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Immunotherapy for malignant glioma

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Abstract

Malignant gliomas (MG) are the most common type of primary malignant brain tumor. Most patients diagnosed with glioblastoma (GBM), the most common and malignant glial tumor, die within 12–15 months. Moreover, conventional treatment, which includes surgery followed by radiation and chemotherapy, can be highly toxic by causing nonspecific damage to healthy brain and other tissues. The shortcomings of standard-of-care have thus created a stimulus for the development of novel therapies that can target central nervous system (CNS)-based tumors specifically and efficiently, while minimizing off-target collateral damage to normal brain. Immunotherapy represents an investigational avenue with the promise of meeting this need, already having demonstrated its potential against B-cell malignancy and solid tumors in clinical trials. T-cell engineering with tumor-specific chimeric antigen receptors (CARs) is one proven approach that aims to redirect autologous patient T-cells to sites of tumor. This platform has evolved dramatically over the past two decades to include an improved construct design, and these modern CARs have only recently been translated into the clinic for brain tumors. We review here emerging immunotherapeutic platforms for the treatment of MG, focusing on the development and application of a CAR-based strategy against GBM.

Key Words: Adoptive cell transfer, brain tumors, central nervous system, glioma, immunotherapy

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INTRODUCTION

Malignant gliomas (MGs) are the most common type of primary malignant brain tumor in the adult population, comprising up to 80% of all cases.[27,79] MGs are classified by histological subtype and include World Health Organization (WHO) grade III and IV tumors, including the most common and deadly, glioblastoma (GBM, WHO grade IV), which accounts for 82% of all MGs.[27] The current standard of care includes surgical resection, followed by adjuvant external beam radiation and chemotherapy. The invasive properties of GBM, however, make complete resection nearly impossible, and tumors almost always recur following initial treatment. MGs are also recognized as highly vascularized tumors, and their unique capacities for regulating angiogenesis contribute to resistance against combined therapies.[105] Moreover, current treatment options are largely incapacitating

and produce a median overall survival (OS) of just 12–15 months.[109]

An impetus for a novel strategy

Cancer immunotherapy is an attractive alternative that broadly aims to harness and redirect a patient's own immune system to recognize and destroy tumors with an astounding degree of specificity. This is of particular importance for patients suffering with primary or secondary MGs, as conventional therapy is nonspecific by nature and often results in crippling damage to healthy brain tissue.[50,71,108] Gross total resection is typically the goal, but can be precluded when tumors are multifocal (unusual) or reside in eloquent areas, where the repercussions of aggressive surgical intervention may be unacceptable. Additionally, these tumors diffusely infiltrate normal brain by single-cell migration along white matter tracts, and can even cross the corpus callosum,[45] making tumor recurrence nearly inevitable even in those patients who undergo resection. In fact, more than 50% of untreated tumors spread to the contralateral hemisphere,[67] making GBM, in essence, a disease of the entire brain. This is underscored by the failure of hemispherectomy to eradicate disease and prevent recurrence when attempted by Walter Dandy in the 1920s.[25]

Following surgery, patients are considered for adjuvant radiotherapy with concomitant administration of the DNA alkylating agent temozolomide (TMZ), which increases median survival from 12 to 15 months when used in combination with radiotherapy.[109] The intractable course for most patients is death within 2 years from the time of diagnosis, and only with the advent of recent technology has there been an ambitious drive toward shifting the treatment paradigm from palliative to curative. The successes of immunotherapy for other cancers[5,35,90] have intersected with this surging impetus, inspiring the hunt for similar applications against brain tumors. Ultimately, the hope is that the immune system will prove capable of directing its specific and highly robust effector mechanisms against tumors residing in the questionably immunoprivileged brain.[12,100,107,117]

Central nervous system immunoprivilege and the blood–brain barrier

Unlike with hematological cancers and solid tumors of the periphery, the central nervous system (CNS) carries unique considerations that may prove encumbering for immunotherapy. The CNS has long been considered an area of immune privilege, and this concept has been historically supported by the presence of a blood–brain barrier (BBB) and the alleged absence of draining lymphatics and resident antigen presenting cells (APCs) within the brain parenchyma.[15,33,41] The absolutes of CNS immune privilege, however, have been largely dismantled over the past 35 years, with increasing observations of systemic immune response to CNS antigens,[117] which likely travel via defined subarachnoid routes and to the cervical nodes via the nasal mucosa.[23] Moreover, recent observations have identified specialized microglia and astrocytes as functional APCs within the CNS, and activated lymphocytes have been shown to frequently traffic through the brain despite the presence of an intact BBB, and can be retained for long periods of time when engaging their cognate antigen.[3,31,38,46,66,72] This all computes to a growing recognition for an existent physiologic immunosurveillance function within the CNS.

Beyond the capacity for immunosurveillance behind an intact BBB, gliomas may facilitate further routes for immune access, as they have disruptive effects on the BBB. Several reports have shown increased permeability of the BBB in the vicinity of tumors, as well as T-cell infiltrates within gliomas, whose presence/degree can correlate with survival.[99,107] A compromised BBB also makes possible the exodus of tumor-associated antigens into the periphery, which should, in theory, stimulate an endogenous immune response against other tumor antigens to potentiate an immune-based strategy. The invasive character of MGs, however, does facilitate their outgrowth into more distant regions of the brain where the BBB remains intact, placing greater dependence on the shoulders of a universally failed cancer immunosurveillance task. It is widely accepted that these invasive, migratory cells are indeed the culprit behind tumor recurrence in patients following resection and adjunctive targeted therapies.[1,82] Immunotherapy, such as with tumor-directed lymphocytes, may offer the best chance at targeting these otherwise ill-accessible microinvasions, by migrating through the brain parenchyma, proffering antigen-dependent clonal expansion, and mounting immunologic memory responses within the brain.

