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Antibody-based immunotherapy for malignant glioma

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Abstract

Conventional therapy for malignant glioma (MG) fails to specifically eliminate tumor cells, resulting in toxicity that limits therapeutic efficacy. In contrast, antibody-based immunotherapy utilizes the immune system to eliminate tumor cells with exquisite specificity. Increased understanding of the pathobiology of MG and the profound immunosuppression present among patients with MG has revealed several biologic targets amenable to antibody-based immunotherapy. Novel antibody engineering techniques allow for the production of fully human antibodies or antibody fragments with vastly reduced antigen-binding dissociation constants, increasing safety when used clinically as therapeutics. In this report, we summarize the use of antibody-based immunotherapy for MG. Approaches currently under investigation include the use of antibodies or antibody fragments to: 1) redirect immune effector cells to target tumor mutations, 2) inhibit immunosuppressive signals and thereby stimulate an immunological response against tumor cells, and 3) provide co-stimulatory signals to evoke immunologic targeting of tumor cells. These approaches demonstrate highly compelling safety and efficacy for the treatment of MG, providing a viable adjunct to current standard-of-care therapy for MG.

Keywords

antigens; antibodies; cancer; central nervous system; immunotherapy

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Malignant primary brain tumors and immunotherapy

Malignant primary brain tumors are the most frequent cause of cancer death in children,[1] are more common than Hodgkin lymphoma, ovarian and testicular cancer and are responsible for more deaths than malignant melanoma.[2] Despite aggressive, image-guided tumor resection;[3] high-dose external beam radiotherapy[4] or brachytherapy;[5] optimized chemotherapy[6] and recent advances in anti-angiogenic treatments,[7] patients with glioblastoma (GBM) live less than an average of 15 months from the time of diagnosis.[6, 8] Standard-of-care therapies for malignant gliomas (MGs) fail to eliminate tumor cells specifically and as a result are limited by incapacitating damage to surrounding normal brain and systemic tissues.[9] In contrast, by virtue of exploiting the inherit specificity of the immune system, anti-cancer immunotherapy provides a promising, highly tumor-specific platform for safe and effective therapy. Pivotal approvals by the United States Food and Drug Administration (FDA) for the immune-based cancer therapies sipuleucel-T and ipilimumab, which demonstrate significant survival benefits in patients with hormone-refractory prostate cancer and metastatic melanoma, respectively,[10, 11] have further validated immunotherapy as a viable treatment modality for cancer.

Antibodies as an immunotherapeutic modality for intracerebral malignancy

The exquisite epitope-binding-specificity imparted by monoclonal antibodies (mAbs) provides an ideal platform for precisely targeted immunotherapy. Intrinsic, high-affinity antigen recognition can further be enhanced with affinity maturation techniques, such as *in vitro* directed evolution; this technique has produced antibody-derived single-chain variable fragments (scFvs) with dissociation kinetics slower than the tightly bound streptavidin-biotin complex.[12] Further advances have also allowed for the production of fully human mAbs via phage display technology, transgenic mouse platforms and, more recently, mRNA and ribosome display,[13, 14] drastically reducing the risk of immunogenicity against the drug and increasing clinical safety. Complications associated with murine antibodies previously used in the clinic, including cytokine release syndrome[15, 16] and human anti-mouse antibody (HAMA) formation leading to rapid clearance from patients' serum[17]; unpredictable dose-response relationships[16, 18] and an acute, potentially severe influenza-like syndrome[16, 18, 19, 20], can be entirely averted.

While antibodies are present in the central nervous system (CNS) in physiologic states,[21] glioma-induced changes render lesions particularly susceptible to antibody-based immunotherapy. Glioma tumor cells induce compositional changes in the basal lamina and astrocytic components of the neurovascular unit (NVU), disrupting the integrity of the blood-brain barrier (BBB). In addition to increasing tumor burden and heightening tumor invasion of the surrounding parenchyma,[22] this allows for enhanced penetrance of large soluble molecules, such as antibodies, from the vascular compartment. For the treatment of GBM, several studies have demonstrated that intravenously (IV) administered antibodies gain access to intracranial (IC) tumors and exert significant therapeutic benefit.[23, 24, 25, 26] In murine GBM models, the antitenascin antibody (81C6) directed against a component of the tumor stroma showed significant localization and therapeutic activity following systemic administration,[23, 24] and in clinical trials, IV administration of radiolabeled 81C6 showed

selective tumor localization.[26] The antibody also accumulated in other tissues expressing high levels of tenascin, including the spleen, bone marrow and liver. As a further example, clinical evaluation of an antibody directed against the entirely tumor-specific mutation of the epidermal growth factor receptor (EGFRvIII), demonstrated higher levels of brain-tumor-specific uptake following IV administration,[25] suggesting that in the absence of cross reactivity with peripherally located epitopes, such as that seen with tenascin, an antibody sink created by the exclusive expression of the target epitope within the CNS may result in enhanced antibody localization to the CNS.

Tumor-specific targets and EGFRvIII

The vast majority of proteins found on the surface of tumor cells are also expressed on normal healthy tissue. While overexpression of specific surface antigens is characteristic of various tumors, most often these antigens are tumor-associated antigens also expressed on the surface of healthy cells. Targeting such tumor-associated antigens via immunotherapeutic methods holds great risk for autoimmunity and thereby undermines the specificity imparted by immunotherapeutic approaches. Tumor-specific antigens, however, occur as a result of mutations in somatic genes and, when targeted therapeutically, are far less likely to be associated with autoimmunity. Most tumor-specific antigens occur randomly due to the genetic instability inherent to human cancers[27] and as a consequence are patient specific.

