frequency of these cells may be relatively low in the entire T cell pool thus minimizing their therapeutic efficacy.

More recent studies sought to isolate and expand tumor infiltrating lymphocytes (TIL), however, no clear therapeutic benefits were observed. According to Quattrocchi et al., six patients with high grade glioma were treated with autologous TIL plus IL-2 in the tumor cavity following surgical resection [120]. Cerebral edema was the only adverse side effect noted. One in six patients demonstrated tumor regression and was tumor free at 45 months. A limitation of this study was the fact that the TIL were simply re-infused without depleting suppressor cells, which have been to be highly suppressive and abundant in TIL. Future trials should seek to deplete Tregs prior to re-infusion and may consider *ex vivo* activation and expansion to increase the cytotoxic function of TIL.

Conclusions

Novel immunotherapeutic strategies have emerged as the understanding of CNS immunobiology and gliomas has progressed. The anatomical location of glioma within the CNS is beneficial for tumor progression and limits the success of many treatment modalities.

Multiple groups, including ours, have demonstrated the therapeutic efficacy of immunotherapy in pre-clinical models of glioma, but these have yet to show clinical efficacy. We suggest that the observed deficiencies of many of these treatment modalities are linked to the poor design of many of the clinical trials. Additionally, large randomized studies are often difficult to conduct since GBM is rare. Moreover, many preclinical trials are conducted in immune comprised animals making extrapolation to immune competent hosts difficult.

In order to truly realize the promise of immunotherapy modalities, there needs to be improvements in study design. To date, EGFRvIII has emerged as the key molecule for tumor targeting. As is the case with other targeted therapies, EGFRvIII vaccination has seen minimal successes in the clinic due to poor patient selection. Further, better end point analyses are required to determine treatment efficacy. In brief, the induction of an immune response does is not always correlative with improved time to tumor progression or overall survival. Therefore, studies should clearly define enrollment criteria and result interpretation prior to study initiation, so that we are able to truly realize the therapeutic efficacy of immunotherapy.

References

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* Mar 10;2005 352(10):987–996. [PubMed: 15758009]
2. Sampson JH, Brady ML, Petry NA, et al. Intracerebral infusate distribution by convection-enhanced delivery in humans with malignant gliomas: descriptive effects of target anatomy and catheter positioning. *Neurosurgery* Feb;2007 60(2 Suppl 1):ONS89–98. [PubMed: 17297371]discussion ONS98-89
3. El Andaloussi A, Han Y, Lesniak MS. Prolongation of survival following depletion of CD4+CD25+ regulatory T cells in mice with experimental brain tumors. *J Neurosurg* Sep;2006 105(3):430–437. [PubMed: 16961139]
4. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro Oncol* Jul;2006 8(3):234–243. [PubMed: 16723631]
5. Fecci PE, Mitchell DA, Whitesides JF, et al. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* Mar 15;2006 66(6):3294–3302. [PubMed: 16540683]
6. Abou-Ghazal M, Yang DS, Qiao W, et al. The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. *Clin Cancer Res* Dec 15;2008 14(24):8228–8235. [PubMed: 19088040]
7. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature* Jan 10-18;1985 313(5998):144–147. [PubMed: 2981413]
8. Bigner SH, Humphrey PA, Wong AJ, et al. Characterization of the epidermal growth factor receptor in human glioma cell lines and xenografts. *Cancer Res* Dec 15;1990 50(24):8017–8022. [PubMed: 2253244]
9. Sonabend AM, Dana K, Lesniak MS. Targeting epidermal growth factor receptor variant III: a novel strategy for the therapy of malignant glioma. *Expert Rev Anticancer Ther* Dec;2007 7(12 Suppl):S45–50. [PubMed: 18076318]
10. Heimberger AB, Sun W, Hussain SF, et al. Immunological responses in a patient with glioblastoma multiforme treated with sequential courses of temozolomide and immunotherapy: case study. *Neuro Oncol* Feb;2008 10(1):98–103. [PubMed: 18079360]
11. Frederick L, Wang XY, Eley G, James CD. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res* Mar 1;2000 60(5):1383–1387. [PubMed: 10728703]
12. Heimberger AB, Hlatky R, Suki D, et al. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. *Clin Cancer Res* Feb 15;2005 11(4):1462–1466. [PubMed: 15746047]

