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Immunotherapy for Glioblastoma: Adoptive T-cell Strategies

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Abstract

Glioblastoma (GBM) is a devastating disease with an extremely poor prognosis. Immune therapy *via* adoptive cell transfer (ACT), especially with T cells engineered to express chimeric antigen receptors (CARs), represents a particularly promising approach. Despite the recent success of CAR T cells for blood cancers, the question remains whether this powerful anti-cancer therapy will ultimately work for brain tumors, and if the primary immunologic challenges in this disease—which include antigenic heterogeneity, immune suppression and T-cell exhaustion—can be adequately addressed. Here, we contextualize these concepts by reviewing recent developments in ACT for GBM, with a special focus on pioneering clinical trials of CAR T-cell therapy.

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Keywords

Glioblastoma; Central nervous system neoplasms; Immunotherapy; T-lymphocytes; Chimeric antigen receptor

Introduction

Glioblastoma (GBM) is the most common and most aggressive primary malignant brain tumor and represents an unmet clinical need. Contrary to conventional notions of central nervous system (CNS) immune privilege, there are now well-described mechanisms by which the immune system interfaces with tumors in the brain. Several first-in-class immune-based therapies for various cancers outside the CNS have been approved by the Food and Drug Administration (FDA) in the last decade. These include a dendritic cell vaccine for prostate cancer in 2010 (1), monoclonal antibody-based immune checkpoint blockade for metastatic melanoma and other cancers beginning in 2011 (2), bispecific T-cell engagers (BiTEs) for acute lymphoblastic leukemia (ALL) in 2014 (3), and oncolytic viral therapy for melanoma in 2015 (4). Perhaps the most promising T-cell technology in development is the chimeric antigen receptor (CAR), which received accelerated approval for hematological malignancies in 2017 (5). In this setting, CAR T cells were directed against a single molecule that is universally expressed on the surface of ALL cells called CD19. By contrast, aggressive solid tumors, including those arising in the brain, are inherently heterogenous (6). This makes targeting them through a single antigen less likely to yield durable, complete remissions. Despite this, early clinical trials of CARs for GBM have reported exciting results, and in at least one case demonstrated complete regression of bulky, multifocal cancer in the brain and spinal canal following intraventricular infusion (7). Here, we discuss recent groundbreaking advances in the development of adoptive T-cell therapy for brain tumors, and summarize emerging opportunities for further investigation.

Immune Biology of Brain Tumors

Despite state-of-the-art advances in treatment for GBM, including a combination of maximal surgical resection, radiation therapy, chemotherapy, anti-angiogenic agents and alternating electrical fields, the prognosis for patients with GBM remains exceedingly grim, with 5-year survival rates of less than 10% (8). In addition, currently available treatments are limited by adverse effects on normal, healthy tissues. As an alternative, immune-based therapies are rapidly evolving, and T cells in particular are thought to be an essential component of an effective antitumor response. Indeed, it has been acknowledged for decades that gliomas are infiltrated by lymphocytes and that their presence in GBM may correlate with improved prognosis (9,10). However, such naturally occurring T cells are observed less frequently than in other tumor types (11,12) and are ostensibly incapable of potentiating tumor regression on their own, due in part to mechanisms of T-cell dysfunction, several of which are idiosyncratic to the natural history of GBM.

In the context of glioma immune biology, T cells face an exceptionally hostile tumor microenvironment. Anatomically, the CNS is considered an immunologically “distinct” site given the absence of conventional lymphatic structures and dearth of resident professional

antigen-presenting cells (13). In the healthy brain, specialized tight junctions between endothelial cells also exist, which impede communication with the systemic circulation (14); however, in the setting of various pathologies including tumors and inflammatory disease, the integrity of the blood-brain barrier (BBB) becomes compromised (15). In addition, nonclassical lymphatic vessels have recently been described along dural venous sinuses, providing a putative gateway for immune cells to travel through the meninges (16). Recent evidence suggests that tumors in the CNS may uniquely potentiate large-scale sequestration of immune effectors in the bone marrow, thus crippling antitumor immunity by harboring T cells in an agnostic anatomical compartment (17). Compounding this GBM-related T-cell deficiency, standard-of-care treatment with temozolomide chemotherapy and high-dose corticosteroids also leads to profound lymphopenia and immune suppression, further depressing the development of effective antitumor immune responses.

