

# Updates On Chimeric Antigen Receptor-Mediated Glioblastoma Immunotherapy

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## ABSTRACT

Glioblastoma multiforme (GBM) is the most malignant of the primary central nervous system (CNS) neoplasms, accounting for nearly 80% of all primary brain tumors and is associated with high morbidity and mortality. Immunotherapy is proving to be a fertile ground for next-generation GBM therapy, with large translational research projects and clinical trials currently underway. One particularly promising area is the chimeric antigen receptors (CARs) in the context of lymphocyte adoptive cell therapy (ACT), which has achieved success in the treatment of hematological malignancies. In this review, we will discuss CARs and review current challenges facing their use in GBM therapy.

**KEYWORDS:** glioblastoma, immunotherapy, CAR

## INTRODUCTION

Glioblastoma (GBM) is the most common and most malignant of all the primary cancers of the central nervous system. It is an aggressive and heterogeneous cancer characterized by densely packed pleomorphic cells with high mitotic activity, necrosis, and high degree of vascularization. GBM most commonly strikes older individuals, with a slight predilection for males over females. The current standard of care therapy for GBM, which consists of maximum allowable surgical resection, focal beam radiation, and chemotherapy with Temozolomide, is not curative. Survival remains abysmal, as fewer than 10% of patients survive after 5 years. The difficulty in treating GBM arises from the cancer's cellular heterogeneity, their diffuse infiltration into the brain, protection provided by the blood brain barrier, the chemo- and radiotherapeutic resistance and regenerative capacity of glioma stem cells [1-3].

With these barriers in mind, immunotherapy may offer an avenue of treatment for GBM that may be both safer and more effective. Immunotherapy offers a targeted approach to treatment, via utilization of unique molecular and genetic signature of tumor cells. It has been noted in several malignancies that cancer patients who had more activated immune systems seem to have better outcomes. Thus, techniques have been developed to enhance a patient's immune response against GBM. Immunotherapy encompasses a wide

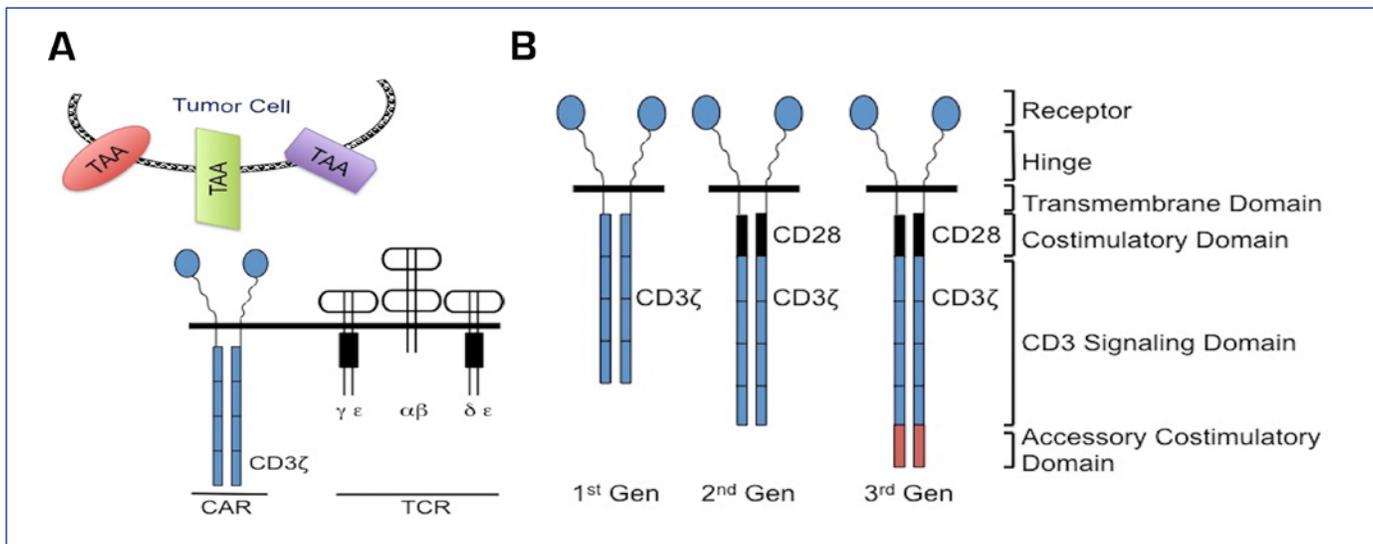
variety of techniques, which are beyond the scope of this review. The most developed of these immunotherapeutic methods is the use of monoclonal antibodies (mAbs). While mAbs have been developed against multiple GBM-specific antigens, they have not yet enjoyed wide success due to a combination of tumor and host factors. Other areas of immunotherapy for GBM under current active investigation include dendritic cell vaccines derived from lysed tumor cells, immunomodulatory checkpoint inhibitors, and engineered T-cell based therapies, which will be the focus of this paper.