CNS tumor immune evasion

Beyond questions of access, novel immune-based strategies must also cope with a uniquely immunosuppressive tumor microenvironment surrounding GBMs. These tumors employ particularly varied and potent means for immune subterfuge, and are capable of secreting factors that suppress $CD8^+$ cytotoxic lymphocytes (CTLs), inhibit T-cell proliferation, and inhibit dendritic cell maturation. Similarly, they can downregulate major histocompatibility complex (MHC) expression, possibly evading cell-mediated immunity altogether.[36,43,84,110] Co-stimulatory signals that are necessary for the functional differentiation of $CD8^+$ CTLs are also significantly reduced or absent within the CNS, conferring a possible immune escape "advantage" to brain tumors.[43,47,68,113] This notion is further advanced by evidence that links the loss of the tumor suppressor PTEN in MGs to enhanced expression of coinhibitory molecules, such as B7-H1,

which contributes to glioma immunoresistance.[81] Finally, the presence of $CD4^+$ $CD25^+$ T regulatory cells (T_{REG}) and myeloid-derived suppressor cells (MDSCs) represents perhaps the biggest impediment of all.

T_{REG} represent a subpopulation of T cells that modulate the activity of the immune system, where their principle duties are to maintain self-tolerance and abrogate autoimmunity. As such, they represent a physiologic means for the curtailing of immunity, whose potency proffers opportunities for usurpation by immune-evasive cancers. They have thus been frequently implicated in the progression of cancer,[24,48,64,119] where their accumulation in tumors or peripheral blood leads to inhibition of $CD4^+$ and $CD8^+$ T cells, dendritic cells, natural killer cells, and B cells.[6,14,63,77,114,115] Such T_{REG}-mediated inhibition has been well documented in patients with MGs, where an increased fraction of these cells correlates with a long-observed reduction in cellular immunity.[34] Implications for their increased representation among T cells (both at tumor and systemically) are immunologically compounded by a puzzling context of severe T-cell lymphopenia.[34] The immunosuppressive effects of T_{REG} may be counteracted by strategies that either inactivate or deplete these cells, with applications in glioma being particularly attractive given the above. Although no specific surface marker of T_{REG} has been identified to date, anti-CD25 antibodies have been used to preferentially deplete these cells following short-term therapy.[39,70,97] Eliminating T_{REG} produces enhanced antitumor responses and may likewise explain the synergy between lymphodepletion/tumor irradiation and immunotherapy.[4,116] Somewhat paradoxically, however, recent studies have also revealed that T_{REG} possess potent cytotoxic activity, which under certain circumstances may be directed toward tumor cells.[16,17]

MDSCs, in turn, represent a collection of macrophages, granulocytes, DCs, and other myeloid cells in varied stages of differentiation. In pathological conditions like cancer, a partial block in the differentiation of immature myeloid cells can result in the expansion of this aberrant population. In mice, MDSCs are defined as $Gr\text{-}1^+$ $CD11b^+$ cells, which, upon activation, upregulate expression of immune-suppressive factors like arginase and inducible nitric oxide synthase (iNOS). Arginase depletes available arginine, and iNOS enriches the local concentration of NO, leading to the suppression of NK and T-cell antitumor function, including against MGs.[26,54,104]

Immunotherapeutic platforms

Active immunotherapy

Cancer immunotherapy aims to harness the potency of the immune system to eradicate neoplasms, and to this end, the field broadly encompasses passive immunotherapy, active immunization, and immune-modulation. The earliest indication of a relationship between the immune system and cancer traces back several centuries, to the observation that infectious disease in cancer-bearing patients led to spontaneous tumor regression in several instances.[80] Infections were later then thought to play a role in stimulating immune-surveillance against other potentially harmful "invaders," including neoplastic cells. These early insights would ultimately pave the way for the use of nonspecific immunostimulatory adjuvants that act on APCs and immune effector cells, upregulating key costimulatory molecules and cytokines – such as interferon γ (IFN γ) and interleukin (IL)-12 – to enhance immunologic responses against tumors, including MGs.[72,89] Eventually, strategies progressed to the direct administration of several cytokines, including IL-2 and IL-12. Though promising in their own right, the majority of these nonspecific adjuvants have been used most effectively in conjunction with other therapies, most notably with immunotherapies that aim to solicit antitumor T-cell activity, such as by 'vaccination' or by adoptive transfer of tumor-specific CTLs.

In principle, both active and passive immunotherapies depend on the sensitization of effector lymphocytes against tumor-associated or -specific antigens (TAA or TSA, respectively). Immunizations using tumor cells, proteins, peptides, DNA, dendritic cells, and recombinant viruses have been widely investigated to date, but have yielded disappointing responses in patients across most trials.[89] In fact, in a recent review by Klebanoff *et al.*, an honest appraisal of cancer vaccine trials conducted between 2004 and 2010 found an overall objective response rate of just 3.6% among 936 patients, who varied widely in their tumor and vaccine type.[60]

Despite these guarded results, immunization against MG has recently gained traction based on promising clinical results with a novel tumor-specific vaccine developed by our group at Duke University Medical Center. PEPvIII-KLH (CDX-110) vaccine is a 14-mer injectable peptide chemically conjugated to keyhole limpet hemocyanin (KLH) and targets the type III tumor-specific mutant of the epidermal growth factor receptor, EGFRvIII. In a recent phase II trial, vaccination with PEPvIII-KLH + granulocyte-macrophage colony-stimulating factor (GM-CSF), administered in coordination with TMZ chemotherapy, lengthened median time to progression to 15.2 months and median survival to 23.2 months compared with 6.4 and 15.2 months for historical controls.[44] This promising vaccine recently entered testing in an international, double-blinded, multicenter Phase III clinical trial.

