

CD19-directed CAR T-cell therapy for treatment of primary CNS lymphoma

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Key Points

- CD19CAR T-cell therapy for patients with primary CNS lymphoma is feasible and safe.
- This case series demonstrates that IV-delivered CD19CAR T cells are promising for the treatment of primary CNS lymphoma.

CD19-directed chimeric antigen receptor (CD19CAR) T-cell therapy has been successful in treating several B-cell lineage malignancies, including B-cell non-Hodgkin lymphoma (NHL). This modality has not yet been extended to NHL manifesting in the central nervous system (CNS), primarily as a result of concerns for potential toxicity. CD19CAR T cells administered IV are detectable in cerebrospinal fluid (CSF), suggesting that chimeric antigen receptor (CAR) T cells can migrate from the periphery into the CNS, where they can potentially mediate antilymphoma activity. Here, we report the outcome of a subset of patients with primary CNS lymphoma (PCNSL; n = 5) who were treated with CD19CAR T cells in our ongoing phase 1 clinical trial. All patients developed grade ≥ 1 cytokine release syndrome and neurotoxicity post-CAR T-cell infusion; toxicities were reversible and tolerable, and there were no treatment-related deaths. At initial disease response, 3 of 5 patients (60%; 90% confidence interval, 19-92%) seemed to achieve complete remission, as indicated by resolution of enhancing brain lesions; the remaining 2 patients had stable disease. Although the study cohort was small, we demonstrate that using CD19CAR T cells to treat PCNSL can be safe and feasible. This trial was registered at www.clinicaltrials.gov as #NCT02153580.

Introduction

CD19-directed chimeric antigen receptor (CD19CAR) T cells have led to remarkable responses in B-cell malignancies, including non-Hodgkin lymphoma (NHL).¹⁻³ Clinical trials evaluating chimeric antigen receptor (CAR) T cells for NHL have largely excluded patients with central nervous system (CNS) lymphoma because of the fear of exacerbating potential neurotoxicity (NT) associated with CAR T cells.^{4,5} Yet, CD19CAR T cells administered IV are detectable in cerebrospinal fluid (CSF),⁶ suggesting that CAR T cells can migrate from the periphery into the CNS and mediate antilymphoma activity.⁷ Recent CD19CAR T-cell trials for NHL increasingly allow patients with secondary CNS lymphoma, and several reports have demonstrated the feasibility of treating patients with secondary CNS lymphoma with CD19CAR T cells.⁶⁻¹⁰ There is also a report describing an individual patient with primary CNS lymphoma (PCNSL) who was treated with a combination of CD19CAR and CD70CAR T cells.¹¹ However, it has not been reported whether CD19CAR T cells delivered IV can expand and/or traffic to the CNS in the absence of systemic lymphoma.

Here, we describe outcomes for a subgroup of patients with PCNSL (n = 5), who were treated at City of Hope (COH) on our ongoing phase 1 clinical trial (NCT02153580), and demonstrate the safety and feasibility of CD19CAR T-cell monotherapy in patients with PCNSL treated on a CD19CAR T-cell clinical trial.

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Data sharing requests should be sent to Tanya Siddiqi (tsiddiqi@coh.org).

The full-text version of this article contains a data supplement.

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Methods

We performed a retrospective analysis on a patient cohort enrolled in our ongoing phase 1 clinical trial that was approved by the COH Institutional Review Board. The study was conducted in accordance with the Declaration of Helsinki. Overall trial eligibility includes adults with recurrent, progressive, or refractory NHL or chronic lymphocytic leukemia with confirmed CD19⁺ disease. Disease response for NHL uses Lugano criteria.¹² Bridging therapy is allowed, including whole brain irradiation; imaging postbridging therapy is not required.

This subanalysis only describes patients with NHL who had PCNSL at enrollment. Patients received a fludarabine/cyclophosphamide-based lymphodepletion regimen prior to receiving CD19CAR T cells generated from autologous T naive/memory cells transduced with a CAR construct containing a CD28 costimulatory domain and coexpressing truncated epidermal growth factor receptor (EGFR).^{13,14} Two dose levels (DLs) were tested: DL1 = 200 million (M) CAR⁺ cells and DL2 = 600M CAR⁺ cells. All patients received levetiracetam for seizure prophylaxis. Initial disease response was assessed by positron emission tomography and brain magnetic resonance imaging (MRI) scans on day 28 post-CAR T-cell infusion; complete resolution of enhancing lesion on MRI was considered complete remission (CR).

