CTIM-29

Clinical Evaluation of Chlorotoxin-Directed CAR T Cells for Patients With Recurrent Glioblastoma

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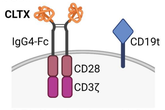
Introduction

- Glioblastoma (GBM) is the most common and aggressive type of malignant glioma.[1] Favorable treatment outcomes have been hindered, in part, by the substantial heterogeneity of these tumors[2]
- While antitumor bioactivity of glioma-targeting chimeric antigen receptor (CAR) T cells has been reported in a subset of patients with malignant glioma (including GBM),[3-5] tumor heterogeneity has limited the potency of adoptively transferred CAR T cells
- Chlorotoxin (CLTX) is a 36-amino acid peptide identified in scorpion venom and shown to selectively bind malignant glioma cells. Clinical administration of CLTX-based biologics has been well tolerated in patients (NCT00114309, 03579602)
- CLTX-directed CAR T cells (CLTX-EQ-28ζ/CD19t+ T cells), a novel immunotherapy developed by City of Hope, exploits the tumor-binding ability of CLTX to recognize a multireceptor complex comprising membrane-bound matrix metalloproteinase 2 (MMP2) on malignant glioma cells[2]
- Here we report the clinical findings of the first four patients treated in our first-in-human phase 1 study (NCT04214392) evaluating the safety and bioactivity of CLTX-CAR T cells in patients with MMP2+ recurrent GBM

Clinical CLTX-CAR

Our CLTX-CAR is comprised of the CLTX peptide, an IgG4-Fc(EQ) spacer, a CD28 transmembrane helix and costimulatory domain, and a CD3ζ cytoplasmic signaling domain. In addition, a T2A ribosomal skip sequence inserted after the CAR open reading frame allows for coexpression of truncated CD19 (CD19t), providing an inert, non-immunogenic surface marker for accurate measurement of gene-modified cells

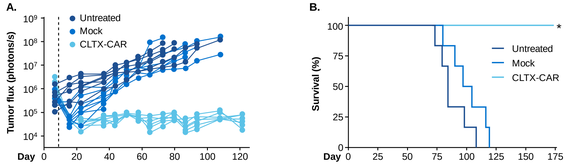


CLTX, chlorotoxin; Ig, immunoglobulin[10]

Preclinical In Vivo Antitumor Activity of CLTX-CAR T Cells

- CLTX peptide displays broad binding to GBM cells, detecting a greater number of GBM tumors and a high percentage of malignant cells within these tumors than other immunotherapy targets,[2] including interleukin (IL)13Rα2,[6] human epidermal growth factor receptor 2 (HER2),[7] and epidermal growth factor receptor (EGFR)[8]
- CLTX-CAR T cells recognize and kill MMP2+ glioma cells with high specificity and potency in vitro and in vivo
- A single intracranial intratumoral (ICT) administration of CLTX-CAR T cells (1×10[6] CAR T cells) demonstrated potent in vivo antitumor activity in orthotopic GBM xenograft mouse models compared with that of mock-transduced (no CAR) T cells[2]
  - Tumor regression as measured by firefly luciferase flux (photons/s) for each mouse treated with CLTX-CAR T cells (Figure 1A)
  - Significantly improved survival in mice treated with CLTX-CAR T cells as indicated by Kaplan-Meier survival curves (p=0.0004; Figure 1B)

Figure 1: Preclinical In Vivo Antitumor Activity



*p=0.0004 using the log-rank (Mantel-Cox) test; p=0.008 for CLTX-CAR T cell versus mock therapy.
CAR, chimeric antigen receptor; CLTX, chlorotoxin.
Wang D, et al. *Sci Transl Med*. 2020;12(533):eaaw2672.

Objectives

Primary: To assess the feasibility, safety, and the maximum tolerated dose (MTD) of dual delivery of CLTX-CAR T cells in patients with MMP2+ recurrent or progressive GBM.

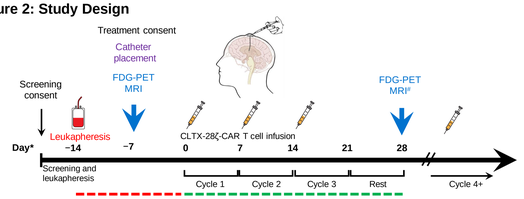
Secondary: To assess determinants of patient response and pathways of resistance, including estimating patient response rates and overall survival, describing persistence and phenotype of CLTX-CAR T cells, evaluating changes in inflammatory cytokines and host immune cells over the treatment time course, and monitoring target and other antigen expression levels in patients undergoing additional tumor resection after treatment.