Adoptive cell transfer

Adoptive cell transfer (ACT), most often with lymphocytes (ALT), has emerged as a highly promising, alternative strategy that enables the augmentation

of antigen-specific immunity without the *in vivo* constraints that are often associated with vaccine strategies.[121] ACT involves the selection, *ex vivo* expansion, and passive transfer of lymphocytes (e.g., tumor infiltrating lymphocytes [TILs]) into patients in order to redirect and promote a heightened immune response against target antigens. ACT was first described decades ago, but failed to mediate objective efficacy until lymphodepletive regimens were introduced as a preparative requisite in 2002.[28,89] Lymphopenic environments are believed to favor the clonal expansion of adoptively transferred cells *in vivo*, at least in part by depleting nontumor specific lymphocytes, namely host immunosuppressive T_{REG}, and by reducing competition for homeostatic cytokines.[28,89] To our knowledge, the vast majority of ACT-based therapies in current trials utilize some variation of lymphodepleting preparative regimens (e.g. nonmyeloablative and/or radiation therapy) to enhance the antitumor response of adoptively transferred cells.

Tumor infiltrating lymphocytes

TILs are that subpopulation of lymphocytes (typically T-lymphocytes comprised of $CD4^+$ helper and $CD8^+$ CTLs) that have successfully exited the bloodstream and migrated into tumors, presumably as a function of antitumor-specific trafficking. Although largely tumor-reactive, nascent unmanipulated TILs are often paralyzed in their cytotoxic functionality and proliferative capacity in the context of suppressive tumor microenvironments, frequently thwarted by counterproductive shifts away from Th1 cytokine production and forced over-activation and exhaustion. Often this comes at the hands of tumor-secreted inhibitory substances and direct contact with T_{REG}, which are frequently present at tumor sites in increased numbers. Nevertheless, adoptive strategies to harvest, manipulate, and employ TILs are historically common, given the concentrated source of lymphocytes with tumor specificity. In one strategy, TILs are isolated from tumor biopsies, expanded *ex vivo* in the presence of IL-2, and peripherally infused into patients.

ACT with TILs has been shown to mediate durable complete tumor regression and is one of the most promising treatments available for melanoma today.[91,92,125] The remarkable antitumor responses observed remain a testament to the power and potential of ACT-based immunotherapy. Although TILs represent one of the first cell populations tapped for this approach, TIL-ACT remains largely infeasible for many cancer types. TILs are exceedingly difficult to isolate and tend to vary in number between cancers, or even patients of the same tumor type. TILs also remain vulnerable to MHC downregulation by tumors, making TIL-based ACT both tedious and limited. Alternative versions of the ACT platform have proven superior in both their efficacy and dynamism.

T-cell receptor gene therapy

The ability to genetically modify T cells to recognize TAAs has improved the TIL platform to avoid the difficulties associated with isolating and expanding tumor-specific lymphocytes from tumor biopsies. Instead, peripheral blood lymphocytes (PBLs) can be retrovirally engineered to express T-cell receptors (TCRs) specific for tumor antigens. ACT employing PBL subjected to TCR gene therapy has proven effective against melanoma and other cancers, but like TILs, genetically modified TCRs remain vulnerable to MHC complex downregulation and impaired antigen-presenting capabilities by tumor cells.[73]

Chimeric antigen receptors

One major goal of T-cell engineering is to generate antitumor lymphocytes by the genetic transfer of tumor-specific receptors. Whereas TCR gene therapy depends on the transfer of physiologic, MHC-restricted TCRs, an alternative paradigm has emerged that circumvents MHC requirements. Chimeric antigen receptors (CARs) are fusion proteins that combine the single chain variable fragment (scFv) of naturally occurring monoclonal antibodies (mAbs) with the signaling molecules that act downstream of TCR engagement. By exploiting the MHC-independent, direct antigen specificity of mAbs, CARs can be easily designed to confer upon T cells the new capacity to simply recognize tumor cell surface antigens of interest and link such recognition to triggered T-cell activation, akin to normal TCR mechanisms. CARs' MHC-independence thus circumvents a major mode of tumor immune evasion. Likewise, as CARs can be plugged into autologous lymphocytes of any prior specificity, whole PBL may be harvested as fodder for engineering, obviating limitations of yield. The same then holds true for *ex vivo* expansion, where all engineered cells possess the desired specificity, and expansion against the desired target need not be a means of finding and cloning the proverbial "needle in a haystack."

The first CARs were produced with antigen receptors fused to either the CD3ζ or FcγRI chain, after several studies demonstrated that inclusion of either signaling domain successfully empowered CARs to redirect T cells and initiate cytotoxicity when engaging cognate antigen.[32,52,88] With this demonstration, an impressive array of CARs quickly emerged specific for a variety of native cell-surface antigens, including proteins, carbohydrates, and glycolipids (an additional advantage is the ability to target nonprotein antigens, given fewer such limitations for antibody-derived specificity). First-generation CARs have since been tested in clinical trials in patients suffering with ovarian cancer,[58] renal cell carcinoma,[61] neuroblastoma,[85] non-Hodgkin and mantle cell lymphomas,[112] and refractory follicular lymphoma[53] with limited but promising responses. Although these CARs successfully redirected cellular cytotoxicity, they

could not persist *in vivo*. The limited lifespan and poor expression profile of CARs has been largely attributed to the absence of any costimulatory signals on target tumor cells (e.g. CD80 and CD86), which likely shuttles CAR T cells into an anergic, quiescent state.

These failures fueled the development of CARs that incorporated co-stimulatory endodomains (e.g. CD28,[65] 4-1BB,[49] OX40[86]) that provided the signals necessary for sustained T-cell activation, growth, and survival, without requiring ligand. The clinical evaluation of these 'second-generation' CARs demonstrated that constructs containing dual signaling domains expanded and persisted far longer than CARs that contained only the CD3ζ chain.[98] Second-generation CARs are currently under clinical investigation for several types of cancer, and have already demonstrated remarkable activity for patients with B cell acute lymphoblastic leukemia.[10]