Patients were monitored for cytokine release syndrome (CRS) using Lee et al criteria¹⁵ and for all other toxicities using the Common Terminology Criteria for Adverse Events v4.0. Patients who developed grade 2 CRS and NT could receive tocilizumab or dexamethasone at the discretion of the treating physician, according to COH standard operating procedures. Blood was collected via central line for correlative assays on day 0 and then weekly following CAR T-cell infusion for 4 of 5 patients. One patient declined all trial-related research studies following CAR T-cell infusion. CSF was collected at 3 time points for 1 of 5 patients. Blood and CSF were assessed for CAR T cells and lymphoma cells by flow cytometry and/or quantitative polymerase chain reaction to estimate lentiviral vector transgene copy number.

Results and discussion

As of October of 2020, we have treated 5 patients with PCNSL with CD19CAR T cells (Table 1). All patients had evidence of PCNSL (diffuse large B-cell lymphoma) at enrollment. The median age was 49 years (range, 42-53); all patients were female. The median number of prior therapies was 5 (range, 2-12; supplemental Table 1). Three patients received CD19CAR T cells at DL1, and 2 patients received CD19CAR T cells at DL2.

All patients developed grade \geq 1 CRS and NT post-CD19CAR T-cell infusion (Table 1), with highest-grade CRS of 2 and highest-grade NT of 3. Two of 5 patients were treated with tocilizumab and dexamethasone. Both patients exhibited grade 2 CRS; 1 patient also had grade 3 NT, and the other patient also had grade 1 NT. The remaining 3 patients did not require treatment for CRS or NT. All 3 patients had grade 1 CRS, whereas 2 had grade 1 NT, and 1 had grade 2 NT. The highest-grade toxicity (grade 3 NT) manifested as headache lasting 3 days, starting at day +5 postinfusion, with severe pain limiting self-care for \geq 1 day; the patient also had concurrent fever and cellulitis. Toxicities were reversible and tolerable, and there were no treatment-related deaths.

At initial disease response evaluation on day 28 postinfusion, 3 of 5 (60%; 90% confidence interval, 19-92%) patients seemed to achieve

Table 1. Patient characteristics and outcomes

Patient UPN	Age, y	Disease location (size at study entry)	KPS at study entry	Prior lines of therapy, n	Bridging therapy	Cell dose, M	CRS max grade	Symptoms (NT max grade)*	Intervention for CRS/NT	Best Response	Duration of response, d	Current status
286	53	Left temporal lobe (0.6 cm)	90	3	High-dose methotrexate-based chemotherapy	200	2	Headache (3), agitation (1), restlessness (1)	Toci and Dex	CR	273	Progressed/alive
310†	53	Posterior corpus callosum (1.6 cm)	80	6	Pomalidomide	115‡	1	Headache (2), dizziness (2), memory impairment (1)	None	SD	13	Off protocol therapy/alive, on maintenance pomalidomide
272	47	Right temporal lobe (3 cm), left temporal lobe (1.5 cm), and right basal ganglia (3 cm)	70	12	Brain radiation; steroids	200	1	Tremor (1), dysarthria (1), hallucinations (1)	None	SD	32	Progressed/lost to follow-up
346	49	Right temporal lobe (0.9 cm)	90	2	None	600	2	Concentration impairment (1), dysphasia (1)	Toci and Dex	CR	520§	CR/alive
475	42	Left basal ganglia (0.5 cm)	90	5	Brain radiation; steroids	600	1	Seizure (1), dizziness (1)	None	CR	43	Off protocol therapy/alive, on maintenance lenalidomide

Dex, dexamethasone; KPS, Karnofsky Performance Status; max, maximum; NA, not applicable; SD, stable disease; Toci, tocilizumab; UPN, unique patient number.

*Highest grade possibly, probably, or definitely related to T-cell infusion. Symptoms experienced by UPN 346 were probably related to T-cell infusion, whereas all other symptoms were possibly related.

†The patient declined all research studies on the trial after receiving CD19CAR T cells and received pomalidomide at day 28, which took them off protocol therapy.

‡The manufactured dose for UPN 310 was 115M.

§Most recent disease assessment based on clinical assessment.