EGFRvIII, however, is a frequent and consistent tumor-specific mutation seen in approximately 31 to 50% of patients with GBM[28, 29, 30, 31, 32, 33, 34, 35] and in a broad array of other cancers.[33, 36, 37, 38, 39, 40, 41] Among patients with EGFRvIII-positive GBM, 37 to 86% of tumor cells express the mutated receptor,[34] indicating that the mutation is translated with significant consistency. The mutation consists of an in-frame deletion of 801 base pairs in the extracellular portion of the wild-type receptor, generating a novel glycine residue at the fusion junction.[42, 43] This produces a highly immunogenic, cell-surface, tumor-specific epitope.[44] Importantly, antibodies directed against EGFRvIII are entirely tumor-specific and do not cross react with the wild-type receptor located on untransformed, healthy cells.[44]

The mutated receptor plays a significant role in tumor pathobiology. EGFRvIII encodes for a constitutively active tyrosine kinase receptor[45, 46] that enhances tumor cell growth[45, 47, 48] and invasion[49, 50] while conferring radiation[51] and chemotherapeutic[52, 53] resistance. Among patients with GBM, expression of EGFRvIII is an independent, negative prognostic indicator.[54] EGFRvIII also enhances the growth of neighboring EGFRvIII-negative tumor cells via cytokine-mediated paracrine signaling[55] and by transferring a functionally active oncogenic receptor to EGFRvIII-negative cells through the release of lipid-raft related microvesicles.[56] Recent research has also found that EGFRvIII is expressed in glioma stem cells (GSC),[57, 58] an important consideration given the paradigm that tumor stem cells (TSCs) represent a subpopulation of cells that give rise to all differentiated tumor cells.[59] Altogether, the specificity, high frequency of surface expression and oncogenicity of the EGFRvIII mutation make it an ideal target for antibody-based immunotherapy.

Bispecific antibody redirected immunotherapy

MG lesions are characteristically heavily infiltrated with T cells,[60, 61] and substantial evidence suggests that, if appropriately redirected, T cells, and in particular, cytotoxic T lymphocytes (CTLs), have the ability to eradicate large, well-established tumors.[62] Although functionally effective, many T cell based therapies have prohibitive limitations. T cells genetically engineered to target tumor antigens, for example, are effective, but limited due to the fact that they rely on heavily-trained laboratory personnel to produce individual, patient-specific vaccines. Furthermore, they require viral transduction, there is a lack knowledge regarding the ideal T-cell phenotype needed, and are difficult to control once infused. Alternatively, activating T cells *in vivo* by single arm agonistic mAbs poses the risk of autoimmunity as a result of global ligation of circulating T cells throughout the body.[63] In direct contrast, bispecific antibody constructs are highly safe and effective by virtue of being able to activate T cells only in proximity to tumor cells expressing a target antigen. Due to their relative ease of manufacture, highly-specific nature and localized mode-of-action, bispecific antibodies overcome many of the limitations associated with the alternative T-cell based therapies described above.

Bispecific antibodies termed bispecific T cell engagers (BiTEs) are monomeric proteins consisting of two antibody-derived, scFvs translated in tandem.[64] These constructs possess one effector-binding arm specific for the epsilon subunit of T-cell CD3 and an opposing target-binding arm directed against an antigen that is expressed on the surface of tumor cells (e.g., EGFRvIII)[64] (Figure 1). This divalent design allows BiTEs to create a molecular tether, resulting in highly-localized and specific T cell activation with concomitant tumor lysis. BiTEs induce immunological synapses between T cells and tumor cells that are indistinguishable in composition, size and subdomain arrangement from native synapses.[65] Following BiTE-mediated synapse formation, T cells proliferate, secrete pro-inflammatory Th1-type cytokines and express surface activation markers[66]. BiTEs are capable of mediating serial rounds of killing[67] and can trigger specific lysis from naïve T cells at exceedingly low concentrations and effector-to-target ratios.[68] BiTEs are also capable of co-opting immunosuppressive regulatory T cells (T_{Regs}), a subset of $CD4^+$ T cells that ordinarily suppress and kill CTLs, redirecting T_{Regs} to efficiently lyse tumor cells.[69, 70] By tethering cytotoxic effectors to target cells without the need for antigen presentation via the major histocompatibility complex (MHC), BiTEs can furthermore overcome tumor immune escape mechanisms, such as the downregulation of MHC.[65]

In a phase I clinical trial among patients with non-Hodgkin lymphoma, 7 of 7 patients receiving CD19-targeted BiTE doses as low as 0.06 mg/m^2/day over a one month continuous infusion period showed objective tumor regression as well as clearance of tumor from the blood, bone marrow and liver.[71] This dose produced serum levels 5-fold lower than effective doses of the CD19-specific antibody rituximab, currently used clinically as standard-of-care therapy.[72] Importantly, no dose-limiting cytokine release syndrome was evident; however, treatment led to the expected depletion of normal CD19-expressing B cells. Thus, a significant limitation of this promising therapeutic platform is the lack of tumor-specific targets.

For the treatment of MG, a recently developed EGFRvIII-CD3 BiTE overcomes this limitation.[73] By retargeting T cells against the entirely tumor specific EGFRvIII antigen, adverse effects associated with lysis of healthy tissue are averted. Pre-clinical assessment of this therapeutic demonstrates the ability to induce polyclonal T-cell proliferation and interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) secretion, exclusively in the presence of EGFRvIII-positive glioma.[74] Specificity was also confirmed in standard *in vitro* cytotoxicity assays where the construct mediated significant tumor-specific lysis of EGFRvIII-positive glioma but did not result in cytotoxicity of EGFRvIII-negative glioma. *In vivo*, as few as 5 daily IV doses of EGFRvIII-targeted BiTE produced complete cures in IC tumor bearing murine models reconstituted with unstimulated human lymphocytes, and treatment of even late-stage disease in moribund mice significantly extended survival ($p<.01$). Interestingly, the EGFRvIII-specific BiTE potently subverted highly purified $CD4^+CD25^+FoxP3^+$ T_{Regs} to induce granzyme-mediated anti-tumor cytotoxicity,[69] suggesting that BiTEs possess the unique ability to overcome mechanisms of intratumoral immunosuppression. Translation of this modality for the safe and effective care of patients with MG awaits clinical trial.