The vastly improved life-span of these CARs inspired groups to question whether the addition of a third signaling domain would further potentiate tumor killing by CAR-engineered lymphocytes. Whereas CD28 signaling is required for the optimal production of IL-2 and cell survival,[40] it is thought to play a lesser role in eliciting T-cell effector functions. This led investigators to consider the incorporation of molecules that would enhance CAR function at these later stages of T-cell activation, such as OX40 (CD134) and 4-1BB (CD137). 4-1BB is a member of the tumor necrosis factor receptor (TNFR) superfamily that is normally expressed by $CD4^+$ and $CD8^+$ T-cells in response to antigen-dependent activation. The signals provided by 4-1BB have been shown to improve $CD8^+$ T-cell proliferation, enhance Th-1 cytokine secretion,[123] amplify cytotoxicity, and inhibit activation-induced cell death (AICD) by upregulation of antiapoptotic genes such as Bcl-xl.[22,102,103,111,123] Third-generation CARs that fused together the intracellular regions of 4-1BB and CD28 with CD3ζ were subsequently designed and are today considered the premier construct for use in CAR therapy. The combination of these three domains has been shown to produce superior antitumor activity when compared with CARs that include one or two of these domains alone.[124] Our group has previously published preclinical data on the use of these third-generation CARs as a highly potent modality against both human and murine MGs.[20,94]

Molecular targets

The success or failure of brain tumor immunotherapy depends on identifying specific antigenic proteins and peptides that are discriminately expressed by tumors and not by normal, healthy tissues. So identified, the remarkably versatile immune system is capable of eliciting an array of innate, humoral, and cellular effector mechanisms that can exquisitely target and eradicate tumors in an antigen-specific manner. There have been several reports to date of severe adverse events and even patient deaths when therapies have been directed against targets also present on normal tissues.[9,75] This is arguably of utmost importance when treating CNS-based cancers, where the normal brain is decidedly intolerant of unintended collateral toxicity. As such, there is growing concern over the induction of potential autoimmunity (e.g. experimental autoimmune encephalomyelitis [EAE] seen in animal models) in the brain as emerging therapies depend on eliciting endogenous immune responses to brain-based tumors.[56] Fortunately, previous studies have identified tumor-specific targets that can be exploited to mitigate this potential for disaster.

Epidermal growth factor variant III

Epidermal growth factor variant III (EGFRvIII) is the type III tumor-specific mutation of the epidermal growth factor receptor that is commonly expressed in gliomas and several other neoplasms, including breast, lung, head, and neck cancers.[37,42,87,106] The mutant protein functions as a constitutively active tyrosine kinase as a result of an in-frame deletion of exons 3-6 from the extracellular domain, bringing distant residues together to form a glycine at the fusion junction.[7,21] EGFRvIII has been shown to be a negative prognostic indicator,[83] to enhance invasiveness[8] and tumorigenicity,[78] and to confer resistance to standard-of-care radio and chemotherapy.[62,106] Importantly, EGFRvIII is commonly expressed on $CD133^+$ brain tumor stem cells,[74] and $EGFRvIII^+$ cells can promote malignant transformation of nearby cells through paracrine signaling of IL-6 family cytokines[51] and through the intercellular transfer of EGFRvIII-positive exosomes.[2] These factors have made EGFRvIII a highly attractive target for novel immunotherapies designed to kill tumors arising *de novo* in the brain.

Several studies have identified highly avid mAbs against EGFRvIII[95,118] and these findings have helped expedite the production of novel therapies with heightened specificity.[18,19] In collaboration with Steve Rosenberg at the National Cancer Institute (NCI), our group has produced EGFRvIII-specific murine and human CAR T cells for preclinical and clinical evaluation. These third-generation (scFv-CD28-4-1BB-CD3ζ) EGFRvIII-specific CARs have proven to be a highly potent treatment modality for brain tumors in both a preclinical human glioma xenograft[20,69] and syngeneic murine model of spontaneous glioma in our laboratory.[94] Similar successes elsewhere have helped precipitate the translation of EGFRvIII-specific CARs into the clinic with the recent approval of a clinical trial for patients with recurrent GBM, which is currently accruing patients (NCT01454596).[74]

Are CARs the answer?

Immunotherapy has evolved dramatically over the past two decades. Among the many strategies put to the test, few have succeeded in producing a robust, long-term response to mediate potent and efficacious tumor-killing in the CNS. Although CAR-based ACT has proven itself as a highly effective strategy for blood-borne and solid cancers, only now is ACT being clinically explored for patients with MGs. This apparent delay in its application for brain tumors is accompanied by serendipitous insights, as previous trials against other cancers have helped mature this strategy for what will hopefully yield potent and specific responses. CARs have already proven their superiority over alternative ACT strategies by circumventing the need of TCR: MHC complex formation, allowing investigators to move CAR-based ACT forward for MGs over TCR or TIL-based strategies. Time has also afforded a dramatic evolution of CAR design to improve versatility, function, and durability *in vivo*. Importantly, CARs of the future may also be conferred the unique potential to offset immune suppression via inclusion of molecules that can selectively inhibit T_{REG} activation or expansion at the site of tumor-recognition.

The enhanced CAR design, in combination with a preparative lymphodepleting regimen, has already shown to produce an impressively robust antitumor and long-term memory phenotype.[57] Moreover, the clinical application of CARs will prove to be a less laborious process compared with competing strategies, as CAR constructs can be used universally between patients bearing the same target antigen. It is important to note, however, that there are potential limitations associated with this strategy, and it remains to be seen if they will become barriers to the clinical success of CARs for patients with MGs. We will discuss here some of the major issues that may arise as CARs continue gaining widespread clinical use.