13. Sok JC, Coppelli FM, Thomas SM, et al. Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. *Clin Cancer Res* Sep 1;2006 12(17):5064–5073. [PubMed: 16951222]
14. Okamoto I, Kenyon LC, Emlet DR, et al. Expression of constitutively activated EGFRvIII in non-small cell lung cancer. *Cancer Sci* Jan;2003 94(1):50–56. [PubMed: 12708474]
15. Belli F, Testori A, Rivoltini L, et al. Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings. *J Clin Oncol* Oct 15;2002 20(20):4169–4180. [PubMed: 12377960]
16. Batra SK, Castellino-Prabhu S, Wikstrand CJ, et al. Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant EGFRvIII gene. *Cell Growth Differ* Oct;1995 6(10):1251–1259. [PubMed: 8845302]
17. Huang HS, Nagane M, Klingbeil CK, et al. The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling. *J Biol Chem* Jan 31;1997 272(5):2927–2935. [PubMed: 9006938]
18. Boockvar JA, Kapitonov D, Kapoor G, et al. Constitutive EGFR signaling confers a motile phenotype to neural stem cells. *Mol Cell Neurosci* Dec;2003 24(4):1116–1130. [PubMed: 14697673]
19. Pedersen MW, Tkach V, Pedersen N, Berezin V, Poulsen HS. Expression of a naturally occurring constitutively active variant of the epidermal growth factor receptor in mouse fibroblasts increases motility. *Int J Cancer* Feb 20;2004 108(5):643–653. [PubMed: 14696090]
20. Lammering G, Hewit TH, Holmes M, et al. Inhibition of the type III epidermal growth factor receptor variant mutant receptor by dominant-negative EGFR-CD533 enhances malignant glioma cell radiosensitivity. *Clin Cancer Res* Oct 1;2004 10(19):6732–6743. [PubMed: 15475464]
21. Nagane M, Coufal F, Lin H, Bogler O, Cavenee WK, Huang HJ. A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer Res* Nov 1;1996 56(21):5079–5086. [PubMed: 8895767]
22. Montgomery RB, Guzman J, O'Rourke DM, Stahl WL. Expression of oncogenic epidermal growth factor receptor family kinases induces paclitaxel resistance and alters beta-tubulin isotype expression. *J Biol Chem* Jun 9;2000 275(23):17358–17363. [PubMed: 10749863]
23. Lammering G, Hewit TH, Valerie K, et al. EGFRvIII-mediated radioresistance through a strong cytoprotective response. *Oncogene* Aug 28;2003 22(36):5545–5553. [PubMed: 12944901]
24. Akasaki Y, Liu G, Chung NH, Ehteshami M, Black KL, Yu JS. Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma. *J Immunol* Oct 1;2004 173(7):4352–4359. [PubMed: 15383564]
25. Shinojima N, Tada K, Shiraishi S, et al. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. *Cancer Res* Oct 15;2003 63(20):6962–6970. [PubMed: 14583498]
26. Heimberger AB, Archer GE, Crotty LE, et al. Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. *Neurosurgery* Jan;2002 50(1):158–164. [PubMed: 11844246]discussion 164-156
27. Heimberger AB, Crotty LE, Archer GE, et al. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. *Clin Cancer Res* Sep 15;2003 9(11):4247–4254. [PubMed: 14519652]
28. 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* Jun;1990 87(11):4207–4211. [PubMed: 1693434]
29. Johns TG, Stockert E, Ritter G, et al. Novel monoclonal antibody specific for the de2-7 epidermal growth factor receptor (EGFR) that also recognizes the EGFR expressed in cells containing amplification of the EGFR gene. *Int J Cancer* Mar 20;2002 98(3):398–408. [PubMed: 11920591]
30. Jungbluth AA, Stockert E, Huang HJ, et al. A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. *Proc Natl Acad Sci U S A* Jan 21;2003 100(2):639–644. [PubMed: 12515857]