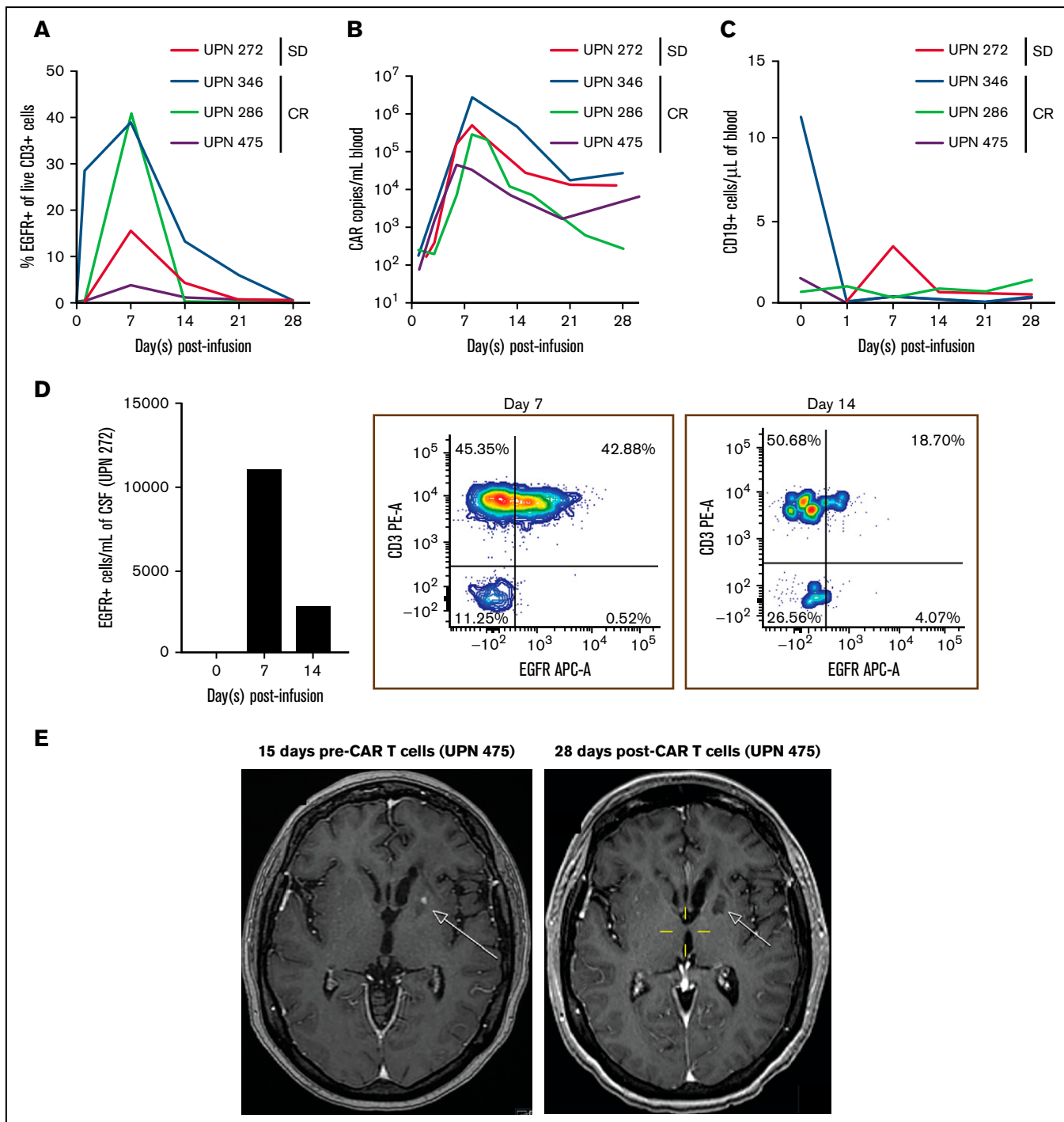


Figure 1. Percentages of CAR T cells in the blood and CSF. (A) Persistence of EGFR⁺ CAR T cells in blood circulation of patients after CAR T-cell infusion on days 0, 1, 7, 14, 21, and 28. The percentage of EGFR⁺ T cells are gated from live CD3⁺ cells. (B) Expansion and persistence of CD19CAR T cells in blood of patients, as measured by Woodchuck post-transcriptional regulatory element (WPRE) copy number per milliliter of blood. (C) Levels of CD19⁺ cells in blood circulation after CAR T-cell infusion. CD19⁺ cells are gated from live cells. (D) Persistence of EGFR⁺ CAR T cells in CSF from unique patient number (UPN) 272 on day 0 and on days 7 and 14 post-T-cell infusion. Absolute cells per milliliter of CSF (left panel) and flow cytometry plots of CD3⁺ cell and EGFR⁺ cells on day 7 (middle panel) and on day 14 (right panel) post-CAR T-cell infusion. (E) Brain MRI series of UPN 475 pre-CAR T-cell infusion (left panel) and post-CAR T-cell infusion (right panel). Images are T1 weighted postcontrast axial. The pretherapy scan shows an enhancing lesion in the left basal ganglia (arrow) that is no longer present at 28 days post-CAR T-cell infusion. SD, stable disease.

CR based on imaging; 2 patients had stable disease. Of those with CR, 1 progressed at day 273, 1 went on maintenance therapy at day 43, and 1 is still in follow-up without maintenance therapy at day 520. At last assessment, 4 of 5 patients were alive, and 1 patient was lost to follow-up. Blood collected from 4 of 5 patients during the 28 days postinfusion demonstrated CAR T-cell expansion by flow cytometry (Figure 1A) and quantitative polymerase chain reaction (Figure 1B), as well as the absence of CD19⁺ B cells or systemic lymphoma (Figure 1C). CSF collected from 1 patient showed CAR T cells by flow cytometry, demonstrating that IV-delivered CAR T cells could traffic to the CSF, despite the absence of systemic lymphoma (Figure 1D). In an image series for 1 patient with CR (Figure 1E), we observed a lesion pre-CAR T-cell infusion that was absent 28 days postinfusion. Patients with CR had small baseline lesions (Table 1), and it is possible that disease burden played a role in the response to IV CD19CAR T-cell therapy in the context of PCNSL.