Target recognition

CAR T-cells depend on recognizing cell-surface molecules, and so are capable of recognizing an array of proteins, sugars, and lipids,[13] but cannot expand their repertoire to include intracellular targets. Unfortunately, this will limit their utility against promising glioma targets such as CMV and IDH-1 mutations. Moreover, as CARs expand in their diversity, newly designed constructs will have to be thoroughly characterized, as their potency may vary based on scFv affinity for target antigen.[76] The use of an unfavorable scFv could, in theory, alter the structural conformation of the chimera and ultimately require modification. These new CARs must also be extensively vetted for immunogenicity, since murine-derived scFv contain conserved mouse regions that could trigger an immune response against engineered T-cells.[93]

'Immunoediting' and antigen escape

The cancer immunosurveillance hypothesis was first put forward by Burnet and Thomas in 1957, in which they proposed the involvement of the immune system in protecting the host from neoplastic disease. This theory would expand over time into a broader description of the immune system's role in relation to cancer, including both its protective and tumor-selective actions within the host. Dunn *et al.* recently proposed the use of 'immunoediting' to describe the three phases in which the immune system exerts its effects on neoplastic cells: (i) Elimination via immunosurveillance, (ii) equilibrium via promotion of select tumor cells, and (iii) tumor escape.[29] These phases are easily applied to and framed in the context of glioma as well.[30] Although immunotherapies aim to target tumorigenic antigens, this inevitably leads to the 'Darwinian selection' of neoplastic variants that do not express the target. The dual actions of protection against and selection of tumor cells present a conundrum that will need to be addressed as immunotherapy is employed against heterogeneous tumors like MGs. Indeed, while the EGFRvIII-specific peptide vaccine PEPvIII-KLH mentioned here previously has been shown to eliminate EGFRvIII-expressing tumor cells in humans, the outgrowth of EGFRvIII-negative antigen loss variants characterized tumor recurrences in the majority (82%) of patients.[96] An ability to circumvent or prevent altogether the selection of antigen-loss escape variants should theoretically help reduce the chances of MG recurrence, which is currently invariable in patients receiving standard of care.[59]

Protective immunity

Although single-antigen targeting should be approached with caution for the reasons described above, our group has recently produced encouraging data that supports the notion of 'protective immunity' against tumor cell variants using the CAR-based platform.[94] We sought to assess whether mice previously treated with EGFRvIII-specific CAR T-cells and cured of an $EGFRvIII^+$ tumor acquired protective immunity against a rechallenge with an $EGFRvIII^-$ tumor. Remarkably, these mice were completely protected, while tumors in control mice quickly reached humane endpoints.

The ability of this T-cell therapy to protect against tumor rechallenge is likely a function of epitope spreading, which can be triggered by an endogenous immune response after an efflux of inflammatory cytokines at sites of tumor. The resulting influx of immune cells into degrading tumor may well lead to immune cell priming against cells that do not express the target antigen. Our group is currently evaluating the role of third-generation CARs in eliciting long-term protection and the relevant mechanisms that might be involved in conferring protective immunity.

An increased understanding of one antigen that may play a central role in eliciting protective immunity is a particular enzyme that has been found to be expressed in GBM and other tumors, but not in any other normal tissues. Isocitrate dehydrogenase-1 (IDH-1) is a key cytosolic Krebs cycle enzyme involved in cellular metabolism, and mutations of this enzyme are consistent and frequent in both low-grade glioma and secondary GBM (>70%).[120] Unlike EGFRvIII mutations, which are present in 30–50% of cells within an EGFRvIII$^+$ tumor, IDH-1 mutations are unilaterally expressed in tumors possessing the mutation (i.e. 100% of cells). An amino acid substitution at position 132 is the most common mutation observed in MGs, where the replacement of an arginine with a histidine affects one allele and results in a marked decrease in wild-type IDH-1 activity by forming an inert heterodimer.[122] The result is an error in Krebs cycle metabolism with the production of a "dead-end" metabolite, 2-hydroxyglutarate. The 132 mutation has likewise been shown to lead to increased oxidative stress and the rapid accumulation of hypoxia-inducible factor 1 alpha (HIF-1α) in MG cells, which may be involved in eliciting malignant transformation by the promotion of angiogenesis and cell survival.[101] Alternatively, the resultant errors in cell metabolism may explain why patients with IDH-1 mutant tumors have markedly better prognoses than those with wild-type tumors. In either event, given that the IDH-1 mutation is limited solely to tumor tissue and are uniformly expressed by tumors when present, it may represent a key mediator in tumor-specific protective immune responses following primary targeting of a surface antigen.

Lastly, an alternative strategy currently being employed by some groups is the design of bispecific or multiantigenic CARs, in parallel with other modalities targeting more than a single antigen. Though these multi-target therapies might reduce the chances of tumor-escape, they are currently hampered by a variety of limitations, including the dearth of appropriate surface-borne tumor-specific antigens. Resultant broadening of target repertoires to include TAAs continues to run the risk of undesirable autoimmunity, and should be approached as a strategy with due caution. To our knowledge, the third-generation EGFRvIII-specific CAR mentioned here is currently the first truly tumor-specific construct to date, and circumvents the toxicities associated with other T-cell therapies that target antigens co-expressed in normal tissues, including gp100,[55] CAIX,[61] ERBB2,[75] or CD19.[11] For the full realization of safe and effective multi-targeted therapy, additional tumor-specific and targetable antigens will likely need to be identified.

Concluding remarks

MGs are an exceptionally dismal group in their occurrence and lethality, and the current standard of care has only marginally improved prognosis. As such, new therapies that can even modestly improve patient outcomes represent important breakthroughs. The advent of immunotherapy has facilitated the development of novel strategies, among which CAR-based approaches are likely to be among the most promising. Although immunotherapy has been avidly explored in cancers residing outside of the brain, CNS-based tumors have been studied to a far less extent. With this in mind, we stand to learn from the limitations and shortcomings of parallel therapies that have made it to advanced phase clinical trials when constructing future preclinical and clinical designs, with continued attention to the peculiarities of directing immune responses in the CNS. CAR-based therapy shows promise not only in the treatment of gliomas, but also in all cancers where tumorigenic and/or tumor-specific antigens exist. Although the functionality of these CARs against MGs in patients is left to be seen, it is clear that this therapy holds tremendous potential and represents a true advance in the rational design of glioma therapies.