Methods

Study Design

- This is a phase 1, single-center, dose-finding, safety and feasibility study of the adoptive transfer of CLTX-CAR T cells for the treatment of patients with MMP2+ recurrent or progressive GBM.
- Data were collected from 4 patients using City of Hope's electronic capture system and entered into protocol-specific electronic case report forms.
- Study treatment began with surgery (~day −7), followed by cycle 1 CAR T cell infusion on day 0 (Figure 2).
- The dose-limiting toxicity (DLT) period was cycle 1 through cycle 3 plus 1 additional week to assess the potential for late subacute adverse events (~28 days).

Figure 2: Study Design



*Days shown are approximate.
#Imaging can occur any time between day 21 and day 35.
CAR, chimeric antigen receptor; CLTX, chlorotoxin; DLT, dose-limiting toxicity; FDG-PET, fluorodeoxyglucose-positron emission tomography; MRI, magnetic resonance imaging.

- Current analysis shows data from 4 patients who had received ≥3 cycles of CLTX-CAR T cells ICT (Arm 1; 3-10 cycles; Figure 4) at dose level 1 (DS1: 4 million [M], 20M, 20M CAR T cells per cycle).

Figure 3: Treatment Plan

Treatment Arm 1 (ICT), involving weekly locoregional infusions of CLTX-CAR T cells directly into the tumor cavity

If safe (0 DLTs in 3 patients or ≤1 DLT in 6 patients)

Treatment Arm 2, involving dual ICT/ICV delivery would be opened at the arm 1 safe dose

CAR, chimeric antigen receptor; CLTX, chlorotoxin; DLT, dose-limiting toxicity; ICT, intracranial intratumoral; ICV, intracranial intraventricular.

- Intrapatient doses were escalated through dose levels labeled A, C, D, and H; inter-cohort doses were escalated per dosing schedules 1 and 2 (Table 1).

Table 1: Treatment Schema for Phase 1

Planned cycle	DS1	DS2	DS3
Cycle 1	A: 4×10[6]	C: 20×10[6]	C: 20×10[6]
Cycle 2	C: 20×10[6]	D: 50×10[6]	H: 100×10[6]
Cycle 3	C: 20×10[6]	D: 50×10[6]	H: 100×10[6]
Total dose/site*	44×10[6]	120×10[6]	220×10[6]
Evaluation/restaging			
Cycle 4+	≤20×10[6]	≤50×10[6]	≤100×10[6]
Cycles unrestricted#	≤20×10[6]	≤50×10[6]	≤100×10[6]

*The dose listed for each cycle is the CAR T cell dose intended for each delivery route. For dual delivery, 2 infusion doses will be prepared per infusion cycle, 1 each for the ICT and ICV delivery routes. To qualify for the dose-escalation portion of the study, the patient product must be able to deliver at least 80% of the total required cell dose for each cycle, 1-3.
#Unrestricted cycles are available after disease progression or if a patient has received a contraindicated drug.
CAR, chimeric antigen receptor; DS, dose schedule; ICT, intracranial intratumoral; ICV, intracranial intraventricular; A,C, D, and H, dose levels.

Key Eligibility Criteria

Inclusion Criteria

- Histopathological verification of WHO grade IV GBM
- Relapsed disease: radiographic evidence of recurrence/progression after standard therapy and ≥12 weeks from completion of front-line radiation therapy
- Age ≥18 years, Karnofsky Performance Status (KPS) ≥60%, and Eastern Cooperative Oncology Group (ECOG) ≤2
- Documented expression (≥20%) of CLTX-targeted antigen MMP2 (moderate and/or high expression [2+/3+])

Exclusion Criteria

- Bevacizumab therapy within 3 months of surgery due to wound-related complications and/or inadequate organ function, concurrent illness, malignancy, or comorbid conditions

Endpoints and Assessments

- **Primary:** DLTs, cytokine release syndrome, and all other toxicities (assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0
- **Secondary:** (i) CAR T cell and endogenous T cell levels (absolute number per µL by flow cytometry), phenotype, and cytokine levels in peripheral blood, tumor cyst fluid, and cerebrospinal fluid; (ii) progression-free survival (PFS) time, disease response by response assessment in neuro-oncology criteria with the need for bevacizumab as an additional indicator of progression, and survival time; (iii) detection of CAR T cells by immunohistochemistry and CLTX antigen expression levels by pathology H-score in tumor tissue; and (iv) biomathematical modeling of tumor growth using perfusion and growth parameters based on serial brain magnetic resonance imaging.

