haematologica
Journal of the Ferrata Storti Foundation

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by Dasom Lee and Saurabh Dahiya

Received: December 3, 2025.
Accepted: December 17, 2025.

Citation: Dasom Lee and Saurabh Dahiya. Mapping the immune orchestra of zamtocabtagene autoleucel in primary CNS lymphoma.
Haematologica. 2025 Dec 24. doi: 10.3324/haematol.2025.289294 [Epub ahead of print]

Mapping the immune orchestra of zamtocabtagene autoleucel in primary CNS lymphoma

Dasom Lee[1], Saurabh Dahiya[2]

[1]Division of Oncology, Department of Medicine, Stanford University, Stanford, CA

[2]Division of Blood and Marrow Transplantation and Cellular Therapy, Department of Medicine, Stanford University, CA

Declarations of Interest

Conflict of Interest

D.L. received honoraria from Kite/Gilead. S.D. Research Funding: Kite Pharma/Gilead. Advisory Board/Consulting: Kite Pharmaceuticals/Gilead Pharma, Bristol Myers Squibb, Incyte, Adaptive Biotechnologies, Novartis Pharmaceuticals, Johnson and Johnson (Jansen Pharmaceuticals)

Authors' Contributions

D.L. and S.D. wrote the manuscript and made the table.

To whom correspondence should be addressed:
Saurabh Dahiya, MD, FACP
Associate Professor
Blood and Marrow Transplantation and Cellular Therapy
Stanford Cancer Center
CJ 250D, 780 Welch Road
Stanford, CA, 94304
tel: +1-650.724.4155
fax: +1-650.498.6919
sdahiya@stanfor.edu

In this report, Hardy et al [1] present a notable case of a 75 year-old patient with Parkinson's Disease treated with zamtocabtagene autoleucel (zamto-cel) for relapsed primary CNS lymphoma (PCNSL) and achieved a clinical response. Zamto-cel is an investigational tandem CD20/CD19 CAR T cell product with dual targeting strategy that may prevent antigen-negative relapse, a well-recognized escape mechanism in B cell lymphomas. In this report, the authors are to be commended for undertaking such comprehensive immune profiling in a single case, yielding insights that extend beyond the anecdote. Serial flow cytometry measurements of peripheral blood and cerebrospinal fluid (CSF) quantified CAR T cell expansion and characterized immunophenotypes and memory subsets (stem cell memory, central memory, effector memory, and effector memory) using multicolor panels at multiple longitudinal timepoints in close intervals. In parallel, cytokine and chemokine dynamics were profiled using a 22-plex CodePlex Secretome technology in both compartments, providing additional insight into CAR T cell CNS trafficking. This combined clinical and correlative framework provides mechanistic insight into CAR T cell kinetics and CNS homing in PCNSL, a disease with substantial unmet need.

At the time of relapse, zamto-cel was available at this institution as a treatment option for relapsed or refractory PCNSL through the DALY II USA/MB-CART2019.1 trial (NCT04792489). However, this patient was ineligible, because the protocol requires a >1-cm measurable lesion, prompting treatment under a single-patient investigational new drug (IND). This scenario underscores a diagnostic and therapeutic challenge in patients with PCNSL on clinical trials. CNS disease is frequently radiographically subtle

or anatomically inaccessible, making confirmation of active disease difficult in both routine care and trial enrollment. MRI of brain and/or spine and lumbar puncture often provide limited assessment of PCNSL; MRI may not distinguish scarring from active disease, and CSF studies lack sensitivity for low-disease burden[2]. Cell-free tumor DNA in CSF and plasma, including PCNSL-specific mutations such as MYD88 L265P, is emerging as a promising diagnostic marker for PCNSL[3]. Further studies are needed to establish their feasibility of cell-free tumor DNA as a reliable biomarker as well as disease monitoring in clinical practice and future trials.

