Chimeric antigen receptor T-cell immunotherapy for glioblastoma: practical insights for neurosurgeons

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The prognosis for glioblastoma (GBM) remains exceedingly poor despite state-of-the-art multimodal therapy. Immunotherapy, particularly with cytotoxic T cells, represents a promising alternative. Perhaps the most prominent T-cell technology is the chimeric antigen receptor (CAR), which in 2017 received accelerated approval from the Food and Drug Administration for the treatment of hematological malignancies. Several CARs for GBM have been recently tested in clinical trials with exciting results. The authors review these clinical data and discuss areas of ongoing research.

<https://thejns.org/doi/abs/10.3171/2018.2.FOCUS17788>

KEYWORDS immunotherapy; glioblastoma; chimeric antigen receptor

GLIOMASTOMA (GBM) is the most common and most lethal tumor of the central nervous system (CNS). Standard treatment includes resection, local and systemic chemotherapy, radiation therapy, antiangiogenic agents, and alternating electric fields. Despite this, the prognosis for patients with GBM remains exceedingly grim, with 5-year survival rates of less than 10%.³² In addition, conventional treatments are often limited due to adverse effects on normal, healthy tissues. As an alternative, immune-based approaches are rapidly emerging, with T cells in particular representing the critical component for antitumor responses. Perhaps the most prominent T-cell technology in development is the chimeric antigen receptor (CAR), an artificial, engineered molecule that has the capacity to redirect cytotoxicity against malignant cells ex-

pressing surface targets of interest. Several CARs for GBM have been recently reported in early clinical studies and have in at least one case mediated the complete regression of multifocal, bulky, invasive tumors. Here we summarize the groundbreaking data from these trials and discuss future directions for CAR therapy in patients with GBM.

CAR T Cells: A Brief Overview

Since their conception in 1989,¹³ CARs have shown remarkable success as an adoptive cell transfer (ACT) immune therapy for cancer, and in 2017 received accelerated approval from the US Food and Drug Administration (FDA) for the treatment of CD19-expressing leukemia. This breakthrough represented not only the first ACT plat-

ABBREVIATIONS ACT = adoptive cell transfer; CAR = chimeric antigen receptor; CNS = central nervous system; EGFRvIII = epidermal growth factor receptor variant III; EphA2 = erythropoietin-producing hepatocellular carcinoma A2; FDA = Food and Drug Administration; GBM = glioblastoma; HER2 = human epidermal growth factor receptor 2; HLA = human leukocyte antigen; IDO1 = indoleamine 2,3-dioxygenase 1; IL-13R α 2 = interleukin receptor 13R α 2; PD-L1 = programmed cell death ligand 1; scFv = single-chain antibody fragment; TCR = transgenic T-cell receptor; TIL = tumor-infiltrating lymphocyte; VST = virus-specific T cell.

SUBMITTED December 28, 2017. **ACCEPTED** February 6, 2018.

INCLUDE WHEN CITING DOI: 10.3171/2018.2.FOCUS17788.

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Nature Reviews | Clinical Oncology

FIG. 1. Chimeric antigen receptor (CAR) structures. (a) The diagram shows the schematic domain structure of a second-generation CAR: a single-chain variable region (scFv) of an antibody, which provides target specificity; hinge and transmembrane regions; a costimulatory domain; and a T-cell-activation domain. (b) The evolution of CAR designs. In contrast to second-generation CARs, first-generation CARs lack a costimulatory domain. In all of the second-generation CARs tested in lymphoma clinical trials to date, the costimulatory domain has been derived from either CD28 or 4-1BB, which are costimulatory receptors expressed on the surface of T cells. Third-generation CARs incorporate two costimulatory domains, derived from different costimulatory proteins, such as CD28 and 4-1BB. Reprinted by permission from Springer Nature: *Nature Reviews Clinical Oncology*, Chimeric antigen receptor T-cell therapies for lymphoma, Brudno JN, Kochenderfer JN, copyright 2018.

form available to patients with cancer but also the first-ever gene therapy approved by the FDA for any indication.²⁴ Despite rapid progress in CAR technology for hematological malignancies, successful translation for solid cancers, including GBM, has yet to be realized.

Structurally, CAR molecules consist of an extracellular, single-chain antibody fragment (scFv) translated in tandem with any number of intracellular T-cell signaling moieties. As such, T cells that are transduced to express CAR molecules have the capacity to release cytotoxic payloads on encounter with specific tumor antigens of interest. A significant advantage of this design is that, unlike ACT with ex vivo expanded tumor-infiltrating lymphocytes (TILs) or T cells expressing transgenic T-cell receptor (TCR), CAR T cells bypass the requirement for interaction with human leukocyte antigen (HLA) molecules, the downregulation of which is a well-characterized mechanism of tumor immune escape.

