Extensive non-clonal CAR T-cell expansion causing fatal hyperinflammatory syndrome with immune effector cell-associated hemophagocytic lymphohistiocytosis, cytokine release syndrome, and neurotoxicity syndrome

Chimeric antigen receptor T-cell (CART) therapy has become the standard of care for patients with relapsed and refractory (R/R) B-cell neoplasia. However, CART-triggered cytokine release syndrome (CRS), immune effector-cell associated neurotoxicity syndrome (ICANS), or immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) may lead to significant non-relapse mortality. IEC-HS is a serious toxicity, defined by pathological macrophage activation associated with IEC therapy, characterized by cytopenias, hyperferritinemia, coagulopathy, and transaminitis.[1] Recently published real-world data on ciltacabtagene autoleucel (cilta-cel) report 5 cases of IEC-HS among 236 analyzed patients (2%).[2] Moreover, IEC-HS is observed not only following CART therapy itself but also in cases involving autologous stem cell boosts for IEC-associated hematotoxicity.[3] The symptom complex cannot be easily distinguished from severe CRS; however, an essential element is its clinical and chronological independence, as it often occurs after CRS and is accompanied by hemophagocytosis in bone marrow or other tissues.[1] We report a patient treated with cilta-cel for R/R myeloma who experienced massive, albeit non-clonal, CART expansion associated with severe hyperinflammatory syndrome characterized by overlapping features of CRS, ICANS, IEC-HS, leading to death 22 days after infusion.

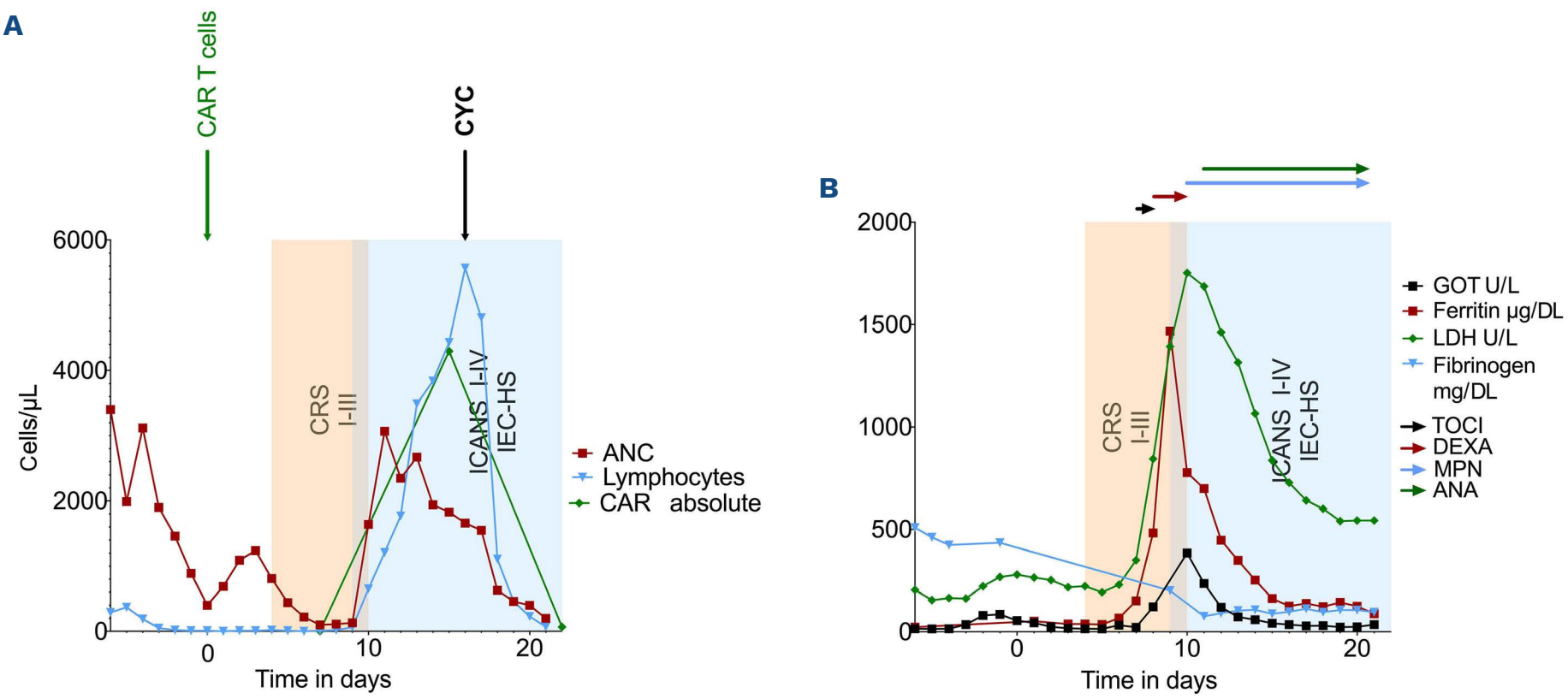
Samples and data were collected in accordance with the principles of the Declaration of Helsinki. Standardized sampling for CART phenotyping is approved by the Ethics Committee of the University of Tübingen, Germany. In accordance with local regulations, no formal ethics committee approval was required for this single case report. Written informed consent for further analyses and publication was obtained from the patient's family.