## CARs

Recently reported success of clinical CAR-T cell therapy of GBM [4] has generated hope in the use of this technology for GBM immunotherapy. CAR-T cell offers an attractive alternative to a limitation inherent in the other T-cell receptor (TCR) dependent cellular immunotherapeutic approaches, namely, that of MHC *independent* antigen presentation. Precluding the need for TCRs, CAR-T cell strategies greatly improve the diversity of antigen targets, and bypasses a mechanism of GBM immune evasion through MHC down-regulation [5]. Engineered CAR-Ts have much higher affinity to their targeted antigens than T cells. The antigen binding can be further modulated in subsequent development of the CAR-T cells to prevent overly strong immune responses that can result in the potentially fatal cytokine release. Since CAR-Ts are artificially constructed from functional polypeptide domains, the receptors can be configured by the addition of new effector domains, adding novel features to the CAR-T cell. Thus, CAR-T cells can be precisely tailored to the goals of therapy, and be individualized to the particular patient and clinical scenario.

CAR-Ts are very similar to TCR in structure and function, as they both combine an antigen-binding domain with downstream signal transduction domains. The antigen-binding domain of CAR can be a heavy chain and the light chain fusion protein (scFv) of a prototypical antibody, and the most common design is an scFv domain joined to a transmembrane CD3 domain. This section of the protein is physically linked to an intracellular CD3- $\zeta$  signaling domain and costimulatory domains that couple extracellular antigen recognition to intracellular signal transduction, which subsequently affect T-cell immune response (**Figure 1**). One can also look beyond the traditional antigen-antibody paradigm

**Figure 1.** Chimeric Antigen Receptors (CARs): Design of CARs versus TCRs (A), and evolution of CARs (B).



for ligand-binding motifs. For example, the natural receptor of a particular tumor specific antigen may be utilized as the ligand-binding domain, thus permitting CARs to bind to non-immunogenic peptides as well as carbohydrate and even lipid-based antigens.

The MHC independent nature of CAR-mediated antigen presentation and their inherent modular properties engineered into these cells make the receptors far more versatile than TCRs. After the CAR design is decided upon, the underlying genetic sequence that encodes for the CAR is encoded and transfected into the T-cell usually via a viral vector. The modified T-cells are then expanded *in vitro* and subsequently re-introduced back to the patient [6, 7].

**CARS FOR GBM IMMUNOTHERAPY**

Initially achieving success for the treatment of hematologic malignancies, adoptive CAR-T cell therapy is now under active investigation for a variety of solid tumors, including GBM. Presently, CARs have been developed against six GBM-associated antigens, with four having passed animal trials and currently in clinical trials. These include: EGFRvIII [8-13], IL13Rα2 [4, 14-16], HER2 [17, 18], and EphA2 [19]. Epidermal growth factor receptor, or EGFR, is directly implicated in GBM through either over-amplification of the gene (HER2/neu) or through constitutively active mutant variants, both of which result in increased growth and survival of the GBM tumor cell [10]. EphA2 is a cell-surface receptor that regulates proliferation, migration and angiogenesis, which ultimately affects the invasive and metastatic potential of GBM [20, 21]. IL13Rα2 is a unique decoy receptor for IL13, and binding of the cytokine to the decoy receptor terminates the chain immuno-stimulatory signals that lead to the generation of an appropriate immune response [22].

Currently, there are three major clinical trials involving

CAR-T cells against EGFRvIII on GBM. All three are in preliminary stages, only one is currently recruiting participants, whereas the two others have not yet started or have suspended patient recruitment [23-25]. There have been two CAR-T-cell clinical trials involving the use of IL13Rα2 as the target and two clinical trials involving anti-HER2 CAR-T cells. Lastly, there is currently one clinical trial with the goal of establishing EphA2 as a safe and feasible CAR-Target, which is presently in recruitment [30] (Table 1).

**Table 1.** List of CAR-T cells currently undergoing clinical trials.

Clinical Trials	CAR Target(s)	Current Status	Special Notes
NCT01454596	EGFRvIII	Suspended recruitment	Status unclear
NCT02664363	EGFRvIII	Not yet started recruitment	Neoadjuvant ACT
NCT02209376	EGFRvIII	In recruitment	Safety/feasibility study
NCT02575261	EphA2	In recruitment	Safety/feasibility study
NCT02442297	HER2	Not yet started recruitment	Inhibitory CAR model
NCT01109095	HER2	Ongoing, not accepting new patients	Modification of preselected CMV specific T cells
NCT01082926	IL-13Rα2	Completed	T cells also engineered with HyTK suicide switch and resistance to steroids
NCT02208362	IL-13Rα2	In recruitment	Safety/feasibility study

## LIMITATIONS OF CARs FOR GBM IMMUNOTHERAPY

So far, the use of adoptive CAR-T cell therapy for GBM has not yet enjoyed the level of success seen in the treatment of lymphoproliferative disorders such as in ALL and CLL. This is due to a combination of tumor and host factors, the most relevant of which is GBM's ability to evade immune surveillance and even achieve active suppression of the local immune response. GBM can actively shield itself from immune assault by concealment of targeted cell-surface antigens (including MHC proteins) and active secretion of various ligands that induce T-cell apoptosis and further inhibit the immune response [31-33]. Furthermore the intrinsic cellular heterogeneity of GBM provides further passive immunoresistance and immune escape mechanisms.