Functionally, the immune system in patients with GBM is suppressed by a network of tightly intertwined, dynamic elements. These include counterproductive cytokine skew (*e.g.*, IL-10, TGF-β), direct inhibition of T cells *via* cell-surface molecules on tumor or extracellular vesicles (*e.g.*, PD-L1, CD95) (18), elaboration of suppressive immune populations (*e.g.*, regulatory T cells [T_{regs}], tumor associated macrophages [TAMs], myeloid-derived suppressor cells [MDSCs]), and production of additional modulatory factors (*e.g.*, indolamine 2,3-dioxygenase). Many of these phenomena are shared observations with other cancers and have been reviewed in greater detail elsewhere (19,20). Notable for GBM, however, is the relative paucity of available tumor-specific and tumor-associated neoantigens that are frequently and homogenously expressed, especially when compared to melanoma and cancers of the lung and colon (21). Perhaps most reflective of the notoriously “cold” immunological milieu associated with GBM is that—despite promising single-arm studies of vaccines and immune checkpoint inhibitors—a randomized trial of immune based therapy for GBM has yet to successfully demonstrate a survival advantage (22,23).

The Promise of Cellular Therapy

Since its introduction in animal models of tumor over 60 years ago (24), adoptive cell transfer (ACT) strategies have emerged as a bona fide treatment for cancer, most recently evidenced by FDA approval of CAR T-cell therapy for acute lymphoblastic leukemia in 2017, which also represents the first ACT approved for cancer of any type. Unlike vaccines and immunomodulatory agents that rely on *in vivo* priming of endogenous tumor-reactive cells, ACT introduces an ability to optimally select or genetically engineer cells with specificity for tumor antigens, and then provide appropriate stimulation to promote proliferation, expansion, and maintenance of potent effector functions to achieve therapeutic goals. In addition, ACT permits favorable manipulation of host immunity prior to cell transfer (*i.e.*, lymphodepletion and removal of T_{regs} or endogenous lymphocytes that may compete with transferred cells for homeostatic cytokines), which may be exploited to create a suitable environment for fostering antitumor immune responses. Indeed, the plasticity of the “living” cellular response, and its ability to both sense and adapt to surroundings, represents one of the most intriguing aspects of this approach. Highlighting this is a recent report of a complete regression achieved in a patient with chronic lymphocytic leukemia,

whose treatment response was, remarkably, attributed to the *in vivo* expansion of a single CAR T-cell clone (25).

ACT therapies for GBM have evolved considerably over time (Summarized in Table 1). Earlier work focused on less specific approaches utilizing natural killer (NK) or lymphokine activated killer (LAK) cells, neither of which rely on human leukocyte antigen (HLA)-restricted mechanisms of killing. Clinical trials for these platforms have become less favored over the past decades, in part due to Phase III evidence from solid tumor settings wherein infusion of cells with IL-2 was not found to be superior to IL-2 alone (26). Local application of allogeneic cytotoxic T lymphocytes (CTLs) for GBM has also been attempted with hopes of redirecting the effects of histocompatibility mismatch against tumor cells. However, as has been characterized in hematological disease, donor lymphocyte infusion typically requires allogeneic hematopoietic stem cell transplantation to prevent elimination by host immunity and ultimately carries with it the potential for graft-versus-host toxicity. To avoid these drawbacks, several strategies based on autologous cells have been conceived and are in active development.

Unlike NK, LAK and allogeneic CTLs, ACT with autologous TILs relies on specific HLA-restricted tumor antigen recognition *via* T-cell receptors (TCRs). Adoptive transfer with TILs targeting tumor-associated somatic mutations has resulted in significant clinical regressions in both primary and metastatic cancers (27–30). Indeed, local infusion of autologous TILs with concomitant recombinant IL-2 has also been attempted in patients with malignant glioma (31), and favorable responses have been achieved with systemic TIL therapy for metastatic melanoma in the brain (32,33). Although providing evidence that peripherally administered lymphocytes may traffic to and effect measurable antitumor activity in the CNS, technical factors—including the ability to isolate and expand TILs from primary brain tumors—have limited the utility of this approach for GBM. Interestingly, a recent analysis demonstrated that TILs may in fact lack specificity for the tumor in which they are found, a phenomenon that appears to be consistent across various types of cancer (34).