Although the study cohort was small, we demonstrated that using CD19CAR T cells to treat PCNSL can be safe and feasible. We showed that CD19CAR T cells delivered IV could expand in the periphery and traffic to the CNS without stimulation by concurrent systemic lymphoma, consistent with Bishop et al, who reported expansion of the CD19CAR T-cell product tisagenlecleucel in patients without disease at infusion.¹⁶ A recent observation of CD19 expression by pericyte populations in the brain raises concern that targeting of these cells by CD19CAR T cells might contribute to NT.¹⁷ Our observation that patients with PCNSL treated with CD19CAR T cells developed reversible and tolerable grade \leq 3 NT suggests that targeting of pericytes was probably not a major issue here, but this will need to be confirmed in a larger cohort of patients.

This trial was originally designed for patients with systemic disease; thus, several aspects of the trial were not ideal for patients with PCNSL, particularly disease response criteria and lack of postbridging imaging. However, data from this preliminary cohort indicates that CD19CAR T cells may be promising for treatment of PCNSL. We observed clinical improvement in 3 of 5 patients, including 1 durable response (Table 1). Therefore, we are planning a prospective trial to assess CD19CAR T cells specifically in patients with PCNSL. Preliminary in vivo mouse models of CNS lymphoma^{13,18} and other CNS disease^{19,20} indicate that intraventricular CAR T-cell administration is more efficacious than IV delivery. Thus, we plan to evaluate

intraventricular delivery of CD19CAR T cells in patients with PCNSL. Other future avenues to investigate could include different targets and manufacturing platforms, among other variables. Overall, our data support further investigation into the use of CD19CAR T cells to treat PCNSL.

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Authorship

Contribution: T.S., X.W., M.S.B., J.R.W., C.E.B., and S.J.F. designed the clinical trial; T.S., S.J.F., L.L.P., L.E.B., M.S.B., and J.R.W. executed the clinical trial; X.W., L.L., and V.V. contributed to correlative studies; T.S., M.C.C., X.W., M.S.B., and T.L.S. prepared the manuscript; and all authors analyzed and interpreted data and reviewed the manuscript.