Immunomodulatory targets

In addition to redirecting T cells to target tumor specific antigens, mAbs can be used as immunomodulatory agents to influence the biology of the tumor immune response. This approach is based on the premise that cancer-bearing hosts have endogenous T cells specific for tumor antigens, but that the activity of these cells is suboptimal, due to cancer-associated immunosuppression known to be particularly pronounced in glioma patients.[60, 75, 76, 77] To overcome this, mAbs can be engineered to possess specificity for surface receptors important in regulating the immune response.

Such immunomodulatory antibodies can serve as either blocking antibodies to prevent binding of the receptor with its endogenous ligand or as agonist antibodies to mimic the role of the receptor's endogenous ligand. In general, blocking antibodies are engineered to target receptors with an immunosuppressive role (e.g., CTLA-4, PD-1), thus preventing inhibition of the T cell response, while agonist antibodies target receptors with a costimulatory role (e.g., CD28, 4-1BB, OX40), thus enhancing immune effector cell activation.

Blocking tumor-mediated immunosuppression with mAbs

The immune system exhibits a number of regulatory strategies to prevent dangerous hyperactivity or autoimmunity. Specifically, T cells express inhibitory receptors on their surface that when activated serve to reduce their response to antigens. These T cell inhibitory pathways are often over-engaged in patients with glioma, due to expression of inhibitory ligands by the tumor[78] and a systemic increase in T_{Regs}.[79] Furthermore, secretion of immunosuppressive cytokines (such as TGF-β or IL-10) by tumor cells[80, 81, 82] causes decreased activity of T cells in the tumor microenvironment and promotes development of a T_{Reg} phenotype by naïve $CD4^+$ T cells. There are a number of strategies currently under investigation that utilize mAbs to curtail these immunosuppressive signals (Figure 2). The use of immunomodulatory mAbs that bind to and block the signaling of inhibitory molecules

expressed on effector T cells and T_{Regs} shows significant clinical promise. Furthermore, mAbs that bind to and block the activity of inhibitory ligands and immunosuppressive cytokines expressed by tumor cells offer the potential to combat immunosuppression directly in the tumor microenvironment.

Perhaps the most promising data in the use of immunomodulatory mAb therapies is observed in clinical trials for PD-1 and CTLA-4 blockade. PD-1 is expressed on activated T-cells and upregulated upon prolonged antigen stimulation.[83] Binding by its ligand, PD-L1, leads to decreased T cell receptor (TCR) signaling as well as downregulation of anti-apoptotic molecules and pro-inflammatory cytokines.[83] Tumor cells, including gliomas, exhibit increased surface expression of PD-L1 and thus shutdown T-cell activity in the tumor microenvironment.[78, 84] Indeed, expression of PD-1L by glioma cells and upregulation of PD-1 on peripheral T cells in glioma patients is associated with immune paralysis[78, 85] and correlates with disease progression.[86, 87] In a model of PD-L1 expressing murine glioma, Zeng *et al.*[88] showed that PD-1 mAb blockade combined with focal radiation lead to an increase in median survival from 30 to 52 days and a 15–40% frequency of long-term survival which corresponded to an increase in the intratumoral effector T cell to T_{Reg} ratio.

Similar to PD-1, binding of CTLA-4 on T cells results in an abrogated immune response. CTLA-4 is expressed on primed T cells and competes with CD28 for binding of B7.1/2 on antigen presenting cells (APCs), thus reducing T cell activation and instead leading to T cell tolerance to antigen.[89, 90, 91, 92] Polymorphisms in CTLA-4 that alter gene expression and increase CTLA-4-mediated downregulation of T cell activity are correlated with increased susceptibility to several malignancies,[93, 94, 95, 96] including gliomas.[97] Furthermore, decreased expression of CTLA-4 on peripheral blood T cells after dendritic cell (DC) vaccination correlates with longer survival in GBM patients.[98] An anti-CTLA-4 mAb (ipilimumab) provides a significant survival benefit in patients with metastatic melanoma.[10] In the context of glioma, CTLA-4 mAb blockade elicited an 80% long-term survival against murine SMA-560 glioma, which corresponded to a reestablished $CD4^+$ T cell compartment following glioma-mediated lymphopenia.[99] Additionally, CTLA-4 blockade following tumor-lysate vaccination resulted in 40% long-term survival and an enhanced antitumor immune response,[100] thus implicating the potential of CTLA-4 blockade to adjuvant existing tumor vaccines. Given the potent ability for CTLA-4 and PD-1 blockade to enhance the antitumor T cell response, a combined anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) phase II trial for treatment of recurrent GBM is currently underway (NCT02017717).