Alternative methods of obtaining tumor-specific lymphocytes from draining lymph nodes, or cultivating these cells by *ex vivo* sensitization with autologous tumor tissue, have also been stymied by inadequate specimens. As such, one intriguing proposal has been ACT with autologous cytomegalovirus (CMV)-specific T cells, which can be readily isolated from peripheral blood or expanded *in vitro* using synthetic peptide epitopes (35,36). Rationale for this approach stems from the discovery of CMV antigen expression in GBM and its absence in normal tissues (37). Although the reliable detection of CMV viral proteins in brain tumors has been confirmed by nine different laboratories, it has been disputed by some when using different techniques than originally published (38–42).

Gene-engineered Cell Therapies

Of all ACT-based immune therapies currently in development for GBM, gene-engineered CAR T cells are at the forefront, with encouraging results reported from several recent clinical trials (43). Structurally, CAR molecules consist of an extracellular, antigen-binding

domain translated in tandem with assorted intracellular signaling regions that have differential effects on T-cell proliferation, effector function and survival (Fig. 1). While first-generation CAR constructs contained CD3ζ in isolation, second- and third-generation constructs included CD3ζ as well as one or two co-stimulatory domains (*e.g.*, CD28, OX40, 4–1BB), respectively. Unlike other gene-engineered ACT platforms such as transgenic TCR, the extracellular portion of CAR T cells is typically composed of an antibody-derived single-chain variable fragment (scFv). This design not only enables recognition of a broad array of antigens (*e.g.*, proteins, carbohydrates) but also obviates the need for presentation in the context of major histocompatibility complex (MHC), downregulation of which represents a well-characterized mechanism of tumor immune escape.

Treatment with CAR T cells directed at the B-cell antigen, CD19, has resulted in remarkable and durable remissions in patients with hematological cancers (5,44), even in cases of extensive disease involvement in the CNS (45). While this certainly provides robust proof-of-concept, CAR T cells have yet to be successfully translated in parallel for solid tumors. One major barrier has been antigenic heterogeneity and the challenge associated with identifying targets that are consistently expressed on cancer cells of interest. To date, four antigens have been pursued in CAR clinical trials for GBM. These include epidermal growth factor receptor variant III (EGFRvIII) (46), human epidermal growth factor receptor 2 (HER2) (47), and interleukin receptor 13Rα2 (IL-13Rα2) (7,48). There is also interest in erythropoietin-producing hepatocellular carcinoma A2 (EphA2), though results from clinical trials investigating this target have not yet been released (NCT02575261).

EGFRvIII

Tumor-specific antigens are those that are present in cancer cells but completely absent from normal tissues. This pattern of expression is ideal, as it confers the immune system with the theoretical capacity to eliminate tumors while minimizing toxicity and leaving healthy cells intact. A classic antigen matching this profile is EGFRvIII. EGFRvIII is a constitutively-activated, mutated form of the wild-type receptor that was first identified in primary human GBM, where its incidence is approximately 30% (49). The EGFRvIII deletion-mutation results in the translation of a novel glycine residue in the extracellular domain, flanked by normally disparate portions of the receptor, thus providing an ideal epitope for surface recognition by CAR T cells.

In 2017, O'Rourke and colleagues reported a series of 10 patients with recurrent GBM who had been treated with a single-dose of intravenous, second-generation (*i.e.*, 4–1BB, CD3ζ) EGFRvIII CAR T cells (NCT02209376) (46). All patients had EGFRvIII-positive tumors confirmed by next-generation sequencing assay. Notably, 7 out of 10 patients in the study underwent post-treatment surgical intervention, which provided the opportunity to directly evaluate the tumor microenvironment following CAR T-cell infusion. Molecular and histopathological analyses of these specimens demonstrated trafficking of peripherally administered CAR T cells to tumors in the brain. Interestingly, this was accompanied by a striking degree of infiltration by new immigrant, unmodified T cells, although a substantial proportion of these were identified as immune suppressive T_{regs}. Post-treatment tumor revealed decreased levels of EGFRvIII expression, consistent with successful elimination of

EGFRvIII-positive tumor. Compared to pre-treatment disease, residual and recurrent tumor displayed enhancement of several immune suppressive pathways, including increased expression of PD-L1, IDO1 and IL-10.