Although clinical trials and real-world series evaluating CAR T cell therapy focusing on PCNSL remain limited (**Table 1**), the data indicate favorable safety profiles, particularly in neurotoxicity. Despite initial concerns about CNS-related neurotoxicity, severe immune effector cell–associated neurotoxicity syndrome (ICANS) only occurs to a few, with no neurotoxicity-related deaths reported. Also, these findings alleviate the concerns fo tumor inflammation-associated neurotoxicity (TIAN)[4]. While this patient did not receive bridging therapy, most of cases in prior studies received bridging therapy such as high-dose methotrexate or cytarabine, radiation, BTK inhibitors, lenalidomide, or steroids before infusion to control CNS disease. Choquet et al reported poor outcomes from CAR T cell therapy in patients who fail to achieve response to bridging therapy[5]. Early results have been encouraging in terms of response rates. However, longer-term follow-up indicates that remissions may not be durable in many patients.[5, 6]. It will be important to determine in future studies whether adding bridging or adjuvant therapies could improve initial response or long-term remission rates after CAR T. Lastly,

the ongoing trial of axicabtagene ciloleucel in relapsed or refractory CNS lymphoma (NCT04608487) shows reassuring safety, durable responses, and with enriched serum and CSF correlative studies, continued follow-up will be critical to validate these findings[7].

In addition to clinical outcomes, immunologic findings from the case provide important clues. A key highlight is the detailed characterization of CAR T cell dynamics in the CSF. Despite the absence of systemic disease, there was CAR T cell expansion in both the peripheral blood and CSF, consistent with prior reports[8-10]. Similar to the peripheral blood, the CSF compartment at day+7 following zamto-cel infusion demonstrated a predominance of CD4+ CAR T cells, followed by CD8+ and a smaller $CD4^+/CD8^+$ double-positive subset. At day +14, despite complete disease clearance by flow cytometry at day+7, the total lymphocyte count in CSF remained elevated; the proportion of CD4+/CD8+ double-positive CAR T cells nearly doubled. Frigault et al has similarly shown preferential CD4+ CAR T cell expansion and the presence of CD4+/CD8+ double-positive CAR T cells in most patients' peripheral blood in the trial, although population analysis in CSF was not performed[9]. Given the small number in this study (n=12), the clinical impact of this double-positive subset on safety and efficacy is yet unclear. As noted by Hardy et al, $CD4^+/CD8^+$ double-positive T cells have been observed in inflammatory and viral settings and demonstrate lytic function and cytokine production, raising the possibility that this subset may contribute to immune modulation in PCNSL[11]. Further investigation in larger cohorts with integrated correlative studies in CSF compartment is needed to clarify their biological and clinical significance.

Lastly, the case report provides insight into how peripheral CAR T cells traffic into CNS disease. In this patient, a marked rise in CXCL10 on day +1, a chemokine that attracts activated T cells to the site of inflammation, suggests early formation of a gradient directing peripheral CAR T cells into the CNS. This observation aligns with Lacan et al showing upregulation of CCR5 and CCR6 in CAR T cells, homing receptors, to tumor antigen stimulation[10]. Frigault et al performed gene-expression analyses in CSF showing that complete responders display an interferon-rich immune profile with lower CCL4L1 and CCL3L1 levels, potentially limiting maladaptive trafficking signals and promoting more effective CAR T cell entry into the CNS[9]. It will be also interesting to see if resurgence of CAR T cell trafficking and homing occurs in CSF when the recurrence of CNS disease occurs. Larger cytokine and transcriptomic analyses will be warranted to confirm and define the mechanisms of CAR T cell therapy in PCNSL.