In addition to its role in counteracting the CD28 costimulatory pathway in effector T cells, CTLA-4 is constitutively expressed on T_{Regs},[101, 102] and CTLA-4 signaling in T_{Regs} enhances their immunosuppressive function.[103] Glioma patients have an increased fraction of systemic T_{Regs}, which corresponds to decreased T cell effector activity and a shift from pro-inflammatory Th1 cytokines to an anti-inflammatory Th2 milieu.[79] In addition to an increase in systemic T_{Regs}, intratumoral T_{Reg} numbers increase profoundly in low and high grade astrocytomas.[79, 104, 105] A number of glioma immunotherapy strategies are thus being explored to directly and specifically block T_{Reg} activity with mAbs, including CTLA-4

blockade. Vom Berg *et al*. showed that CTLA-4 expression increases on T_{Regs} in glioma-bearing mice[106] and that intratumoral CTLA-4 mAb blockade, combined with intratumoral IL-12, leads to enhanced survival compared to either therapy alone. This prolonged survival was accompanied by an intratumoral shift from a high population of $FoxP3^+$ T_{Reg} cells to a high population of pro-inflammatory IFN-γ-producing $CD4^+$ cells.

Antibodies against CD25, a subunit of the IL-2 receptor present on the surface of T_{Regs}, also shows promise. El Andaloussi *et al.* showed that anti-CD25 mAb blockade leads to a decrease in the intratrumoral T_{Reg} compartment from 46% to 6%, corresponding to an increase in median survival from 27 to 40 days in mice harboring IC glial (GL261) tumors.[107] Further studies confirmed that CD25 blocking mAb leads to prolonged survival in glioma-bearing mice, and that CD25 blockade works not only by reducing T_{Reg} numbers, but also by inhibiting the suppressive activity of the remaining T_{Reg} compartment.[79] Of note, combined IC and systemic administration of anti-CD25 lead to complete survival in GL261-bearing mice, compared to a lower cure rate of 40% in mice receiving only systemic anti-CD25,[108] suggesting that direct targeting of intratumoral T_{Regs} may be integral for optimal antitumor efficacy. CD25 blockade also synergizes with CTLA-4 blockade, reducing T_{Reg} numbers and prolonging survival in glioma-bearing mice.[109] The enhanced immune response observed upon combination therapy was tumor-specific, sparing surrounding areas of healthy, eloquent brain tissue.

Importantly, the proportion of systemic functional T_{Regs} increases significantly following standard-of-care temozolomide (TMZ) therapy,[110] a treatment known to provide a significant survival benefit among patients with newly diagnosed GMB.[6, 8] When combined with TMZ, however, mAb mediated CD25 blockade curbs the increased T_{Reg} fraction, resulting in enhanced antitumor immune responses in mice and humans.[111] Immunotherapeutic approaches for the treatment of MG can also result in an increase in the proportion of functional T_{Regs}. For example, administration of an RNA-loaded DC vaccine against murine glioma was shown to increase the splenic T_{Reg} compartment by 12%, but T_{Reg} depletion via an anti-CD25 mAb in combination with the DC vaccine lead to increased antitumor efficacy compared to vaccine alone.[112] Clinical trials have likewise demonstrated that the addition of a humanized anti-CD25 mAb (daclizumab) to immunotherapeutic platforms for the treatment of GBM leads to a decreased proportion of T_{Regs} and more effective outcomes. Daclizumab given 3 weeks after the first TMZ cycle, concomitant with adoptive transfer of naïve lymphocytes and a pp65 DC vaccine for example, leads to sustained T_{Reg} depletion and an increase in antigen-specific T cells with progression free survival exceeding 24 months in 4 of 6 patients.[111] Additionally, daclizumab combined with EGFRvIII peptide vaccination[113] was well tolerated in a randomized pilot study and resulted in a significant decrease in the peripheral T_{Reg} compartment.[114]

Alternative strategies to combat the potent immunosuppression observed in patients with MG attempt to modify the immunosuppressive climate of the tumor microenvironment. To this end, TGF-β has surfaced as a promising target for mAb blockade. TGF-β, secreted by glioma cells and microglia, promotes tumor growth via enhancing tumor cell migration and angiogenesis.[115, 116] TGF-β furthermore contributes to the immunosuppressive tumor microenvironment by inducing a T_{Reg} phenotype in naïve $CD4^+$ cells[117, 118, 119] and

suppresses cytotoxic immune responses.[82, 120, 121] In preclinical studies, TGF-β blockade with a murine mAb, 1D11, increased antitumor efficacy of a glioma-associated-antigen (GAA) vaccine, and lead to increased infiltration and persistence of anti-GAA CTLs, decreased the proportion of T_{Regs}, and shifted the intratumoral cytokine production to that of a pro-inflammatory Th1 profile.[122] 1D11 administration was additionally shown to reduce infiltration and invasion of glioma cells into normal brain tissue.[123] A humanized TGF-β mAb (fresolimumab) currently under clinical investigation for breast cancer, mesothelioma and melanoma has likewise shown promising results.[124] The use of fresolimumab among patients with MG is currently under investigation in a phase II clinical trial (NCT01472731).

Given the pre-clinical and clinical data that continues to accumulate, it is evident that abrogating glioma-mediated immunosuppression will undoubtedly be a critical component of therapeutic intervention among patients with MG. Given their exquisite target binding specificity, mAbs are particularly well suited for this role. As we continue to uncover details of the complex relationship between IC malignancies and systemic immunosuppression, new therapeutic targets will likely surface.

Agonist mAbs for T cell costimulatory pathways

Optimal activation of naïve T cells requires both a strong interaction between the TCR and peptide-bound MHC, as well as interaction with costimulatory ligands expressed by APCs. Costimulation in the presence of TCR engagement results in increased proliferation, cytokine production and enhanced survival (for a review on T cell costimulatory pathways, see reference[125]). During a functional immune response, costimulatory ligands are upregulated on activated APCs and displayed during antigen-presentation to T cells.[126, 127] However, APC costimulatory activity is often abrogated in cancer-bearing hosts, which can result in T cell apoptosis or anergy to tumor antigens.[128, 129] Agonist mAbs for costimulatory receptors on T cells offer a promising strategy for overcoming aberrant APC activity and ensuring delivery of the vital costimulatory signal (Figure 3).