31. Kuan CT, Wikstrand CJ, Archer G, et al. Increased binding affinity enhances targeting of glioma xenografts by EGFRvIII-specific scFv. *Int J Cancer* Dec 15;2000 88(6):962–969. [PubMed: 11093822]
32. Perera RM, Narita Y, Furnari FB, et al. Treatment of human tumor xenografts with monoclonal antibody 806 in combination with a prototypical epidermal growth factor receptor-specific antibody generates enhanced antitumor activity. *Clin Cancer Res* Sep 1;2005 11(17):6390–6399. [PubMed: 16144944]
33. Sampson JH, Crotty LE, Lee S, et al. Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. *Proc Natl Acad Sci U S A* Jun 20;2000 97(13):7503–7508. [PubMed: 10852962]
34. Wikstrand CJ, Stanley SD, Humphrey PA, et al. Investigation of a synthetic peptide as immunogen for a variant epidermal growth factor receptor associated with gliomas. *J Neuroimmunol* Jul;1993 46(12):165–173. [PubMed: 8360327]
35. Ochiai H, Archer GE, Herndon JE 2nd, et al. EGFRvIII-targeted immunotoxin induces antitumor immunity that is inhibited in the absence of CD4+ and CD8+ T cells. *Cancer Immunol Immunother* Jan;2008 57(1):115–121. [PubMed: 17634939]
36. Wu AH, Xiao J, Anker L, et al. Identification of EGFRvIII-derived CTL epitopes restricted by HLA A0201 for dendritic cell based immunotherapy of gliomas. *J Neurooncol* Jan;2006 76(1):23–30. [PubMed: 16155724]
37. Purev E, Cai D, Miller E, et al. Immune responses of breast cancer patients to mutated epidermal growth factor receptor (EGF-RvIII, Delta EGF-R, and de2-7 EGF-R). *J Immunol* Nov 15;2004 173(10):6472–6480. [PubMed: 15528389]
38. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Bigner DD. Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. *Semin Immunol* Oct;2008 20(5):267–275. [PubMed: 18539480]
39. Eguchi J, Hatano M, Nishimura F, et al. Identification of interleukin-13 receptor alpha2 peptide analogues capable of inducing improved antiglioma CTL responses. *Cancer Res* Jun 1;2006 66(11):5883–5891. [PubMed: 16740728]
40. Okano F, Storkus WJ, Chambers WH, Pollack IF, Okada H. Identification of a novel HLA-A*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. *Clin Cancer Res* Sep;2002 8(9):2851–2855. [PubMed: 12231526]
41. Joshi BH, Leland P, Asher A, Prayson RA, Varricchio F, Puri RK. In situ expression of interleukin-4 (IL-4) receptors in human brain tumors and cytotoxicity of a recombinant IL-4 cytotoxin in primary glioblastoma cell cultures. *Cancer Res* Nov 15;2001 61(22):8058–8061. [PubMed: 11719427]
42. Joshi BH, Leland P, Silber J, et al. IL-4 receptors on human medulloblastoma tumours serve as a sensitive target for a circular permuted IL-4-Pseudomonas exotoxin fusion protein. *Br J Cancer* Jan 21;2002 86(2):285–291. [PubMed: 11870521]
43. Kawakami K, Leland P, Puri RK. Structure, function, and targeting of interleukin 4 receptors on human head and neck cancer cells. *Cancer Res* Jun 1;2000 60(11):2981–2987. [PubMed: 10850446]
44. Obiri NI, Hillman GG, Haas GP, Sud S, Puri RK. Expression of high affinity interleukin-4 receptors on human renal cell carcinoma cells and inhibition of tumor cell growth in vitro by interleukin-4. *J Clin Invest* Jan;1993 91(1):88–93. [PubMed: 8423237]
45. Obiri NI, Siegel JP, Varricchio F, Puri RK. Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells. *Clin Exp Immunol* Jan;1994 95(1):148–155. [PubMed: 8287600]
46. Puri RK, Leland P, Kreitman RJ, Pastan I. Human neurological cancer cells express interleukin-4 (IL-4) receptors which are targets for the toxic effects of IL4-Pseudomonas exotoxin chimeric protein. *Int J Cancer* Aug 15;1994 58(4):574–581. [PubMed: 8056454]
47. Wheeler CJ, Das A, Liu G, Yu JS, Black KL. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. *Clin Cancer Res* Aug 15;2004 10(16):5316–5326. [PubMed: 15328167]
48. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* Jul 15;2004 64(14):4973–4979. [PubMed: 15256471]