Conflict-of-interest disclosure: T.S. has served on an Advisory Board for Juno Therapeutics, Celgene, Bristol Myers Squibb, and Kite Pharma. M.S.B. has received funding from Mustang Bio. L.L.P. has conflicts of interest with Pfizer Inc., Novartis Pharmaceuticals, and F. Hoffmann-La Roche AG. C.E.B. receives royalty payments and research support from Mustang Bio and Chimeric Therapeutics. L.E.B. has received research support from Mustang Therapeutics, Genentech, Inc., Merck, and Amgen and has acted as a consultant for Gilead and Novartis. S.J.F. has received Grant support and is a stock/shareholder and has intellectual property for Mustang Bio and is a Stock/shareholder for Lixte Bio. The remaining authors declare no competing financial interests.

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References

1. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
2. Schuster SJ, Bishop MR, Tam CS, et al; JULIET Investigators. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45-56.
3. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396(10254):839-852.
4. Karschnia P, Jordan JT, Forst DA, et al. Clinical presentation, management, and biomarkers of neurotoxicity after adoptive immunotherapy with CAR T cells. *Blood*. 2019;133(20):2212-2221.
5. Gust J, Hay KA, Hanafi LA, et al. Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discov*. 2017;7(12):1404-1419.
6. Abramson JS, McGree B, Noyes S, et al. Anti-CD19 CAR T cells in CNS diffuse large-B-cell lymphoma. *N Engl J Med*. 2017;377(8):783-784.
7. Frigault MJ, Dietrich J, Martinez-Lage M, et al. Tisagenlecleucel CAR T-cell therapy in secondary CNS lymphoma. *Blood*. 2019;134(11):860-866.

8. Abbasi A, Peeke S, Shah N, et al. Axicabtagene ciloleucel CD19 CAR-T cell therapy results in high rates of systemic and neurologic remissions in ten patients with refractory large B cell lymphoma including two with HIV and viral hepatitis. *J Hematol Oncol*. 2020;13(1):1.
9. Rafelson W, Olszewski A. What a headache! Double-hit lymphoma with CNS recurrence - role of chimeric antigen receptor (CAR) T-cell therapy. *Leuk Lymphoma*. 2020;61(4):757-762.
10. Abramson JS, Palomba ML, Arnason JE, et al. Lisocabtagene maraleucel (liso-cel) treatment of patients (pts) with relapsed/refractory (R/R) B-cell non-Hodgkin lymphoma (NHL) and secondary CNS lymphoma: Initial results from TRANSCEND NHL 001. *J Clin Oncol*. 2019;37(15 suppl):7515.
11. Tu S, Zhou X, Guo Z, et al. CD19 and CD70 dual-target chimeric antigen receptor T-cell therapy for the treatment of relapsed and refractory primary central nervous system diffuse large B-cell lymphoma. *Front Oncol*. 2019;9(1350):1350.
12. Cheson BD, Fisher RI, Barrington SF, et al; United Kingdom National Cancer Research Institute. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3067.
13. Wang X, Huynh C, Urak R, et al. The cerebroventricular environment modifies CAR T cells for potent activity against both central nervous system and systemic lymphoma. *Cancer Immunol Res*. 2020;9(1):75-88.
14. Wang X, Popplewell LL, Wagner JR, et al. Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL. *Blood*. 2016;127(24):2980-2990.
15. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome [published correction appears in *Blood*. 2015;126(8):1048]. *Blood*. 2014;124(2):188-195.
16. Bishop MR, Maziarz RT, Waller EK, et al. Tisagenlecleucel in relapsed/refractory diffuse large B-cell lymphoma patients without measurable disease at infusion. *Blood Adv*. 2019;3(14):2230-2236.
17. Parker KR, Migliorini D, Perkey E, et al. Single-cell analyses identify brain mural cells expressing CD19 as potential off-tumor targets for CAR-T immunotherapies. *Cell*. 2020;183(1):126-142.e17.
18. Wang X, Huynh C, Urak R, et al. The cerebroventricular environment reprograms locally infused CAR T cells for superior activity against both CNS and systemic B cell lymphoma. *Blood*. 2018;132(suppl 1):965.
19. Priceman SJ, Tilakawardane D, Jeang B, et al. Regional delivery of chimeric antigen receptor-engineered T cells effectively targets HER2⁺ breast cancer metastasis to the brain. *Clin Cancer Res*. 2018;24(1):95-105.
20. Brown CE, Aguilar B, Starr R, et al. Optimization of IL13R α 2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against glioblastoma. *Mol Ther*. 2018;26(1):31-44.