A 72-year-old male patient received cilta-cel in February 2024 for the treatment of refractory and rapidly progressive serological and morphological IgGκ myeloma. The initial diagnosis was made in May 2015, and six prior treatment lines had been administered before cilta-cel. Due to a substantial serological and morphological progression, the patient was admitted to our center for leukapheresis and to plan cilta-cel therapy in October 2023. In contrast to the stable serological parameters, a CT scan carried out in December 2023 showed massive progression affecting the entire axial skeleton, significantly jeopardizing the patient's physical stability. Extramedullary lesions were not observed. The last biopsy from July 2023 had shown an infiltration of 15-20%. According to these results, the CART therapy initially planned for December 2023 was postponed and radiotherapy was initiated to stabilize the osteolytic lesions. Before initiation of the therapy, the patient again showed significant serological progressive disease. His performance status was Eastern Cooperative Oncology Group grade 0. No cardiac disease was observed, and no comorbidities were known except for arterial hypertension.

CAR T cells were administered on day 0 after lymphodepletion with fludarabine and cyclophosphamide (30 mg/m^2 and 300 mg/m^2, day -5 to day -3). On day +4, the patient developed a fever, suggesting an infection or grade I CRS. Antibiotic therapy was escalated to meropenem and linezolid and later supplemented with levofloxacin. However, none of the microbiological investigations, including blood cultures (days -3/+4/+6/+22), urine cultures (days -3/+4/+6/+14) and viremia testing (days +1/+8/+16) identified a causative pathogen. Given the patient's cardiopulmonary stability and the fact that fever was the only initial symptom of CRS, we decided not to administer pre-emptive tocilizumab, consistent with the guidelines of the German Society for Hematology and Oncology. On day +7, there was a rapid deterioration in the patient's clinical condition, with hemodynamic instability and a requirement for vasopressor developing within a few hours, consistent with the diagnosis of a grade III CRS. Consequently, treatment with tocilizumab (800 mg, 4 times/day, days +7/+8) and dexamethasone (10 mg and then later 20 mg, 4 times/day, days +8 to +10) was initiated (Figure 1A, B). On day +9 and day +10 the patient developed renal failure requiring dialysis and peripheral necrosis, most likely as a consequence of disseminated intravascular coagulation (DIC) (Figure 1C). On day +9, blood tests showed strongly elevated inflammatory (IL-6: 73,072 ng/L) and altered IEC-HS markers (fibrinogen: 76 mg/dL; triglycerides: 581 mg/dL; bilirubin: 6.2 mg/dL; LDH: 1,752 U/L), resulting in an HScore of 195 points (reactive HS probability: 80-93%).[4] Despite CRS therapy, the patient developed grade II ICANS on day +9. Subsequently, therapy was escalated to methylprednisolone (initially 1,000 mg daily, day +10 to day +21) and anakinra (100 mg 4 times/day, day +11 to day +21). Initially, this led to a transient stabilization of the clinical situation, but no sustained improvement in neurological symptoms or renal function was observed. Due to the persistent and rapid

expansion of CART (day +15: 97.3% of $CD3^+$; absolute: 4,297/μL), cyclophosphamide (1,500 mg/m²) was administered on day +17, followed by dialysis for re-lymphodepletion.[5] Ruxolitinib was not considered as a treatment alternative, as it is only available as oral medication. The patient had persistent neurological impairment and did not tolerate a gastric tube. After administration of cyclophosphamide, the lymphocyte counts declined to 70/μL by day +21. However, the patient continued to deteriorate and by day +21, ICANS had progressed to grade IV. Neither MRI nor EEG revealed an explanation for the symptoms. In accordance with the family's wishes, we chose not to proceed with a lumbar and bone marrow puncture. The patient succumbed to multiple organ failure on day +22.