In summary, this case report by Hardy et al describes an elderly patient with Parkinson's disease and relapsed PCNSL treated with zamto-cel, demonstrating the safety and potential efficacy of this investigational product in CNS lymphoma. Hardy et al performed serial profiling of peripheral blood and CSF using multiparameter flow cytometry and multiplex cytokine assays, enabling detailed immunomonitoring throughout treatment. Clinically, the case aligns with emerging evidence that zamto-cel and other CAR T cell products exhibit encouraging safety, particularly with low rates of severe ICANS, and early efficacy in PCNSL. Serial flow cytometry revealed CAR T cell expansion in both peripheral blood and CSF, characterized by predominant CD4+

expansion and an increase in CD4+/CD8+ double-positive CAR T cells, the significance of which remains yet unclear. The observed day +1 surge in CXCL10 supports a chemokine-driven homing mechanism consistent with prior studies. Collectively, these findings provide biologic support for peripheral-to-CNS CAR T cell trafficking and highlight the need for larger correlative studies incorporating longitudinal cytokine, CSF, and transcriptomic analyses to clarify mechanisms of durable response and improve outcomes for patients with PCNSL.

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Table 1. Summary of key clinical and real-world evidence on the safety, outcomes, and correlative analyses of CAR T cell therapy in relapsed or refractory primary central nervous system lymphoma.

Study	Source	Product	Bridging Therapy[a] (%, n)	Neurotoxicity (%, n)	Efficacy	Correlative Assays
Siddiqi et al[8]	Phase 1 clinical trial	Autologous CD19 CAR product (CD28 costim; truncated EGFR: n = 5)	80% (4/5) (2 chemo-immunotherapy; 2 XRT + steroids)	Any gr: 100% (5/5) Gr ≥3: 20% (1/5)	3/5 achieved response (all CR); 1 later progressed. (Median f/u: not specified)	Flow cytometry and qPCR for CAR T cells in blood and CSF
Frigault et al[9]	Phase 1/2 clinical trial	Tisa-cel (n=12)	100% (12/12) (not specified)	Any gr: 50% (6/12) Gr ≥3: 8% (1/12)	7/12 achieved response (6 CR, 1PR); 4 later progressed. (Median f/u: 12.2 mo)	Flow cytometry and phenotyping for CAR T cells in blood and CSF; CSF gene-expression profiling; Serum/CSF cytokine profiling
Alcantara et al[12]	RWE (French network)	Tisa-cel (n=7) Axi-cel (n=2)	89% (8/9) (all chemo-immunotherapy)	Any gr: 56% (5/9) Gr ≥3: 22% (2/9)	- 6/9 achieved response (5 CR, 1 PR); 2 later progressed. - 6-month PFS rate of 44% and OS rate of 89%, (Median f/u: 8.5 mo)	None
Choquet et al[5]	RWE (French network)	Tisa-cel (n=16) Axi-cel (n=9)	96% (26/27[b]) (all chemo-immunotherapy)	Any gr: 68% (17/25) Gr ≥3: 20% (5/25)	- 20/25 achieved response (16 CR, 4 PR); 6 later progressed. - 12-month PFS rate of 46% and OS rate of 55% (Median f/u: 19.4 mo)	None
Mercadal et al[6]	RWE (CIBMTR)	Tisa-cel (n=21) Axi-cel (n=3)	25% (5/20[c]) (all chemo-immunotherapy)	Any gr: 33% (8/24) Gr ≥3: 8% (2/24)	- 14/23[c] achieved response by day+100 (11 CR; 3 PR) - 2-year PFS rate of 28% and OS rate of 50% (Median f/u: 26 mo)	None

[a]Chemotherapy regimens include high-dose methotrexate based regimen, high-dose AraC based regimen, pomalidomide, BTK inhibitors, lenalidomide, temozolomide, anti-PD1 therapy. [b]Of 27 patients, 2 did not receive CAR T cell therapy due to neurologic decline. Bridging therapy was administered in 26 of 27 patients. [c]Total number of patients may differ depending on the availability of evaluable data. EGFR, epidermal growth factor receptor; XRT, radiation therapy; gr, grade; CR, complete response; f/u, follow-up; CSF, cerebrospinal fluid; tisa-cel, tisagenlecleucel; PR, partial response; Mo, month; RWE, real-world experience; axi-cel, axicabtagene ciloleucel; PFS, progression-free survival; OS, overall survival; CIBMTR, Center for International Blood & Marrow Transplant Research