HER2

Another member of the epidermal growth factor receptor family, HER2, has well-documented overexpression in a wide variety of cancer types and is also found in approximately 80% of GBMs (50). However, this antigen is also present in healthy epithelial cells; thus, CAR T cells specific for HER2 carry a theoretical risk of on-target, off-tumor autoimmune effects. Indeed, this phenomenon was manifest in a 2010 case report of fatal toxicity in a patient with metastatic colon cancer, with signs of severe multiple organ dysfunction occurring just minutes after a single intravenous infusion of third-generation HER2 CAR (51).

Nonetheless, in 2017, Ahmed and colleagues performed a subsequent study which successfully demonstrated safety without dose-limiting toxicity, this time with an alternative HER2 CAR T-cell product in a trial of 17 patients with GBM (NCT01109095) (47). Important differences that may have contributed to a more favorable toxicity profile included the use of a second-generation (*i.e.*, CD28, CD3ζ) CAR with a different scFv, as well as the absence of concomitant IL-2 and lymphodepletive chemotherapy, both of which were administered in the aforementioned case report. Of the 17 patients treated with peripheral infusions of HER2 CAR T cells, three had stable disease for 24 months, and one patient with an unresectable thalamic GBM was noted to have a partial response.

IL-13Rα2

Unlike mutations in somatic genes or overexpression of otherwise normal proteins, cancer-germline antigens represent a class of immunogenic targets best described as having shared expression in both normal gametogenic cells as well as in human cancer. One such cancer-germline antigen is a cytokine receptor known as IL-13Rα2, which is found in both glioma cells and the testes. As a caveat, reports of its detection in several normal adult human tissues suggest that IL-13Rα2 may in fact be more accurately categorized as a tumor-associated antigen. It is estimated that IL-13Rα2 has relative overexpression in approximately 50% of GBMs, although its presence can vary somewhat within individual tumors (52).

A distinguishing feature of IL-13Rα2 CARs to date has been the use of a membrane-tethered, mutated IL-13 ligand for target antigen recognition, rather than a traditional scFv as has been typical with other constructs. Employing this design, data from two clinical trials studying IL-13Rα2 CAR T cells in patients with GBM have been published. In 2015, Brown and colleagues published first-in-human experience with a first-generation (*i.e.*, CD3ζ) IL-13Rα2 CAR in three patients with recurrent GBM (NCT00730613) (48). Patients received multiple intracavitary infusions *via* indwelling catheter, a strategy that was found to be both feasible and safe. Tumor specimen was available for analysis after therapy and in one instance demonstrated evidence of reduced IL-13Rα2 expression. These findings led to a subsequent study of a second-generation (*i.e.*, 4–1BB, CD3ζ) IL-13Rα2 CAR, during

which the authors observed an extraordinary response to treatment after serial intraventricular administration in one patient with multifocal GBM (NCT02208362) (7). In this case, IL-13Rα2 CAR was noted to mediate the complete regression of several bulky lesions in the brain and spinal canal. Given this response, perhaps most intriguing is that the patient's GBM did not homogeneously express the target antigen, with no verified staining of IL-13Rα2 in approximately 30% of the original tumor. These results raise the possibility that CAR T cells successfully targeted cells with low levels of IL-13Rα2 or triggered immunity to other targets through epitope spreading.

Future Challenges and Opportunities

Over the past decades, CAR technology has become an especially intriguing area of research in GBM. Clinical experience with ACT frequently provides new insights that have broader implications for general principles in brain tumor immune therapy. For example, a common theme presented throughout CAR T-cell trials is a repeated account of CNS immune access —namely that peripherally administered, tumor-specific T cells have the capacity to infiltrate lesions beyond the BBB. Moreover, mounting data support that introduction of these activated cells into the CNS can be accomplished with acceptable safety, even when administered directly into the brain. Certainly, there is some evidence that route-of-delivery may significantly impact outcome, since, in at least one case, infusion into the intraventricular space appeared to be necessary for tumor regression, whereas prior attempts by intracavitary administration in the same patient led to disease progression (7). While intraventricular approaches may theoretically increase the risk of neurosurgical complications such as hydrocephalus (53), potential advantages include enhanced access to multifocal disease throughout the CNS as well as the ability to achieve increased effector-to-target ratios at the tumor bed. Furthermore, local administration may also prove to mitigate off-tumor toxicity attributable to first-pass clearance in the lung, a mechanism which was implicated in the case of lethal toxicity following systemic infusion with HER2 CAR (51).