Many costimulatory pathways have been described over the past 30 years, with the highly characterized CD28 signal canonically considered to be the most potent and necessary for a functional T cell response.[130] The early therapeutic promise of enhancing this costimulatory pathway was observed in clinical studies evaluating the efficacy of adding exogenously delivered CD28 signals to tumor vaccine platforms.[131, 132, 133, 134, 135, 136] The results of such studies lead to the development of a CD28 superagonist mAb (TGN1412), capable of fully activating T cells via the CD28 receptor in the absence of TCR and peptide bound MHC interaction, and its evaluation as a therapy for B cell lymphoma. All patients enrolled in the first phase I trial of TGN1412, however, suffered severe and life-threatening adverse effects due to CD28-mediated cytokine storm.[63] While this finding has curtailed further evaluation of CD28 mAbs, given the promise of earlier studies implicating the role of costimulatory signals as potential tumor vaccine adjuvants, mAbs targeting alternative costimulatory pathways are cautiously being pursued.

In the context of immunotherapy for MG, the evaluation of costimulatory mAbs is still in its infancy. Such agonist antibodies, however, demonstrate great promise in clinical trials for

the treatment of other cancers and may prove especially useful in the treatment of MG. Targeting costimulatory members of the tumor necrosis factor receptor (TNFR) superfamily specifically upregulated on activated T cells, OX40 on $CD4^+$ cells[137, 138] and 4-1BB on $CD8^+$ cells[139, 140] has proven particularly effective in pre-clinical glioma models. The use of mAbs for OX40[141] and 4-1BB[142] as monotherapies for murine glioma resulted in a 50% cure rate and increased median survival from 31 days to 42 days, respectively. In a separate study, addition of an anti-4-1BB antibody enhanced the antitumor activity of T cells primed with glioma-lysate pulsed DCs.[143] In a recent study by Murphy *et al.*,[144] four costimulatory targets were compared as treatments for murine glioma. The ligands for OX40, CD28, 4-1BB, and glucocorticoid-induced tumor necrosis factor receptor (GITR) were fused to the Fc portion of human Ig to generate surrogate agonist antibodies, and these agonist treatments were combined with tumor lysate vaccines. The authors found that the OX40L-Fc/lysate combined treatment had the highest efficacy (70% cure rates) compared to the other three fusion proteins, which yielded no enhanced survival. The efficacy in targeting OX40 and 4-1BB is likely due to their role in augmenting the tumoricidal activity of activated tumor-specific T cells. Interestingly, given these data, it appears that targeting costimulatory pathways specific to either $CD4^+$ or $CD8^+$ T cells may yield an enhanced antitumor response. Strikingly, the addition of standard-of-care TMZ therapy to the OX40LFc/lysate vaccine regiment resulted in 100% tumor regression.[144] Similarly, the antitumor effect of the 4-1BB agonist was significantly enhanced by the addition of radiotherapy (median survival was increased to 114 days).[142] TMZ and radiation are presumed to enhance tumor antigen presentation via upregulation of tumor MHC expression[145, 146] and shedding of antigens by dead tumor cells;[147, 148, 149] this putative increase in antigen presentation would provide one of the two necessary signals for an immune response, while the agonist mAb ensures delivery of the second signal.

Indeed, given promising preclinical findings,[150, 151, 152, 153] there are a number of agonist OX40 and 4-1BB mAbs currently in clinical development. Two clinical trials were recently initiated to investigate anti-OX40 mAb therapy in combination with standard-of-care chemotherapy and radiation therapy in prostate and breast cancer patients (NCT01303705; NCT01862900). A humanized anti-4-1BB mAb, BMS-663513, was tested in a phase I study in patients with advanced melanoma and yielded partial responses in 3 out 4 patients, as well as an increased percentage of activated $CD8^+$ T cells in peripheral blood.[154] Another humanized 4-1BB agonist is currently in a clinical trial as a single agent in patients with solid tumors (NCT01307267). It will be of interest to evaluate these mAbs as treatments for human gliomas.

There are a number of other costimulatory molecules for which agonist mAbs have yet to be thoroughly evaluated in the setting of MG, but that have shown promise in other tumor types, including GITR, CD27 and CD40, additional members of the TNFR superfamily. Similar to OX40 and 4-1BB, GITR is expressed on activated T cells.[155] Anti-GITR agonist mAbs have been shown to induce rejection of several murine syngeneic tumors,[156, 157] but like OX40, GITR agonism may proceed in a CD4-mediated fashion,[158] a facet that is not yet clearly understood in the context of glioma immunotherapy. Indeed, an anti-human GITR

mAb, TRX518, is currently in a phase I trial for advanced stage melanoma and other solid tumors, including CNS malignancies (NCT01239134).

Unlike OX40, 4-1BB and GITR, CD27 is expressed on both naïve and activated T cells,[159] suggesting its role in T cell priming early in the immune response. Keller *et al.*[160] showed that constitutive expression of the CD27 ligand (CD70) on DCs enabled T cell priming in the absence of any adjuvant, suggesting that this costimulatory pathway is perhaps one of the more critical ones for adaptive immunity. In the context of GBM, Miller *et al.*[161] have demonstrated that intratumoral soluble CD70 significantly prolonged survival of tumor-bearing mice in a CD8-dependent fashion. Furthermore, a CD27 agonist mAb was reported to have efficacy as a monotherapy in a variety of murine tumor models[162] and is now in early clinical trials for the treatment of hematologic malignancies and solid tumors (NCT01460134). Our group is currently investigating CD27 agonist mAb therapy for the treatment of MG; we have observed promising results in preclinical murine glioma models.