49. Liao LM, Black KL, Martin NA, et al. Treatment of a patient by vaccination with autologous dendritic cells pulsed with allogeneic major histocompatibility complex class I-matched tumor peptides. Case Report. *Neurosurg Focus* 2000;9(6):e8. [PubMed: 16817691]
50. Heimberger AB, Crotty LE, Archer GE, et al. Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. *J Neuroimmunol* Feb 1;2000 103(1):16–25. [PubMed: 10674985]
51. de Vleeschouwer S, Rapp M, Sorg RV, et al. Dendritic cell vaccination in patients with malignant gliomas: current status and future directions. *Neurosurgery* Nov;2006 59(5):988–999. [PubMed: 17143233]discussion 999–1000
52. Wheeler CJ, Black KL. DCVax-Brain and DC vaccines in the treatment of GBM. *Expert Opin Investig Drugs* Apr;2009 18(4):509–519.
53. Parajuli P, Mathupala S, Sloan AE. Systematic comparison of dendritic cell-based immunotherapeutic strategies for malignant gliomas: in vitro induction of cytolytic and natural killer-like T cells. *Neurosurgery* Nov;2004 55(5):1194–1204. [PubMed: 15509326]
54. Srivastava PK, Maki RG. Stress-induced proteins in immune response to cancer. *Curr Top Microbiol Immunol* 1991;167:109–123. [PubMed: 1711433]
55. Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. *J Exp Med* Oct 1;1993 178(4):1391–1396. [PubMed: 8376942]
56. Udono H, Srivastava PK. Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90, and hsp70. *J Immunol* Jun 1;1994 152(11):5398–5403. [PubMed: 8189059]
57. Bethke K, Staib F, Distler M, et al. Different efficiency of heat shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60. *J Immunol* Dec 1;2002 169(11):6141–6148. [PubMed: 12444117]
58. Binder RJ, Harris ML, Menoret A, Srivastava PK. Saturation, competition, and specificity in interaction of heat shock proteins (hsp) gp96, hsp90, and hsp70 with CD11b+ cells. *J Immunol* Sep 1;2000 165(5):2582–2587. [PubMed: 10946285]
59. Parsa, A.; Crane, C.; Wilson, S., et al. Autologous tumor derived gp96 evokes a tumor specific immune response in recurrent glioma patients that correlates with clinical response to therapy. Paper presented at: AACR-NCI-EORTC International Conference Molecular Targets and Cancer Therapeutics (C274); 2007;
60. Cohen L, de Moor C, Parker PA, Amato RJ. Quality of life in patients with metastatic renal cell carcinoma participating in a phase I trial of an autologous tumor-derived vaccine. *Urol Oncol* May-Jun;2002 7(3):119–124. [PubMed: 12474545]
61. Cohen L, Parker PA, Sterner J, De Moor C. Quality of life in patients with malignant melanoma participating in a phase I trial of an autologous tumour-derived vaccine. *Melanoma Res* Oct;2002 12(5):505–511. [PubMed: 12394193]
62. Pilla L, Patuzzo R, Rivoltini L, et al. A phase II trial of vaccination with autologous, tumor-derived heat-shock protein peptide complexes Gp96, in combination with GM-CSF and interferon-alpha in metastatic melanoma patients. *Cancer Immunol Immunother* Aug;2006 55(8):958–968. [PubMed: 16215718]
63. Li G, Hu YS, Li XG, Zhang QL, Wang DH, Gong SF. Expression and switching of TH1/TH2 type cytokines gene in human gliomas. *Chin Med Sci J* Dec;2005 20(4):268–272. [PubMed: 16422258]
64. Roussel E, Gingras MC, Grimm EA, Bruner JM, Moser RP. Predominance of a type 2 intratumoural immune response in fresh tumour-infiltrating lymphocytes from human gliomas. *Clin Exp Immunol* Aug;1996 105(2):344–352. [PubMed: 8706344]
65. Bodmer S, Strommer K, Frei K, et al. Immunosuppression and transforming growth factor-beta in glioblastoma. Preferential production of transforming growth factor-beta 2. *J Immunol* Nov 15;1989 143(10):3222–3229. [PubMed: 2809198]
66. Tada T, Yabu K, Kobayashi S. Detection of active form of transforming growth factor-beta in cerebrospinal fluid of patients with glioma. *Jpn J Cancer Res* May;1993 84(5):544–548. [PubMed: 8320172]
67. de Martin R, Haendler B, Hofer-Warbinek R, et al. Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-beta gene family. *Embo J* Dec 1;1987 6(12):3673–3677. [PubMed: 3322813]