Although several modifications have been made to the original CAR constructs over time, the most important distinction between each “generation” to date has been the utilization of various signaling endodomains. Initial development of first-generation CARs employed the CD3ζ

chain in isolation, given its known role as the primary mediator of signals downstream of endogenous TCR. Subsequent generations have sought the addition of T-cell co-stimulatory signals (e.g., CD28, OX40, 4-1BB) with the goal of enhancing desired features such as cytokine production, proliferation, and persistence (Fig. 1). Occasionally, tumor-specific scFvs may be interchanged with alternative domains responsible for antigen recognition, such as the natural ligand for receptors with known expression on the surface of target cells.

Ultimately, manufacturing of CAR T cells can be labor-intensive, and currently available applications require harvesting a patient’s T cells, which are then gene-modified ex vivo with a given CAR molecule, expanded, and finally reintroduced either peripherally or directly into the CNS of patients with GBM.

CAR T Cells for GBM: Clinical Studies

Four GBM antigens have been targeted by CAR T cells in clinical studies, three of which have recently released results from clinical trials: epidermal growth factor receptor variant III (EGFRvIII),²⁶ human epidermal growth

TABLE 1. CAR T-cell clinical trials for glioblastoma

Study	No. of Pts, Institution	Registry No.	Generation	Route	Results
EGFRvIII					
O'Rourke et al., 2017	10, University of Pennsylvania	NCT02209376	2 (BBζ)	IV	5 SD
HER2					
Ahmed et al., 2017	17, Baylor University	NCT01109095	2 (28ζ)	IV	1 PR, 7 SD
IL-13Rα2					
Brown et al., 2015	3, City of Hope	NCT00730613	1 (ζ)	IC	1 PR
Brown et al., 2016	1, City of Hope	NCT02208362	2 (BBζ)	IC, IVT	1 CR

CR = complete response; IC = intracranial; IV = intravenous; IVT = intraventricular; PR = partial response; pts = patients; SD = stable disease.

factor receptor 2 (HER2),¹ and interleukin receptor 13Rα2 (IL-13Rα2).⁴ These studies are outlined in Table 1. Targeting erythropoietin-producing hepatocellular carcinoma A2 (EphA2) is also in clinical trials, but results have not yet been released.

EGFRvIII

The ideal target antigen for immune therapy is one that is present in tumor cells but is completely absent everywhere else in the body. The only known surface antigen with this desired expression pattern is EGFRvIII. EGFRvIII results from an in-frame deletion of the wild-type protein; this produces both a constitutively active receptor (i.e., ligand independent) and a unique epitope from the union of 2 normally distant portions of the extracellular domain. EGFRvIII was first identified in primary human GBM, where it can be found in approximately 30% of specimens.³³

In 2017, O'Rourke et al. reported on 10 patients with recurrent GBM and confirmed EGFRvIII positivity by next-generation sequencing assay, who were treated with a single-dose peripheral infusion of second-generation EGFRvIII CAR T cells (NCT02209376).^{17,26} The treatment was safe, with no dose-limiting toxicity. Following infusion, 1 patient in the trial was noted to have stable disease for more than 18 months without additional treatment. Despite having a small sample size, the study was optimized to obtain biological information from patients after receiving CAR T cells. Toward this end, 7 of 10 patients had posttherapy surgical intervention, which permitted direct examination of the tumor microenvironment by histopathological and molecular analyses.

Several important findings were noted from data obtained in this study. First, contrary to conventional notions of CNS immune privilege, posttherapy tumor specimens from 4 patients demonstrated efficient CAR T-cell trafficking to tumors in the brain. This was accompanied by vigorous infiltration of new-immigrant, nonmodified, endogenous T cells. Following treatment, tumor specimens were notable for decreased EGFRvIII expression, implying successful elimination of EGFRvIII-positive tumor cells. Residual and recurrent tumors, when compared to preinfusion specimens, also displayed a marked increase of several well-described immune-suppressive molecules, including programmed cell death ligand 1 (PD-L1), indoleamine 2,3-dioxygenase 1 (IDO1), IL-10, and FoxP3, the principal marker for suppressive, regulatory T cells. Additional studies will be needed to characterize the func-

tional significance of non-CAR-modified T-cell infiltrates, and to address the harsh, counterproductive immune microenvironment revealed in posttreatment specimens.