REFERENCES

1. Agarwal S, Manchanda P, Vogelbaum MA, Ohlfest JR, Elmquist WF. Function of the blood-brain barrier and restriction of drug delivery to invasive glioma cells: Findings in an orthotopic rat xenograft model of glioma. Drug Metab Dispos 2013;41:33-9.
2. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, *et al*. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 2008;10:619-24.
3. Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by CNS antigen-presenting cells: Different roles for microglia and astrocytes. Immunol Today 2000;21:141-7.
4. Antony PA, Piccirillo CA, Akpinarli A, Finkelstein SE, Speiss PJ, Surman DR, *et al*. CD8+T cell immunity against a tumor/self-antigen is augmented by CD4+T helper cells and hindered by naturally occurring T regulatory cells. J Immunol 2005;174:2591-601.
5. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, *et al*. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999;17:2105-16.
6. Azuma T, Takahashi T, Kunisato A, Kitamura T, Hirai H. Human CD4+CD25+regulatory T cells suppress NKT cell functions. Cancer Res 2003;63:4516-20.
7. Batra SK, Castelino-Prabhu S, Wikstrand CJ, Zhu X, Humphrey PA, Friedman HS, *et al*. Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant EGFRvIII gene. Cell Growth Differ 1995;6:1251-9.
8. Boockvar JA, Kapitonov D, Kapoor G, Schouten J, Counelis GJ, Bogler O, *et al*. Constitutive EGFR signaling confers a motile phenotype to neural stem cells. Mol Cell Neurosci 2003;24:1116-30.
9. Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: Case report of an unforeseen adverse event in a phase I clinical trial. Mol Ther 2010;18:666-8.
10. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, *et al*. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 2013;5:177ra138.
11. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, *et al*. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 2011;118:4817-28.

12. Bullard DE, Gillespie GY, Mahaley MS, Bigner DD. Immunobiology of human gliomas. Semin Oncol 1986;13:94-109.
13. Cartellieri M, Bachmann M, Feldmann A, Bippes C, Stamova S, Wehner R, *et al*. Chimeric antigen receptor-engineered T cells for immunotherapy of cancer. J Biomed Biotechnol 2010;2010:956304.
14. Chen W. Dendritic cells and (CD4+) CD25+T regulatory cells: Crosstalk between two professionals in immunity versus tolerance. Front Biosci 2006;11:1360-70.
15. Choi BD, Archer GE, Mitchell DA, Heimberger AB, McLendon RE, Bigner DD, *et al*. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol 2009;19:713-23.
16. Choi BD, Gedeon PC, Herndon JE 2nd, Archer GE, Reap EA, Sanchez-Perez L, *et al*. Human regulatory T cells kill tumor cells through granzyme-dependent cytotoxicity upon retargeting with a bispecific antibody. Cancer Immunol Res 2013;1:163.
17. Choi BD, Gedeon PC, Sanchez-Perez L, Bigner DD, Sampson JH. Regulatory T cells are redirected to kill glioblastoma by an EGFRvIII-targeted bispecific antibody. Oncoimmunology 2013;2:e26757.
18. Choi BD, Kuan CT, Cai M, Archer GE, Mitchell DA, Gedeon PC, *et al*. Systemic administration of a bispecific antibody targeting EGFRvIII successfully treats intracerebral glioma. Proc Natl Acad Sci U S A 2013;110:270-5.
19. Choi BD, Pastan I, Bigner DD, Sampson JH. A novel bispecific antibody recruits T cells to eradicate tumors in the "immunologically privileged" central nervous system. Oncoimmunology 2013;2:e23639.
20. Choi BD, Suryadevara CM, Gedeon PC, Herndon Ii JE, Sanchez-Perez L, Bigner DD, *et al*. Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. J Clin Neurosci 2014;21:189-90.
21. Chu CT, Everiss KD, Wikstrand CJ, Batra SK, Kung HJ, Bigner DD. Receptor dimerization is not a factor in the signalling activity of a transforming variant epidermal growth factor receptor (EGFRvIII). Biochem J 1997;324:855-61.
22. Croft M. Co-stimulatory members of the TNFR family: Keys to effective T-cell immunity? Nat Rev Immunol 2003;3:609-20.
23. Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: A new view. Immunol Today 1992;13:507-12.
24. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, *et al*. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942-9.
25. Dandy WE. Removal of right cerebral hemisphere for certain tumors with hemiplegia-Preliminary report. JAMA 1928;90:823-5.
26. Dolcetti L, Marigo I, Mantelli B, Peranzoni E, Zanovello P, Bronte V. Myeloid-derived suppressor cell role in tumor-related inflammation. Cancer Lett 2008;267:216-25.
27. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 2012;14 Suppl 5:v1-49.
28. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, *et al*. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002;298:850-4.
29. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
30. Dunn GP, Fecci PE, Curry WT. Cancer immunoediting in malignant glioma. Neurosurgery 2012;71:201-22.
31. Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: Anatomical sites and molecular mechanisms. Trends Immunol 2005;26:485-95.
32. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci U S A 1993;90:720-4.
33. Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: The dialect of the immune response in the CNS. Immunol Today 1994;15:218-24.
34. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, *et al*. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res 2006;66:3294-302.
35. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995;13:688-96.
36. Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, *et al*. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages *in vivo*. Blood 1998;92:4150-66.
37. Ge H, Gong X, Tang CK. Evidence of high incidence of EGFRvIII expression and coexpression with EGFR in human invasive breast cancer by laser capture microdissection and immunohistochemical analysis. Int J Cancer 2002;98:357-61.
38. Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: Intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 1995;20:269-87.
39. Golovina TN, Vonderheide RH. Regulatory T cells: Overcoming suppression of T-cell immunity. Cancer J 2010;16:342-7.
40. Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature 1992;356:607-9.
41. Hart DN, Fabre JW. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J Exp Med 1981;154:347-61.
42. Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, *et al*. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. Clin Cancer Res 2005;11:1462-6.
43. Heimberger AB, Sampson JH. Immunotherapy coming of age: What will it take to make it standard of care for glioblastoma? Neuro Oncol 2011;13:3-13.
44. Heimberger AB, Sampson JH. The PEPvIII-KLH (CDX-110) vaccine in glioblastoma multiforme patients. Expert Opin Biol Ther 2009;9:1087-98.
45. Hentschel SJ, Lang FF. Current surgical management of glioblastoma. Cancer J 2003;9:113-25.
46. Hickey WF. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. Brain Pathol 1991;1:97-105.
47. 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 2006;8:261-79.
48. 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 2003;9:4404-8.
49. Imai C, Mihara K, Andreansky M, Nicholson IC, Pui CH, Geiger TL, *et al*. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. Leukemia 2004;18:676-84.
50. Imperato JP, Paleologos NA, Vick NA. Effects of treatment on long-term survivors with malignant astrocytomas. Ann Neurol 1990;28:818-22.
51. Inda MM, Bonavia R, Mukasa A, Narita Y, Sah DW, Vandenberg S, *et al*. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev 2010;24:1731-45.
52. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell 1991;64:891-901.
53. Jensen MC, Popplewell L, DiGiusto D, Kalos M, Raubitschek A, Forman SJ. A first-in-human clinical trial of adoptive therapy using CD19-specific chimeric antigen receptor re-directed T-cells for recurrent/refractory follicular lymphoma. Blood 2007;110:92a.
54. Jia W, Jackson-Cook C, Graf MR. Tumor-infiltrating, myeloid-derived suppressor cells inhibit T cell activity by nitric oxide production in an intracranial rat glioma+vaccination model. J Neuroimmunol 2010;223:20-30.
55. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, *et al*. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009;114:535-46.
56. Johnson LA, Sampson JH. Immunotherapy approaches for malignant glioma from 2007 to 2009. Curr Neurol Neurosci Rep 2010;10:259-66.
57. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, *et al*. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011;3:95ra73.
58. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, *et al*. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 2006;12:6106-15.
59. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of

vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest 2010;120:694-705.
60. Klebanoff CA, Acquavella N, Yu Z, Restifo NP. Therapeutic cancer vaccines: Are we there yet? Immunol Rev 2011;239:27-44.
61. Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, *et al.* Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: First clinical experience. J Clin Oncol 2006;24:e20-2.
62. Lammering G, Valerie K, Lin PS, Hewit TH, Schmidt-Ullrich RK. Radiation-induced activation of a common variant of EGFR confers enhanced radioresistance. Radiother Oncol 2004;72:267-73.
63. Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: Direct suppression of B cells by CD4+CD25+regulatory T cells. J Immunol 2005;175:4180-3.
64. Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, *et al.* Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 2002;169:2756-61.
65. Maher J, Brentjens RJ, Gunset G, Riviere I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. Nat Biotechnol 2002;20:70-5.
66. Masson F, Calzascia T, Di Berardino-Besson W, de Tribolet N, Dietrich PY, Walker PR. Brain microenvironment promotes the final functional maturation of tumor-specific effector CD8+T cells. J Immunol 2007;179:845-53.
67. Matsukado Y, Maccarty CS, Kernohan JW. The growth of glioblastoma multiforme (astrocytomas, grades 3 and 4) in neurosurgical practice. J Neurosurg 1961;18:636-44.
68. Matyszak MK, Denis-Donini S, Citterio S, Longhi R, Granucci F, Ricciardi-Castagnoli P. Microglia induce myelin basic protein-specific T cell anergy or T cell activation, according to their state of activation. Eur J Immunol 1999;29:3063-76.
69. Miao H, Choi BD, Suryadevara CM, Sanchez-Perez L, Yang S, De Leon G, *et al.* EGFRvIII-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma. PloS One 2014;9:e94281.
70. Mitchell DA, Cui X, Schmittling RJ, Sanchez-Perez L, Snyder DJ, Congdon KL, *et al*. Monoclonal antibody blockade of IL-2 receptor alpha during lymphopenia selectively depletes regulatory T cells in mice and humans. Blood 2011;118:3003-12.
71. Mitchell DA, Fecci PE, Sampson JH. Adoptive immunotherapy for malignant glioma. Cancer J 2003;9:157-66.
72. Mitchell DA, Fecci PE, Sampson JH. Immunotherapy of malignant brain tumors. Immunol Rev 2008;222:70-100.
73. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, *et al.* Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126-9.
74. Morgan RA, Johnson LA, Davis JL, Zheng Z, Woolard KD, Reap EA, *et al.* Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. Hum Gene Ther 2012;23:1043-53.
75. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther 2010;18:843-51.
76. Moritz D, Groner B. A spacer region between the single chain antibody- and the CD3 zeta-chain domain of chimeric T cell receptor components is required for efficient ligand binding and signaling activity. Gene Ther 1995;2:539-46.
77. Murakami M, Sakamoto A, Bender J, Kappler J, Marrack P. CD25+CD4+T cells contribute to the control of memory CD8+T cells. Proc Natl Acad Sci U S A 2002;99:8832-7.
78. 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 1996;56:5079-86.
79. Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: A clinical review. JAMA 2013;310:1842-50.
80. Parish CR. Cancer immunotherapy: The past, the present and the future. Immunol Cell Biol 2003;81:106-13.
81. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, *et al.* Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007;13:84-8.
82. Peereboom DM, Shepard DR, Ahluwalia MS, Brewer CJ, Agarwal N, Stevens GH, *et al.* Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. J Neurooncol 2010;98:93-9.
83. Pelloski CE, Ballman KV, Furth AF, Zhang L, Lin E, Sulman EP, *et al.* Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma. J Clin Oncol 2007;25:2288-94.
84. Platten M, Wick W, Weller M. Malignant glioma biology: Role for TGF-beta in growth, motility, angiogenesis, and immune escape. Microsc Res Tech 2001;52:401-10.
85. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, *et al.* Virus-specific T cells engineered to coexpress tumor-specific receptors: Persistence and antitumor activity in individuals with neuroblastoma. Nat Med 2008;14:1264-70.
86. Pule MA, Straathof KC, Dotti G, Heslop HE, Rooney CM, Brenner MK. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. Mol Ther 2005;12:933-41.
87. Purev E, Cai D, Miller E, Swoboda R, Mayer T, Klein-Szanto A, *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 2004;173:6472-80.
88. Romeo C, Amiot M, Seed B. Sequence requirements for induction of cytolysis by the T cell antigen/Fc receptor zeta chain. Cell 1992;68:889-97.
89. Rosenberg SA. Raising the bar: The curative potential of human cancer immunotherapy. Sci Transl Med 2012;4:127ps128.
90. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, *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 1985;313:1485-92.
91. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008;8:299-308.
92. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, *et al*. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17:4550-7.
93. Sadelain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 2003;3:35-45.
94. Sampson JH, Choi BD, Sanchez-Perez L, Suryadevara CM, Snyder DJ, Flores CT, *et al.* EGFRvIII mCAR-modified T cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clin Cancer Res 2014;20:972-84.
95. Sampson JH, Crotty LE, Lee S, Archer GE, Ashley DM, Wikstrand CJ, *et al.* Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. Proc Natl Acad Sci U S A 2000;97:7503-8.
96. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, *et al.* Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol 2010;28:4722-9.
97. Sampson JH, Schmittling RJ, Archer GE, Congdon KL, Nair SK, Reap EA, *et al.* A pilot study of IL-2Ralpha blockade during lymphopenia depletes regulatory T-cells and correlates with enhanced immunity in patients with glioblastoma. PloS One 2012;7:e31046.
98. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, *et al.* CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest 2011;121:1822-6.
99. Schneider SW, Ludwig T, Tatenhorst L, Braune S, Oberleithner H, Senner V, *et al.* Glioblastoma cells release factors that disrupt blood-brain barrier features. Acta Neuropathol 2004;107:272-6.
100. Sehgal A, Berger MS. Basic concepts of immunology and neuroimmunology. Neurosurg Focus 2000;9:e1.
101. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 2010;29:625-34.
102. Shuford WW, Klussman K, Tritchler DD, Loo DT, Chalupny J, Siadak AW, *et al.* 4-1BB costimulatory signals preferentially induce CD8+T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. J Exp Med 1997;186:47-55.