68. Huettner C, Paulus W, Roggendorf W. Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas. *Am J Pathol* Feb;1995 146(2):317–322. [PubMed: 7856743]
69. Colombo F, Barzon L, Franchin E, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: biological and clinical results. *Cancer Gene Ther* Oct;2005 12(10):835–848. [PubMed: 15891772]
70. 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* Oct;1997 45(2):77–87. [PubMed: 9390198]
71. Wrann M, Bodmer S, de Martin R, et al. T cell suppressor factor from human glioblastoma cells is a 12.5-kd protein closely related to transforming growth factor-beta. *Embo J* Jun;1987 6(6):1633–1636. [PubMed: 3497030]
72. 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* Summer;2007 17(2):201–212. [PubMed: 17638524]
73. Olson JJ, McKenzie E, Skurski-Martin M, Zhang Z, Brat D, Phuphanich S. Phase I analysis of BCNU-impregnated biodegradable polymer wafers followed by systemic interferon alfa-2b in adults with recurrent glioblastoma multiforme. *J Neurooncol* Dec;2008 90(3):293–299. [PubMed: 18679580]
74. Buckner JC, Brown LD, Kugler JW, et al. Phase II evaluation of recombinant interferon alpha and BCNU in recurrent glioma. *J Neurosurg* Mar;1995 82(3):430–435. [PubMed: 7861221]
75. Buckner JC, Schomberg PJ, McGinnis WL, et al. A phase III study of radiation therapy plus carmustine with or without recombinant interferon-alpha in the treatment of patients with newly diagnosed high-grade glioma. *Cancer* Jul 15;2001 92(2):420–433. [PubMed: 11466698]
76. Yong Z, Chang L, Mei YX, Yi L. Role and mechanisms of CD4+CD25+ regulatory T cells in the induction and maintenance of transplantation tolerance. *Transpl Immunol* Feb;2007 17(2):120–129. [PubMed: 17306743]
77. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol Rev* Jun;2008 223:371–390. [PubMed: 18613848]
78. Bayer AL, Yu A, Malek TR. Function of the IL-2R for thymic and peripheral CD4+CD25+Foxp3+ T regulatory cells. *J Immunol* Apr 1;2007 178(7):4062–4071. [PubMed: 17371960]
79. Ichihara F, Kono K, Takahashi A, Kawaida H, Sugai H, Fujii H. Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers. *Clin Cancer Res* Oct 1;2003 9(12):4404–4408. [PubMed: 14555512]
80. Liyanage UK, Moore TT, Joo HG, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* Sep 1;2002 169(5):2756–2761. [PubMed: 12193750]
81. Okita R, Saeki T, Takashima S, Yamaguchi Y, Toge T. CD4+CD25+ regulatory T cells in the peripheral blood of patients with breast cancer and non-small cell lung cancer. *Oncol Rep* Nov;2005 14(5):1269–1273. [PubMed: 16211295]
82. Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro Oncol* Jul;2006 8(3):261–279. [PubMed: 16775224]
83. Grauer OM, Nierkens S, Bennink E, et al. CD4+FoxP3+ regulatory T cells gradually accumulate in gliomas during tumor growth and efficiently suppress antiglioma immune responses in vivo. *Int J Cancer* Jul 1;2007 121(1):95–105. [PubMed: 17315190]
84. Takahashi Y, Onda M, Tanaka N, Seya T. Establishment and characterization of two new rectal neuroendocrine cell carcinoma cell lines. *Digestion* 2000;62(4):262–270. [PubMed: 11070410]
85. Manzotti CN, Tipping H, Perry LC, et al. Inhibition of human T cell proliferation by CTLA-4 utilizes CD80 and requires CD25+ regulatory T cells. *Eur J Immunol* Oct;2002 32(10):2888–2896. [PubMed: 12355442]
86. Tang Q, Boden EK, Henriksen KJ, Bour-Jordan H, Bi M, Bluestone JA. Distinct roles of CTLA-4 and TGF-beta in CD4+CD25+ regulatory T cell function. *Eur J Immunol* Nov;2004 34(11):2996–3005. [PubMed: 15468055]