HER2

Most well-characterized tumor antigens are overexpressed proteins that are also present in normal cells (i.e., tumor-associated antigens). The HER2 tumor-associated antigen, also named HER2/neu after its original discovery in rodent neuroblastomas,²⁷ is a member of the human epidermal growth factor receptor family that has well-documented overexpression in several cancer types, with varying levels of expression in approximately 80% of GBM specimens.²² The gene encoding HER2 is ubiquitous, and the corresponding protein has been detected on the surface of a wide array of normal, healthy epithelial cell types.²⁸ Accordingly, treatments directed at HER2 carry the theoretical risk of on-target, off-tumor toxicity. One illustration is a 2010 case report of fatal toxicity in a patient receiving peripherally administered HER2-specific CAR T cells—thought to be due to antigen recognition during first-pass clearance in the lung—for the treatment of metastatic colon cancer to the lung and liver.²³

In 2017, Ahmed et al.¹ reported their initial clinical experience with a second-generation, HER2-specific CAR T cell in 17 patients with recurrent, HER2-positive GBM (NCT01109095). In this trial, CARs were transduced into T cells with endogenous TCR specificity for viral proteins (virus-specific T cells [VSTs]) in an attempt to optimize ACT persistence. Patients received one or more infusions of HER2 CAR T cells, which, in contrast to the aforementioned study, were well tolerated without dose-limiting toxicity. Notable differences that likely contributed to improved safety included 1) the use of a different scFv, 2) the use of second-generation signaling domains—as opposed to third-generation domains that resulted in fulminant autoimmunity, and 3) the absence of immune potentiation with concomitant IL-2 and preceding lymphodepletive chemotherapy.

Of the 17 patients treated, 1 patient with an unresectable, thalamic GBM was noted to have a partial response after the first infusion, and 3 patients in the study remained alive with stable disease for at least 24 months. Despite the incorporation of VSTs into the study design, infused CAR T cells were not noted to expand in the periphery. Certainly, however, these results lend further credence to the idea that peripherally administered CAR T cells might

be used to mediate safe and therapeutic effects against tumors in the CNS.

IL-13R α 2

Somewhat unexpectedly, the receptor for an immune-regulatory cytokine has emerged as an important surface marker for the selective targeting of GBM. In the immune system, IL-13 normally regulates immune responses along with its homolog, IL-4, through a shared receptor that is present in several normal tissues. Early studies revealed that nearly all GBM specimens abundantly expressed a receptor for IL-13, but unlike the IL-13 receptor found in other tissues, the receptor found on the surface of GBM was strictly IL-4 independent.¹⁰ This led to the discovery of the IL-13R α 2 protein chain, a tumor-associated component of the native receptor. While prominently expressed in the testes, this so-called cancer testis antigen (CTA) has been shown to have negligible expression at the transcript level in normal human adult tissues, including the CNS.⁹ To date, CARs directed at IL-13R α 2 are somewhat unique in that they have employed a membrane-tethered IL-13 ligand for cognate antigen recognition, rather than the traditional scFv, which has also been mutated at a single site (E13Y) to reduce affinity for the more widely expressed IL-13/IL-4 receptor complex.

First-in-human clinical experience with a first-generation CAR T cell targeting IL-13R α 2 was reported in 2015 by Brown et al. in 3 patients with recurrent GBM (NCT00730613).⁵ Notably, unlike the aforementioned clinical trials for EGFRvIII and HER2 in which patients received intravenous infusions, in the IL-13R α 2 study CAR-transduced T cells were delivered intracranially. Patients received up to 12 intracavitary infusions via an implanted Rickham reservoir and indwelling catheter placed at the time of surgery. The catheter tip was partially embedded into the resection wall in order to facilitate delivery into both the cavity and peritumoral brain tissue. Cells were manually injected using a 21-gauge butterfly needle in a total of 2 ml over 5–10 minutes. Treatment was well tolerated without serious adverse event associated with the device (e.g., malfunction or occlusion) or the biological itself. Following CAR T-cell infusion, a tumor specimen from 1 patient was obtained and was noted to have reduced overall IL-13R α 2 expression.