Statistical Analysis

- The toxicity equivalence range design of Blanchard and Longmate (2011)[9] will be used to determine the MTD. Rates (90% confidence intervals [CIs]) of PFS at 6 months, overall survival (OS) at 9 months, and disease response will be evaluated in patients who received the full schedule of 3 doses of CLTX-CAR T cells.
- A sample size of 12 at the MTD is expected to achieve (i) a maximum margin of error of 0.25 for a 90% CI for the DLT rate and (ii) the ability to detect a toxicity with a true rate of 0.20 in 93% of trials.
- The recommended phase 2 dose (RP2D) will be determined based on the MTD, toxicities in later cycles, and activity data. Rate and associated Clopper-Pearson binomial 90% CI will be estimated for patients experiencing DLTs in the RP2D schedule.

Results for Arm 1

- No patient experienced a DLT during the 28-day period for DLT evaluation (treated, n=4; evaluable, n=4; DLT=0)
- One patient experienced a serious adverse event of grade 3 cerebral edema, possibly attributed to CAR T cells (Table 2)

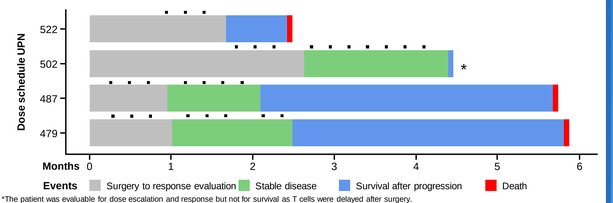
Table 2: Results From Arm 1 (ICT) Safety Lead-in

Arm	Grade ≥2 adverse events*	Best response
Arm 1 ICT (safety lead-in)	Grade 2 confusion (n=1) Grade 2 dysphasia (n=1) Grade 2 somnolence (n=1) Grade 2 hypertension (n=3) Grade 3 cerebral edema[#] (n=1)	Unconfirmed stable disease (n=3) Disease progression (n=1)

*Maximum grade toxicity attributed at the possible or above level to CAR T cells. Counts represent the number of patients experiencing the event.
#This event was an SAE but not a DLT as the attribution was only possible to CAR T cells.
CAR, chimeric antigen receptor; DLT, dose-limiting toxicity; ICT, intracranial intratumoral; SAE, severe adverse event.

- Of the 4 patients enrolled at the lowest dose level, the overall best response (overall response rate [ORR]) observed was stable disease (SD) in 3 patients, leading to an overall disease response rate (DRR) of 75% (90% CI, [25%, 99%]; Figure 4).

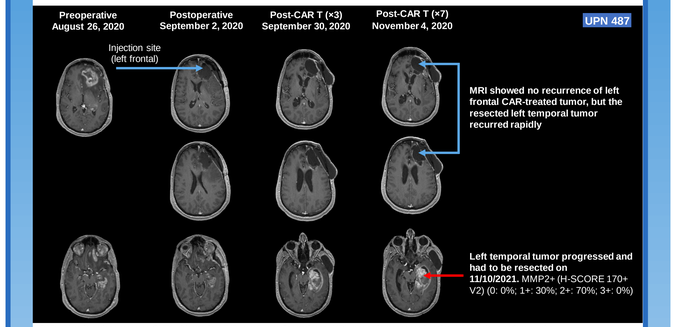
Figure 4: Treatment Response Time Course for the Intratumoral Arm



*The patient was evaluable for dose escalation and response but not for survival as T cells were delayed after surgery.
Events are color coded in the column chart; the dots above each column represent individual T cell infusions.
UPN, unique patient number.