103. Sica G, Chen L. Modulation of the immune response through 4-1BB. Adv Exp Med Biol 2000;465:355-62.
104. Sinha P, Clements VK, Miller S, Ostrand-Rosenberg S. Tumor immunity: A balancing act between T cell activation, macrophage activation and tumor-induced immune suppression. Cancer Immunol Immunother 2005;54:1137-42.
105. Soda Y, Myskiw C, Rommel A, Verma IM. Mechanisms of neovascularization and resistance to anti-angiogenic therapies in glioblastoma multiforme. J Mol Med 2013;91:439-48.
106. Sok JC, Coppelli FM, Thomas SM, Lango MN, Xi S, Hunt JL, *et al*. Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. Clin Cancer Res 2006;12:5064-73.
107. Stevens A, Kloter I, Roggendorf W. Inflammatory infiltrates and natural killer cell presence in human brain tumors. Cancer 1988;61:738-43.
108. Stupp R, Dietrich PY, Ostermann Kraljevic S, Pica A, Maillard I, Maeder P, *et al*. Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol 2002;20:1375-82.
109. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
110. Tada M, Suzuki K, Yamakawa Y, Sawamura Y, Sakuma S, Abe H, *et al*. Human glioblastoma cells produce 77 amino acid interleukin-8 (IL-8 (77)). J Neurooncol 1993;16:25-34.
111. Takahashi C, Mittler RS, Vella AT. Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. J Immunol 1999;162:5037-40.
112. Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, *et al*. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood 2008;112:2261-71.
113. Tirapu I, Huarte E, Guiducci C, Arina A, Zaratiegui M, Murillo O, *et al*. Low surface expression of B7-1 (CD80) is an immunoescape mechanism of colon carcinoma. Cancer Res 2006;66:2442-50.
114. Trzonkowski P, Szmit E, Mysliwska J, Dobyszuk A, Mysliwski A. CD4+CD25+T regulatory cells inhibit cytotoxic activity of T CD8+and NK lymphocytes in the direct cell-to-cell interaction. Clin Immunol 2004;112:258-67.
115. Trzonkowski P, Szmit E, Mysliwska J, Mysliwski A. CD4+CD25+T regulatory cells inhibit cytotoxic activity of CTL and NK cells in humans-impact of immunosenescence. Clin Immunol 2006;119:307-16.
116. Wei S, Egenti MU, Teitz-Tennenbaum S, Zou W, Chang AE. Effects of tumor irradiation on host T-regulatory cells and systemic immunity in the context of adoptive T-cell therapy in mice. J Immunother 2013;36:124-32.
117. Wikstrand CJ, Bigner DD. Immunobiologic aspects of the brain and human gliomas. A review. Am J Pathol 1980;98:517-68.
118. Wikstrand CJ, Hale LP, Batra SK, Hill ML, Humphrey PA, Kurpad SN, *et al*. Monoclonal antibodies against EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. Cancer Res 1995;55:3140-8.
119. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, *et al*. Regulatory CD4(+) CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res 2001;61:4766-72.
120. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, *et al*. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765-73.
121. Yee C, Thompson JA, Byrd D, Riddell SR, Roche P, Celis E, *et al*. Adoptive T cell therapy using antigen-specific CD8+T cell clones for the treatment of patients with metastatic melanoma: *In vivo* persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci U S A 2002;99:16168-73.
122. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, *et al*. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 2009;324:261-5.
123. Zhao Y, Wang QJ, Yang S, Kochenderfer JN, Zheng Z, Zhong X, *et al*. A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity. J Immunol 2009;183:5563-74.
124. Zhong XS, Matsushita M, Plotkin J, Riviere I, Sadelain M. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI3kinase/AKT/Bcl-XL activation and CD8+T cell-mediated tumor eradication. Mol Ther 2010;18:413-20.
125. Zippel DB, Besser M, Shapira R, Ben-Nun A, Goitein D, Davidson T, *et al*. Adoptive cell therapy with autologous tumor-infiltrating lymphocytes and high-dose interleukin-2 for metastatic melanoma: The surgeon's perspective. Exp Ther Med 2012;3:898-902.