Based on these data, a second-generation IL-13R α 2 CAR T cell (i.e., 4-1BB, CD3 ζ) was pursued clinically, resulting this time in an astonishing antitumor response documented in a 2016 case report in *The New England Journal of Medicine* (NCT02208362).⁴ A 50-year-old patient had presented with multifocal GBM with leptomeningeal spread after having recently undergone resection of a dominant right temporal mass. A catheter had been placed at the time of surgery into the right temporal region, and the patient was treated with 6 weekly intracavitary infusions of second-generation IL-13R α 2 CAR T cells. During this time, there was no evidence of local disease progression; however, several distant intracranial lesions progressed and there was also development of a symptomatic, intradural metastatic focus in the spine at T-8. The patient was then administered 10 intraventricular infusions into the right lateral ventricle without further

therapeutic intervention. By the fifth intraventricular infusion, all tumors had decreased dramatically, and by the tenth infusion the tumors (including the T-8 lesion) were no longer measurable by MRI. This complete response was sustained for 7.5 months in the absence of serious treatment-related toxicity or hydrocephalus.

The investigators of these studies made several critical observations. Interestingly, the complete response observed after administration of second-generation IL-13R α 2 CAR was achieved despite heterogeneous target antigen receptor expression. That is to say, prior to treatment, the patient had no verified staining of the IL-13R α 2 antigen in 30% of his tumor. While the mechanisms underlying this outcome are still unclear, the ability to elicit broader effects against even tumor cells that do not express the target antigen of interest certainly highlights the potential involvement of endogenous immune responses. In addition, the route of administration appeared to be a crucial determinant in therapeutic success; whereas intracavitary infusion successfully abrogated local tumor progression, intraventricular infusion led to an impressive albeit temporary regression of even distant metastases.

Discussion

Results from these emerging clinical studies in patients with GBM suggest that CAR T cells may offer a feasible, safe, and effective strategy for eliciting antitumor responses in the CNS. Despite this promise, several obstacles and unresolved areas of future study remain. As trials advance into the next phase, perhaps a few of the central questions now posed will be addressed. For example, what will be the optimal mode of delivery for CAR T cells targeting GBM? How will antigenic heterogeneity and mechanisms of tumor immune evasion be addressed? Also, which imaging modalities and techniques may be optimal for assessing CAR T-cell localization and activity?

More than ever before, a number of state-of-the-art technologies are available and primed to help answer these questions. Collaborators in molecular imaging are now developing noninvasive techniques to monitor both T-cell viability and trafficking to GBMs through positron emission tomography (PET) (NCT00730613, NCT01082926).¹⁸ Such efforts, in conjunction with the elaboration of specific immune-related response criteria (irRC),^{25,34} may aid in the definition of more accurate end points in CAR T-cell clinical trials for GBM, given that standard radiographic assessment of local inflammation and treatment-related changes are often indistinguishable from tumor progression.

Advances in preclinical modeling and molecular biology may also lend significant insight into strategies for CAR T-cell delivery and mechanisms of tumor escape. Both intravenous and more recently orthotopic intracranial infusion⁷ with CAR T cells have been performed successfully in rodents. In addition, syngeneic or immune-competent preclinical systems,²⁹ while employed less frequently, offer an innovative strategy to assess for the potential involvement of endogenous immune responses, or alternatively, the impact of concomitant lymphodepletive effects of standard-of-care temozolomide chemotherapy. Numerous modifications and improvements of existing

CAR T-cell constructs for GBM have already been proposed; conceptually, these include CAR T cells that have the ability to target multiple surface antigens,^{3,12,14,15} express immune-potentiating cytokines or transgenes,^{18,19,30} and be imaged noninvasively without the use of radiotracers.^{20,21} Additional antigens that have been proposed as GBM-associated targets for CAR T cells include EphA2,⁸ chondroitin sulfate proteoglycan 4 (CSPG4),^{2,11} podoplanin (PDPN),³¹ the cancer stem cell antigen CD133,^{35,36} and the immune-suppressive ligand CD70.¹⁶ Clinical data are not yet available for CAR constructs targeting these antigens, though several are currently under investigation.

Conclusions

The translation of CAR T-cell therapy for patients with GBM is in its infancy. However, the revolutionary adoption of this technology for hematological malignancies and data from the early clinical trials summarized here suggest a bright future with fruitful areas of ongoing research. Almost certainly, the involvement of the neurosurgeon will remain paramount as direct, intracranial delivery methods continue to be refined. It is our hope that continued familiarity with this exciting therapeutic platform will foster additional collaboration toward fulfilling the great potential for CAR T-cell therapy in patients with GBM.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Choi. Drafting the article: Choi. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Choi. Study supervision: Choi.

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