87. Pae HO, Oh GS, Choi BM, Chae SC, Chung HT. Differential expressions of heme oxygenase-1 gene in CD25- and CD25+ subsets of human CD4+ T cells. *Biochem Biophys Res Commun* Jul 4;2003 306(3):701–705. [PubMed: 12810075]
88. Choi BM, Pae HO, Jeong YR, Kim YM, Chung HT. Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression. *Biochem Biophys Res Commun* Feb 25;2005 327(4):1066–1071. [PubMed: 15652505]
89. Choi BM, Pae HO, Jeong YR, et al. Overexpression of heme oxygenase (HO)-1 renders Jurkat T cells resistant to fas-mediated apoptosis: involvement of iron released by HO-1. *Free Radic Biol Med* Apr 1;2004 36(7):858–871. [PubMed: 15019971]
90. Song R, Zhou Z, Kim PK, et al. Carbon monoxide promotes Fas/CD95-induced apoptosis in Jurkat cells. *J Biol Chem* Oct 22;2004 279(43):44327–44334. [PubMed: 15280387]
91. Curtin JF, Candolfi M, Fakhouri TM, et al. Treg depletion inhibits efficacy of cancer immunotherapy: implications for clinical trials. *PLoS ONE* 2008;3(4):e1983. [PubMed: 18431473]
92. Bromberg J. Stat proteins and oncogenesis. *J Clin Invest* May;2002 109(9):1139–1142. [PubMed: 11994401]
93. Brantley EC, Benveniste EN. Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas. *Mol Cancer Res* May;2008 6(5):675–684. [PubMed: 18505913]
94. Chung CD, Liao J, Liu B, et al. Specific inhibition of Stat3 signal transduction by PIAS3. *Science* Dec 5;1997 278(5344):1803–1805. [PubMed: 9388184]
95. Rakesh K, Agrawal DK. Controlling cytokine signaling by constitutive inhibitors. *Biochem Pharmacol* Sep 1;2005 70(5):649–657. [PubMed: 15936728]
96. Pillemer BB, Xu H, Oriss TB, Qi Z, Ray A. Deficient SOCS3 expression in CD4+CD25+FoxP3+ regulatory T cells and SOCS3-mediated suppression of Treg function. *Eur J Immunol* Aug;2007 37(8):2082–2089. [PubMed: 17621372]
97. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat Rev Immunol* Jan;2007 7(1):41–51. [PubMed: 17186030]
98. Turkson J, Zhang S, Palmer J, et al. Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent antitumor activity. *Mol Cancer Ther* Dec;2004 3(12):1533–1542. [PubMed: 15634646]
99. Zhang L, Alizadeh D, Van Handel M, Kortylewski M, Yu H, Badie B. Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. *Glia*. Mar 20;2009
100. Jing N, Li Y, Xu X, et al. Targeting Stat3 with G-quartet oligodeoxynucleotides in human cancer cells. *DNA Cell Biol* Nov;2003 22(11):685–696. [PubMed: 14659041]
101. Leong PL, Andrews GA, Johnson DE, et al. Targeted inhibition of Stat3 with a decoy oligonucleotide abrogates head and neck cancer cell growth. *Proc Natl Acad Sci U S A* Apr 1;2003 100(7):4138–4143. [PubMed: 12640143]
102. Siddiquee K, Zhang S, Guida WC, et al. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc Natl Acad Sci U S A* May 1;2007 104(18):7391–7396. [PubMed: 17463090]
103. Gu J, Li G, Sun T, et al. Blockage of the STAT3 signaling pathway with a decoy oligonucleotide suppresses growth of human malignant glioma cells. *J Neurooncol* Aug;2008 89(1):9–17. [PubMed: 18415045]
104. Konnikova L, Kotecki M, Kruger MM, Cochran BH. Knockdown of STAT3 expression by RNAi induces apoptosis in astrocytoma cells. *BMC Cancer* Sep 17;2003 3:23. [PubMed: 13678425]
105. Ren W, Duan Y, Yang Y, Ji Y, Chen F. Down-regulation of Stat3 induces apoptosis of human glioma cell: a potential method to treat brain cancer. *Neurol Res* Apr;2008 30(3):297–301. [PubMed: 17716392]
106. Rahaman SO, Harbor PC, Chernova O, Barnett GH, Vogelbaum MA, Haque SJ. Inhibition of constitutively active Stat3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells. *Oncogene* Dec 5;2002 21(55):8404–8413. [PubMed: 12466961]
107. Blaskovich MA, Sun J, Cantor A, Turkson J, Jove R, Sefti SM. Discovery of JSI-124 (cucurbitacin D), a selective Janus kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. *Cancer Res* Mar 15;2003 63(6):1270–1279. [PubMed: 12649187]