## OVERCOMING THE LIMITATIONS

One of the most obvious ways to counteract the problem of tumor heterogeneity is the use of CAR T cells that recognize multiple tumor antigens. By designing CARs with multiple distinct antigen-binding domains and through the introduction of multiple different CARs onto the surface of a single T cell, there is more opportunity to overcome intratumor antigen heterogeneity [34]. CAR-T cells can be further modified to render them unresponsive to the various immunosuppressive and apoptotic-inducing signals secreted by GBM [35, 36]. In addition, checkpoint inhibitors such as nivolumab, which suppress such interaction between the inhibitory ligand and its associated receptor on the T cell, can be given in conjunction with CAR-T cell administration to impair GBM's immunosuppressive capabilities [37-39].

Further engineering of the CAR-T cells allows for potential improvement in their immune response. Examples include the addition of co-stimulatory domains to the intracellular portion of the CAR construct to amplify the immune response generated by the CAR-T cell (**Figure 1B**). Further amplification may include designing CARs that can independently secrete critical pro-inflammatory cytokines [40], priming CAR-T cells with viral particles, and/or the appropriate pre-selection of T cells for CAR engraftment, such as the use of particular T cell subsets that have greater capacity to replicate *in vivo* after exposure to GBM antigens. In addition, CAR-T cells can also be rendered resistant to various chemotherapeutic agents, so that chemotherapy may be given in conjunction with adoptive T cell therapy [41-43].

One of the major issues confronting CAR-T cells is stimulating the appropriate level of immune response that kills the tumor, while causing minimal damage to normal tissues. While *in vitro* CAR-T cells demonstrate specificity for target antigen, *in vivo* studies have shown that CAR-T cells have resulted in systemic toxicity. Three classes of toxicities are seen with CAR-T cell therapy. Off-target-off-tumor toxicity is cross-reactive due to qualitative defects in scFv

design and/or production. Since these antibody fragments are often derived from murine immune systems, human toxicity resulting from murine derived antigen cross-reactivity will likely become less of an issue. A more serious problem is the on-target, but off-tumor toxicity observed with CAR-T cell therapy, as the tumor antigens are sometimes expressed in or cross react with non-neoplastic cells [44]. One way to offset this type of toxicity is designing a CAR-T cell that becomes activated only when it interacts with multiple different GBM antigens on the surface of the glioma cell [45, 46]. Because there is a dearth of antigens that are only expressed in GBM and nowhere else, investigators have turned to utilizing antigens that are highly expressed in glioma cells, but are also expressed at lower levels elsewhere in the body. By modulating the affinity of the CAR so that T cell activation occurs only after a defined threshold of binding is reached between the T-cell and its target, this type of toxicity may be abrogated [47]. The last category of toxicity due to a dysregulated immune response can range from a mild systemic flu-like illness to a massive overwhelming cytokine release that can result in multiorgan failure, and ultimately death. This may be addressed through the introduction of safety suicide switches into the adoptive T cell, which, when turned on by an exogenous signal (usually introduced by a clinician), results in the death of the T cell.

## CONCLUSION

The unique modular nature of CARs allows them to be tailor-made to match the need of any particular clinical scenario and nature makes CAR-T cell therapy a good potential complement to existing conventional therapies. Protocols are in place to determine their efficacy when used in conjunction with Temozolomide. However, the full potential of CAR-T therapy will not be realized until they are designed for the individual patient. Because technology now exists to allow for rapid genome-wide sequencing of individual cells, CAR-T cell therapy allows for targeting of targets unique to the individual cancer patient. And as the patient's GBM evolves over time, due to selection pressure during therapy, new CAR-T cells may be prepared and reintroduced to the patient in response to the antigenic shift. Of course, to identify these shifts, fresh tumor samples and genetic sequencing will be required to identify antigenic changes over time in the tumor organ.

Currently, there are still major challenges facing CARs that prevent their widespread use; however, solutions are in development that address most of these hurdles. With the cost of genomic sequencing rapidly decreasing due to technical innovations and economy of scale, a personalized approach to glioblastoma therapy is nearing. CAR-T cells represent a novel therapeutic option that may soon be ready for widespread use in this and other diseases.

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## Updates On Chimeric Antigen Receptor-Mediated Glioblastoma Immunotherapy

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