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

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.\textsuperscript{[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.\textsuperscript{[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,\textsuperscript{[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,\textsuperscript{[107]} 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.\textsuperscript{[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.\textsuperscript{[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\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[115,35,41]} 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.\textsuperscript{[117]} which likely travel via defined subarachnoid routes and to the cervical nodes via the nasal mucosa.\textsuperscript{[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.\textsuperscript{[5,31,35,46,66,72]} 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.\textsuperscript{[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.\textsuperscript{[11,82]} 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.\textsuperscript{[16,45,84,110]} 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.\textsuperscript{[41,47,78,111]}

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 immuno-resistance. 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, 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. 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. 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. 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. Eliminating T_{reg} produces enhanced antitumor responses and may likewise explain the synergy between lymphodepletion/tumor irradiation and immunotherapy. 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.

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-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.

**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. 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. 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. 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.

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. 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. 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. 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 Tregs and by reducing competition for homeostatic cytokines. 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 a 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 Treg, 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. 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 TAA 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.

**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. 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, renal cell carcinoma, neuroblastoma, non-Hodgkin and mantle cell lymphomas, 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,\textsuperscript{65} 4-1BB,\textsuperscript{49} OX40\textsuperscript{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.\textsuperscript{99} 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.\textsuperscript{100}

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,\textsuperscript{101} 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,\textsuperscript{122} amplify cytotoxicity, and inhibit activation-induced cell death (AICD) by upregulation of antiapoptotic genes such as Bcl-xl.\textsuperscript{122,102,103,111,123} “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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{975} 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.\textsuperscript{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.\textsuperscript{157,428,106} 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.\textsuperscript{721} EGFRvIII has been shown to be a negative prognostic indicator,\textsuperscript{83} to enhance invasiveness\textsuperscript{84} and tumorigenicity,\textsuperscript{73} and to confer resistance to standard-of-care radio and chemotherapy.\textsuperscript{62,106} Importantly, EGFRvIII is commonly expressed on CD133+ brain tumor stem cells,\textsuperscript{74} and EGFRvIII+ cells can promote malignant transformation of nearby cells through paracrine signaling of IL-6 family cytokines\textsuperscript{51} and through the intercellular transfer of EGFRvIII-positive exosomes.\textsuperscript{82} 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\textsuperscript{95,113} and these findings have helped expedite the production of novel therapies with heightened specificity.\textsuperscript{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\textsuperscript{20,99} and syngeneic murine model of spontaneous glioma in our laboratory.\textsuperscript{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).\textsuperscript{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. 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, 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. 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.

‘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. These phases are easily applied to and framed in the context of glioma as well. 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. 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.

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. 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%). Unlike EGFRvIII mutations, which are present in 50–50% 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. 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. 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-targeted 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, CAIX, ERBB2, or CD19. 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.

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