In addition to providing costimulatory signals directly to T cells, promoting activation of APCs through the use of agonist mAbs for CD40 is yet another attractive strategy for enhancing the antitumor immune response. CD40 is constitutively expressed by immature DCs, and its ligation by CD40L results in DC maturation and upregulation of costimulatory ligands, making it crucial for functional antigen presentation.[163, 164] Indeed, CD40 agonist mAbs have anti-tumor effects in a variety of murine tumor models.[165, 166, 167] Interestingly, CD40 stimulation in gliomas was found to directly inhibit cell proliferation through NFκB signaling and TNFα production.[168] These studies make mAb targeting of CD40 a promising strategy from both an immunomodulatory perspective and as a direct cancer therapeutic. An anti-CD40 mAb, CP-870,893, has shown anti-tumor activity mediated by CD40-expressing macrophages; a clinical trial evaluating the effect of CP-870,893 in combination with anti-CTLA-4 (tremilumumab) in melanoma patients is currently underway (NCT01103635).

Discussion

Due to their inherent capacity to be engineered for specificity against nearly any biological target, antibodies provide a particularly promising immunotherapeutic modality for MG. Capable of being formulated as an off-the-shelf therapeutic, antibodies furthermore offer a distinct advantage over many other forms of anti-cancer immunotherapy that require costly preparation of patient-specific vaccines. Three general approaches for antibody based immunotherapy for MG have emerged: 1) redirecting immune effector cells against tumor antigens to mediate tumor cell death, through bispecific antibodies such as BiTEs directed against tumor specific mutations for example, 2) blocking immunosuppressive signals by targeting of surface molecules on immune cells or soluble mediators, and 3) and enhancing immune effector cell activity via antibodies agonistic for co-stimulatory receptors. Preclinical and clinical studies in MG have brought validation to each of these approaches.

Bispecific antibody based therapy against the tumor specific antigen EGFRvIII elicits a highly potent and specific tumoricidal response and clinical trials investigating bispecific antibody based therapy[71, 169, 170] demonstrate safety and efficacy against forms of cancer highly refractory to conventional therapy. This approach is limited only by the paucity of

glioma-specific targets. As more glioma-specific targets, such as EGFRvIII, are uncovered there will be additional opportunity for therapeutic intervention. Still, we have seen that that in addition to eradicating well-established EGFRvIII-positive GBM, EGFRvIII-specific antibody-redirected T cells produce long-lasting immunity against EGFRvIII-negative tumor cells without evidence of autoimmune toxicity,[171] suggesting that this approach is superior to EGFRvIII-targeted vaccines that are limited by antigen escape,[172] and thus warranting further investigation through clinical trial.

Using mAbs to modulate the biology of the anti-tumor immune response likewise holds promise for the treatment of MG. This approach has proven safe and effective in the treatment of a variety of different solid tumors. In particular, combined blockade of the immunosuppressive CTLA-4 and PD-1 pathways has garnered much attention recently given the success in clinical trials for the treatment of advanced melanoma;[173, 174, 175] data generated from the ongoing clinical study of such combination therapy for MG is awaited. Antibody based CD25 blockade will also potentially play a significant role in immunotherapeutic intervention for MG, given its ability to decrease the number and activity of T_{Regs}, a highly immunosuppressive cell fraction that increases with standard-of-care TMZ therapy for MG.[110] Several co-stimulatory molecules on the surface of immune effector cells are also attractive targets for the reversal of immunosuppression and antibody based therapy for MG. Indeed, clinical trials investigating this approach in several forms of solid tumors are underway and pre-clinical data in the context of therapy for MG demonstrate encouraging results. The availability of transgenic animal models expressing functional human co-stimulatory receptors will be critical, however, in order to allow for comprehensive pre-clinical assessment of safety in fully immunocompetent, pharmacologically responsive animals.

It is evident that given the profound immunosuppression among patients with MG, a multitude of immunosuppressive pathways are amenable to antibody based blockade or activation. Large-scale clinical trials are necessary in order to ascertain which therapeutic targets provide the safest and most effective anti-tumor immunotherapy for patients with MG. It is likely that optimal therapy for MG may be obtained via synergistic immunotherapeutic combinations. There is also great potential for personalized therapy, tailored to target patient specific tumor mutations (ex. EGFRvIII-specific BiTE) and to reverse the immunosuppressive factors most prevalent for a given patient.

Despite advances in the standard of care for MG, the survival statistics among patients remains dismal. Antibody-based immunotherapy, however, by virtue of allowing for tumor cell specific lysis, permits for therapy aggressive and prolonged enough to safely eliminate all malignant cells while sparing eloquent, healthy CNS and peripheral tissue. Continued clinical investigation is necessary in order to avail antibody based immunotherapeutic options to patients with MG.