108. 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* Oct 15;2007 67(20):9630–9636. [PubMed: 17942891]
109. Iwamaru A, Szymanski S, Iwado E, et al. A novel inhibitor of the STAT3 pathway induces apoptosis in malignant glioma cells both in vitro and in vivo. *Oncogene* Apr 12;2007 26(17):2435–2444. [PubMed: 17043651]
110. Cuevas P, Diaz-Gonzalez D, Gimenez-Gallego G, Dujovny M. Dihydroxy-2,5 benzenesulphonate (dobesilate) elicits growth arrest and apoptosis in glioma cells. *Neurol Res* Dec;2005 27(8):797–800. [PubMed: 16354538]
111. Rayner AV, O'Brien JG, Schoenbachler B. Behavior disorders of dementia: recognition and treatment. *Am Fam Physician* Feb 15;2006 73(4):647–652. [PubMed: 16506707]
112. Iwasaki K, Kikuchi H, Miyatake S, Oda Y, Yamashita J, Yamasaki T. Clonal analysis in the ultrastructure of cell-to-cell interaction between a human glioma cell line and autologous tumor-specific cytotoxic T lymphocytes. *Cell Immunol* Mar;1990 126(1):164–175. [PubMed: 2302738]
113. Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* Dec 5;1985 313(23):1485–1492. [PubMed: 3903508]
114. 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* Apr;1986 46(4 Pt 2):2101–2104. [PubMed: 3512079]
115. 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* Sep 1;1995 76(5):840–852. [PubMed: 8625188]
116. Yamasaki T, Kikuchi H. An experimental approach to specific adoptive immunotherapy for malignant brain tumors. *Nippon Geka Hokan* Nov 1;1989 58(6):485–492. [PubMed: 2519184]
117. 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]
118. Tsurushima H, Liu SQ, Tuboi K, et al. Reduction of end-stage malignant glioma by injection with autologous cytotoxic T lymphocytes. *Jpn J Cancer Res* May;1999 90(5):536–545. [PubMed: 10391094]
119. Plautz GE, Miller DW, Barnett GH, et al. T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin Cancer Res* Jun;2000 6(6):2209–2218. [PubMed: 10873070]
120. 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]

Figure 1.