Figure 5: Regional Control of Tumor Recurrence Following CLTX-CAR T Cell Therapy

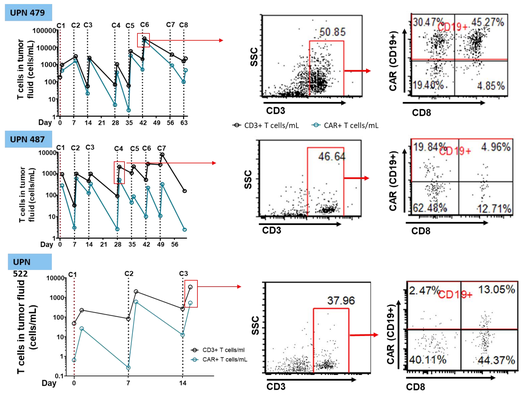
- Infusion of CAR T cells in the tumor cavity showed lack of tumor recurrence. (Figure 5)
- Tumor recurrence at sites without CAR T cell infusion further supports initiation of Arm 2 study involving dual intracranial intratumoral (ICT)/intracranial intraventricular (ICV) infusions.



CAR, chimeric antigen receptor; CLTX, chlorotoxin; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; UPN, unique patient number.

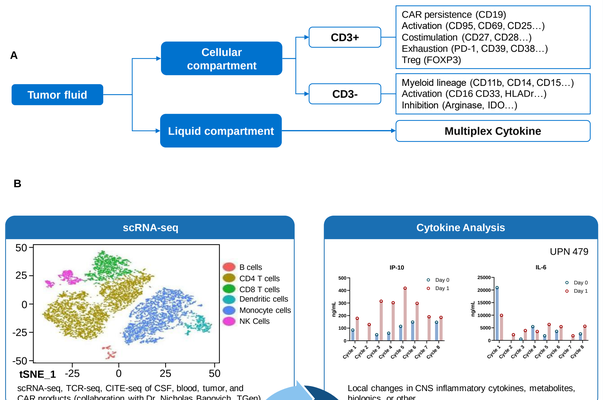
- Persistence of CAR T cells and endogenous immune cells in tumor fluid (TF) during CLTX-CAR T cell treatment was evaluated through flow cytometry (Figure 6A)
- CLTX-CAR T cell persistence in TF suggests the absence of immune rejection of the therapeutic cells (Figure 6B).

Figure 6: CAR T Cells Persist in TF Suggesting Non-Immunogenicity of Adoptively Transferred Cells



*Total T cell and CLTX-CAR T cell counts in the tumor fluid during CAR T cell therapy for UPN 479, UPN 487 and UPN 522 (left graphs). Dotted lines represent CAR-T cell infusion of 4M at cycle 1 (C1) and 20M at cycles 2+ (C2-C8). Tumor fluid could not be collected for UPN 522.
CAR, chimeric antigen receptor; CD, cluster of differentiation; CLTX, chlorotoxin; SSC, side scatter; UPN, unique patient number.

Figure 7: Correlative Studies



CAR, chimeric antigen receptor; CD, cluster of differentiation; CLTX, chlorotoxin; CNS, central nervous system; GBM, glioblastoma; MMP, matrix metalloproteinase; NK, natural killer; scRNA-seq, single-cell RNA sequencing.

Conclusions and Future Directions

- Overall, Arm 1-DS1 therapy using CLTX-CAR T cells was well tolerated, with no patient experiencing a DLT.
- Of the 4 patients enrolled in Arm 1 at the lowest dose level, the overall best response (ORR) observed was stable disease in 3 patients, leading to an overall disease response rate of 75%.
- Tumor recurrence occurred away from the CLTX-CAR T cell ICT infusion site, suggesting that Arm 2 dose levels (dual delivery of CLTX-CAR T cells with ICT and ICV catheters at higher doses) may offer hope to patients in the future.

References

1. Thakkar JP, et al. *Cancer Epidemiol Biomarkers Prev.* 2014;23(10):1985-96; **2.** Wang D, et al. *Sci Transl Med.* 2020;12(533):eaaw2672; **3.** Brown CE, et al. *N Engl J Med*. 2016;375(26):2561-9; **4.** Durgin JS, et al. *Front Oncol.* 2021;11:669071; **5.** Ahmed N, et al. *JAMA Oncol.* 2017;3(8):1094-101; **6.** Brown CE, et al. *Clin Cancer Res*. 2015;21(18):4062-72; **7.** Ahmed N, et al. *Clin Cancer Res*. 2010;16(2):474-85; **8.** O'Rourke DM, et al. *Sci Transl Med*. 2017;9(399):eaaa0984; **9.** Blanchard MS, Longmate JA. *Contemp Clin Trials*. 2011;32(1):114-21. **10.** Figure created with BioRender

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