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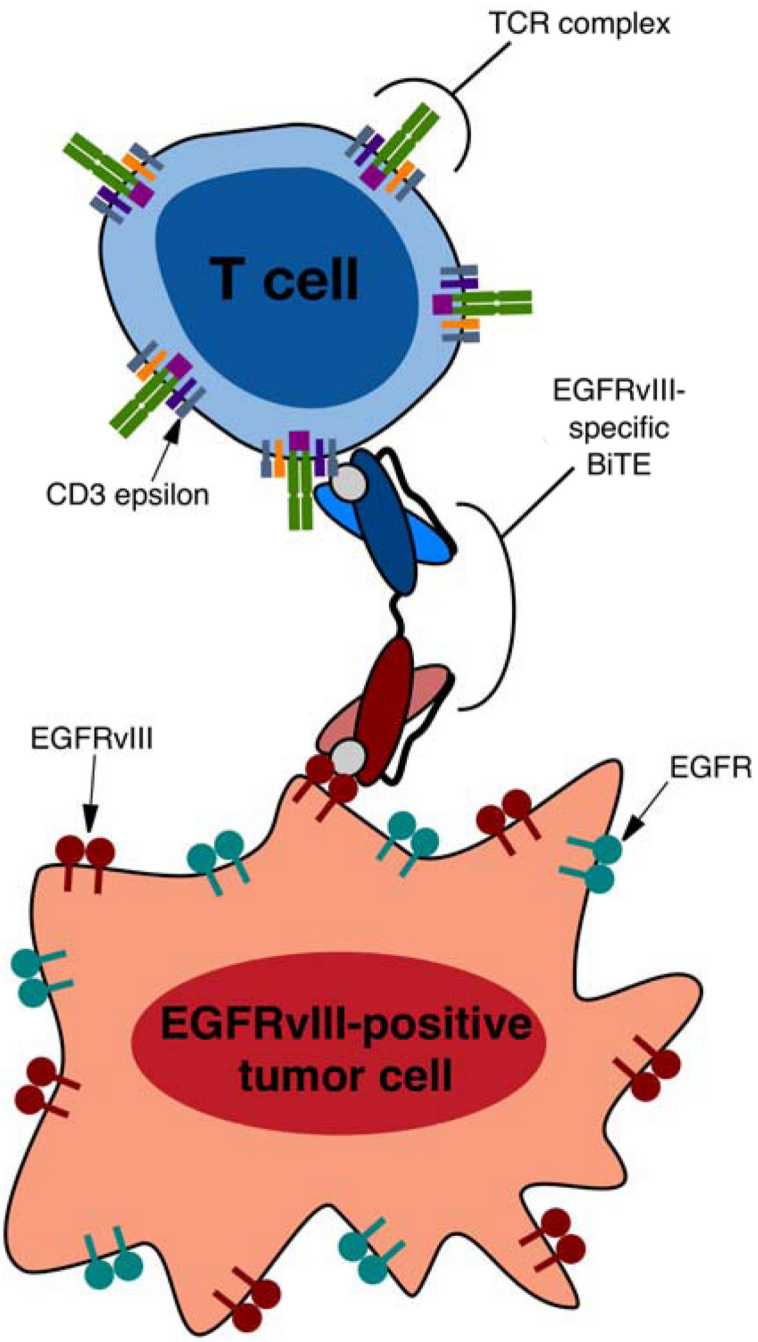

Figure 1.

A schematic representing EGFRvIII-specific BiTE creating an immunologic synapse by binding to a tumor cell via the tumor specific antigen EGFRvIII and a T cell via CD3-epsilon. Note that the EGFRvIII binding portion does not bind to the wild-type EGFR, thus mediating tumor cell specific targeting.

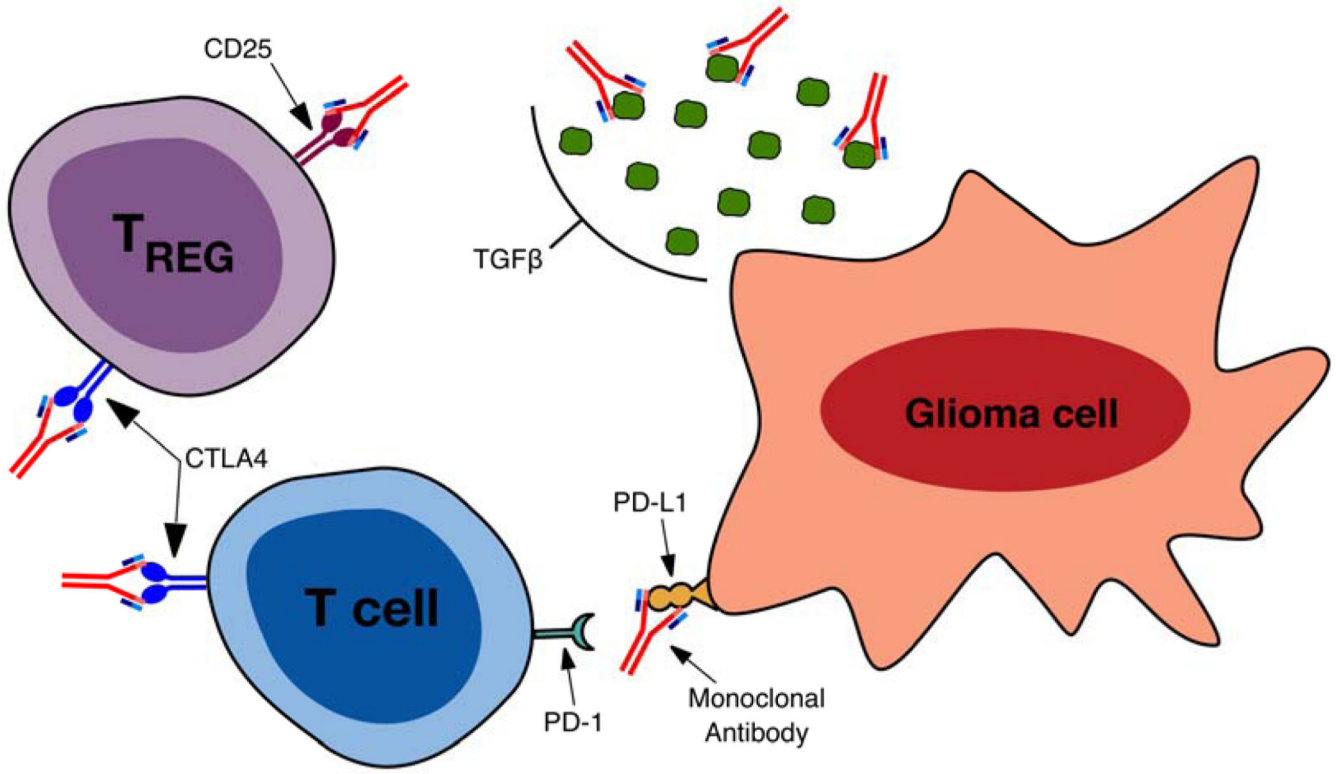

Figure 2.