Despite this promise, substantial gaps in our understanding remain. Although CAR T cells efficiently eliminate cancer cells expressing their cognate antigen of interest, whether this approach will ultimately treat tumors that heterogeneously express these targets is unknown. Indeed, reports of CD19-negative escape variants in the setting of ALL suggest that CAR T cells may be limited in their ability to efficiently prime the immune response and protect against antigen loss. At least one contributing factor in the resistance to therapy is T-cell exhaustion, related in part to excessive CD3ζ phosphorylation within nonnative, CAR-mediated signaling complexes. Lastly, it is still unclear how to best optimize ACT in general, and conceptualize this therapy in the context of aforementioned suppressive glioma biology and iatrogenic effects of chemotherapy or steroid use. Ongoing advances in preclinical modeling have provided some insight into these issues. Although used less frequently than immune-compromised rodents bearing xenografted tumors, syngeneic systems have also been developed in order to better capture phenomena such as epitope-spreading *via* endogenous immunity (54) and the potential to enhance ACT responses through lymphodepletive host-conditioning (55). More recently, robust humanized models have offered an exciting alternative for screening ACT products, and have even been used to successfully recapitulate the complexities of CAR-mediated cytokine release syndrome (56).

CAR T cells are not only the first ACT treatment approved by the FDA, but they also represent the first gene-modified therapy made available for any indication. Now more than ever, the manipulation of genetic material has taken its place at the forefront of viable options in the battle against cancer. In fact, further modification of CAR T cells for GBM is already underway and has yielded a wide range of permutations; these include changes that allow CAR T cells to target multiple surface antigens at once (57–60), to be used as allogeneic products “off-the-shelf,” or to alter expression of cytokines and other immune-modulating molecules (61). The prospect of combination therapy has also been suggested between ACT and other treatments currently under investigation for GBM such as immune checkpoint blockade or oncolytic viruses (62,63). Localized treatment including radiosurgery, laser ablation, and that of various therapeutic devices also offer potential synergy with immune therapy (64), and the degree to which these interventions may be used to enhance adoptive therapy has yet to be seen.

Given the potential for significant crosstalk between cellular effectors, tumor cells, and accompanying treatment modalities, it will become increasingly important to implement appropriate clinical tools to assess surrogates of response to ACT, such as *in vivo* engraftment, trafficking and persistence. Regardless of route-of-delivery, advances in molecular imagining may offer noninvasive techniques to monitor the location and viability of adoptively transferred cells, as has been demonstrated in GBM through positron emission tomography (PET) (NCT00730613, NCT01082926) (65). These efforts, in conjunction with guidelines proposed by the response and assessment in neuro-oncology (RANO) criteria, will be vital in defining accurate endpoints for future clinical trials, given that local inflammation triggered by ACT may be indistinguishable from tumor progression using standard radiographic assessment (66).

Conclusions

Results from clinical trials for ACT immune therapy—particularly with CAR T cells—suggest a safe and feasible strategy for eliciting effective immune responses in GBM. The introduction of CAR T cells for the treatment of hematological malignancies has been transformative in the field of cancer immune therapy; however, as detailed here, GBM poses a unique set of challenges that must be addressed before the full potential of ACT can be realized. Continued familiarity with ACT may offer significant insight into general mechanisms of cellular immunity and their role in GBM. Given early successes in translation, there appears to be a bright future with abundant opportunity for research.