Schematic diagram of the EGFR wild-type protein showing the area of in-frame deletion which forms EGFRvIII. During the deletion amino acids 6 and 273 are split forming a novel glycine at the junction of amino acids 5 and 274. PEPvIII is a 13 amino acid peptide with a terminal cysteine added to facilitate conjugation to KLH. (Reprinted with permission from- Sampson *et al.*, (2008) Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. *Sem. Immunol.* **20(5)**: 267-275)

Figure 2.

Inhibition of STAT-3 signal transduction. A variety of endogenous and pharmacologic inhibitors can attenuate STAT-3 signaling. SOCS-3, PIAS3, and various protein tyrosine phosphatases (PTP) inhibit STAT-3 activity endogenously. STAT-3 – specific siRNA degrades STAT-3 mRNA. Pharmacologic inhibition of JAK activity by AG490 and WP1066 dampens the signals that result in STAT-3 activation. Attenuation of FGF signaling by doberisate also inhibits STAT-3 – mediated gene expression by attenuating kinase signals upstream of STAT-3 activation. (Reprinted with permission from- Brantley and Benveniste, (2008) Signal transducer and Activator of Transcription-3: A molecular hub for signaling pathways in gliomas, *Mol. Cancer Res.* 6(5): 675-684)

Current clinical trials for patients with malignant glioma.

Table 1

Protocol ID	Immunotherapeutic Strategy	Phase	Treatment Strategy
NCT00089427	IL-13- <i>Pseudomonas</i> Exotoxin fusion protein (IL13-PE38QQR)	I	Specifically targets IL-13R overexpression on tumor cells to deliver an immunotoxin
NCT00509301	Radiolabeled Antibody	I	Utilizes an antibody to deliver a radioactive drug to specifically target tumor cells
NCT00576641	Peptide-pulsed PBMC	I	Autologous PBMC are loaded with autologous tumor peptides in vitro and used to activate the patient's immune cells
NCT00694330	Irradiated tumor cells plus GM-CSF secreting cells	I	GM-CSF matures dendritic cells which present autologous tumor antigens to activate the patient's immune cells
NCT00639639	Tumor lysate-pulsed PBMC and anti-CD3 activated lymphocytes	I/II	In vitro activation of patient's T cells and autologous tumor antigen presentation by PBMC specifically stimulates tumor antigen specific T cells
NCT00293423	Gp96-tumor peptide vaccination	I/II	Gp96 is associated with multiple peptide antigens in tumor cells and stimulates tumor antigen specific immune cells
NCT00766753	Tumor peptide-pulsed DC vaccination	I/II	Autologous DC are loaded with autologous tumor peptides in vitro and used to activate the patient's immune cells
NCT00797940	IL-4- <i>Pseudomonas</i> Exotoxin fusion protein (IL-4(38-37)-PE38KDEL)	II	Specifically targets IL-4R overexpression on tumor cells to deliver an immunotoxin
NCT00045698	Tumor peptide-pulsed DC vaccination (DC-Vax®)	II	Autologous dendritic cells are loaded with autologous tumor peptides in vitro and used to activate the patient's immune cells
NCT00458601	EGFRvIII (CDX-110)	II	Specifically targets EGFRvIII overexpression on tumor cell
NCT00814593	LAK cells infusion	II	Autologous lymphocytes activated/stimulated in vitro with IL-2
NCT00068510	Tumor lysate-pulsed PBMC	II	Autologous PBMC are loaded with autologous tumor lysate in vitro and used to activate the patient's immune cells to multiple tumor antigens
NCT00431561	phosphorothioate antisense human TGFβ-2 mRNA (AP 12009)	IIb	Inhibits the expression of TGFβ-2, thus relieving tumor cell mediated immunosuppression