A depiction of strategies under clinical investigation to enhance immune mediated tumor cell rejection via mAb mediated blockade of immunosuppressive signals. CTLA-4 blockade inhibits signaling via the receptor, blocking the induction of effector T cell tolerance to tumor antigens and reducing the immunosuppressive function of T_{Regs}. CD25 blockade inhibits the development of the T_{Reg} linage, resulting in a reduction in the maintenance and survival of $T^{Regs.}$ mAb binding to TGF-β reduces the level of extracellular protein available to act on receptors, diminishing TGF-β induced tumor cell migration and angiogenesis, inhibition of cytotoxic T lymphocyte (CTL) generation and the generation of a T_{Reg} phenotype in naïve $CD4^+$ T cells. Blockade of interaction of PD-1 with its ligand increases T cell receptor (TCR) signaling, up-regulates expression of pro-inflammatory cytokines and reduces T cell apoptosis.

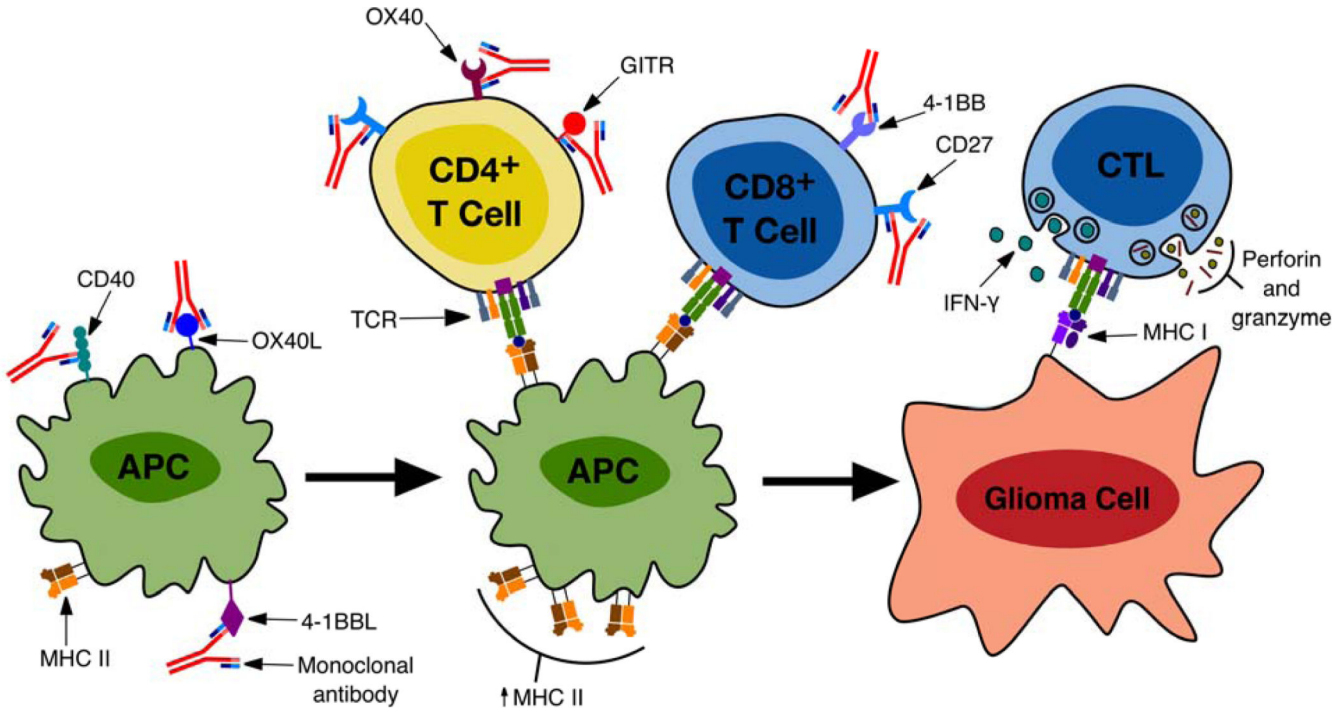

Figure 3.

A summary of stages of the immune response against tumor cells where mAbs can be used to enhance glioma cell specific lysis. mAbs agonistic for CD40, OX40L and 4-1BBL present on antigen presenting cells (APCs) result in an increase in pro-inflammatory cytokine production and MHC expression on APCs. CD27 stimulation enhances proliferation and survival of effector and memory T cells, increases IL-2 production and increases the secretion of effector cytokines. mAb mediated stimulation of OX40 results in clonal expansion and survival of effector T cells, activation and survival of memory T cells, blocks the activity of T_{Regs} and antagonizes the generation of inducible T_{Regs}. mAbs agonistic for 4-1BB enhance the survival of activated and memory T cells, maintain effector function during prolonged antigen stimulation and increase tumoricidal activity. mAbs agonistic for GITR likewise increase the tumoricidal immune response. These pathways converge with the generation of cytotoxic T lymphocytes (CTLs) which upon interaction with glioma cells mediate tumor cell lysis via the release of interferon-gamma (IFNγ) and perforin and granzyme.