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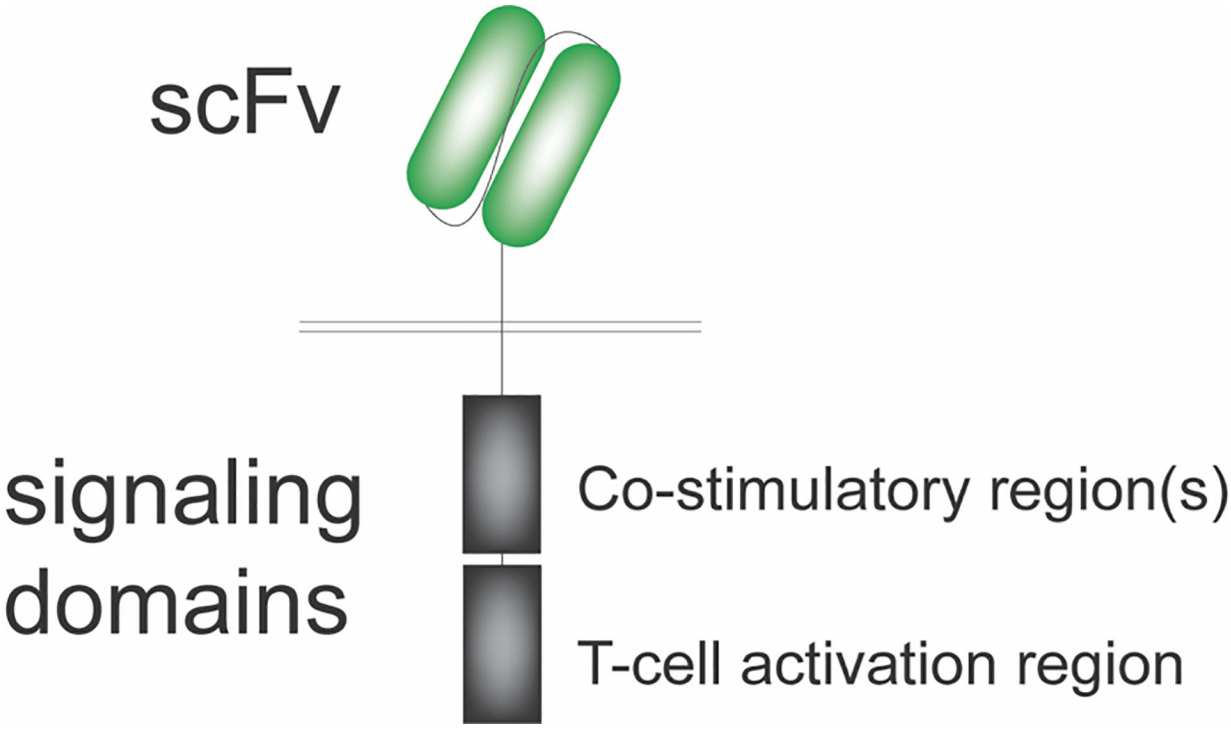

Figure 1.

Chimeric antigen receptors are composed of an extracellular antigen-binding domain, typically in the form of an antibody-derived single-chain variable fragment (scFv), translated in tandem with assorted intracellular signaling regions that have differential effects on T-cell proliferation, effector function and survival.

Table 1:

Adoptive T-cell Therapies for Glioblastoma in Development

Type	Mechanism	Sponsor Institution: Identifier
Lymphokine-activated killer cells	Unknown, likely MHC-independent	Hoag Memorial Hospital Presbyterian: NCT00331526
Allogeneic donor lymphocyte infusion	Graft versus tumor HLA-mismatch, may include viral antigens (CMV)	City of Hope Medical Center: NCT01082926 Jonsson Comprehensive Cancer Center: NCT01144247 Milton S. Hershey Medical Center: NCT00990496 University of Colorado: NCT00002572
Autologous lymphocytes	MHC-restricted TCR recognition, may include viral antigens (CMV)	Baylor College of Medicine: NCT01205334 CytoVac A/S: NCT01588769 Duke University Medical Center: NCT00693095 Green Cross Cell Corporation: NCT00807027 Huashan Hospital: NCT03347097 M.D. Anderson Cancer Center: NCT02661282 TVAX Biomedical: NCT01290692, NCT01081223
Tumor infiltrating lymphocytes	MHC-restricted TCR recognition	National Cancer Institute: NCT01174121
Transgenic T-cell receptor T cells	MHC-restricted TCR recognition	National Cancer Institute: NCT03412877
Antibody-armed T cells	MHC-independent antigen recognition	Barbara Ann Karmanos Cancer Institute: NCT02521090 University of Virginia: NCT03344250
Chimeric antigen receptor T cells	MHC-independent antigen recognition coupled with intracellular signaling domains	Baylor College of Medicine: NCT01109095, NCT02442297 Beijing Sanbo Brain Hospital: NCT02844062, NCT02937844 City of Hope Medical Center: NCT00730613, NCT03389230, NCT02208362 Duke University Medical Center: NCT02664363, NCT03283631 Fuda Cancer Hospital: NCT02575261 National Cancer Institute: NCT01454596 RenJi Hospital: NCT02331693 Shenzhen Geno-Immune Medical Institute: NCT03170141 University of Pennsylvania: NCT02209376

Abbreviations: MHC = major histocompatibility complex; HLA = human leukocyte antigen; CMV = cytomegalovirus, TCR = T-cell receptor