For flow cytometric analyses, isolated peripheral blood mononuclear cells were washed and incubated in a serum-human IgG solution (1 μg/mL, Merck). Following incubation and washing, anti-BCMA CAR reagent (MiltenyiBiotec) was added. Subsequently, Streptavidin-PE (ThermoFisher) was added along with panel-specific antibodies. Data acquisition was conducted using a LSR Fortessa (BD Biosciences). Phenotyping analyses of the peripheral blood showed massive CART expansion (day +10: 80.6%; day +15: 97.3% CAR^+) among $CD3^+$ cells (4,297 μL) (Figure 2A), predominantly of $CD4^+$ subtype (77.2%) (CD4/CD8 ratio: 3.61), along with a shift toward Th1 and Tc1 effector phenotypes (Figure 2B). In contrast, pharmacokinetic data from CARTITUDE-4 indicated a lower mean peak expansion of 1,451 CART/μL (SD 6169 CART/μL), while data from CARTITUDE-1 suggest a preferential expansion of $CD8^+$ central memory T cells.[6,7] Analysis of T-cell exhaustion revealed elevated expression of TIM3, PD1, and LAG3 on CAR^+ cells compared to CAR^- cells, while TIGIT expression was reduced (Figure 2C). Activation markers (CD40L, IFN-γ, CD69) showed no differences between T-cell populations. Additionally, the CAR^- population exhibited a higher proportion of $CD25^+$/$FOXP3^+$ regulatory T cells (Tregs), whereas only a minor fraction of CART was identified as Tregs (Figure 3A). This pattern highlights dysregulated CART activation. Supporting this, findings from Good *et al.* describe CAR Tregs with low cytotoxic properties, immunosuppressive function, characteristics associated with progressive disease, reduced



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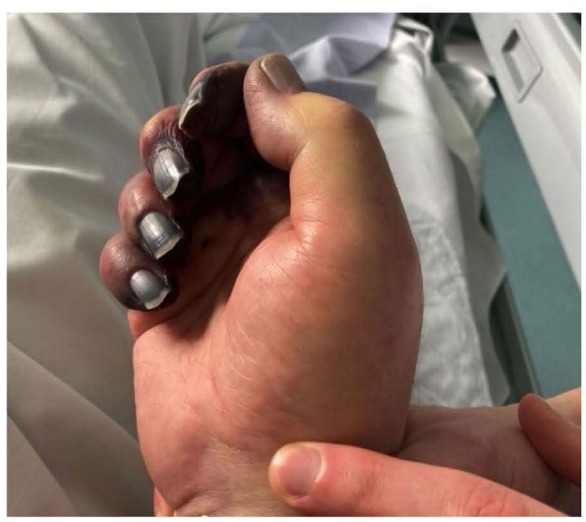

Figure 1. Clinical course and treatments. (A) Course of absolute neutrophil count (ANC), lymphocytes and chimeric antigen receptor positive (CAR^+) cells. Due to rapid expansion of CAR T cells, cyclophosphamide (CYC, 1,500 mg/m²) was administered on day 17 for re-lymphodepletion. Arrows indicate the time points of CAR T-cell and CYC application. The colored background represents the time period during which cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) or immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) were observed. (B) Courses of IEC-HS markers (GOT, ferritin, LDH, fibrinogen) reveal a chronological independence from CRS, with a renal failure requiring dialysis on day 9, and peripheral necrosis most likely as a consequence of disseminated intravascular coagulation (DIC) on day 10, prompting heparin treatment. Arrows indicate the therapies used to treat CRS, ICANS and IEC-HS. TOCI: tocilizumab; DEXA: dexamethasone; MPN: methylprednisolone; ANA: anakinra. (C) Severe peripheral necrosis as part of an IEC-HS-induced disseminated intravascular coagulation.

neurotoxicity, and lower expansion.[8] In this context, our observations suggest a more cytotoxic and subsequently exhausted phenotype of CART, most likely as a consequence of strong transient activation and enhanced initial efficacy.[9]

In November 2023, the US Food and Drug Administration (FDA) investigated the risk of secondary T-cell malignancies in patients treated with CART therapies.[10] At that time, over 20 cases of T-cell lymphoma associated with CART treatments had been documented. In several cases, CAR transgenes or transgenic products were detected in lymphoma samples.[11,12] Notably, Kobbe *et al*. described a case of IEC-HS arising from a $CD4^{-}CD8^{-}$ CAR^{+} peripheral T-cell lymphoma (PTCL), following treatment with tisagenlecleucel.[12] Due to the malignant-like characteristics of CART expansion, we performed T-cell clonality analysis in peripheral blood using flow cytometry (BD FACSLyric™) spectratyping of TCR Vb usage with the IO Test Beta Mark Kit (Beckman Coulter). The analysis demonstrated a diverse T-cell receptor repertoire among CART, suggesting robust expansion driven by strong activation rather than a rogue clone (Figure 3B).

Whole genome sequencing (WGS) was performed after cell sorting of CAR^{+} and CAR^{-} cells (Sony MA 900). Genomic DNA was isolated using the Monarch Genomic DNA Purification Kit (NEB) and quantified with Qubit dsDNA HS assay kit and Qubit fluorometer (Thermo Fisher). Libraries were prepared using the NEBNext FFPE DNA Library Prep kit (NEB), with 30-100 ng DNA, followed by fragmentation, amplification and double-sided bead purification. Library size (~500 bp) was assessed using the Fragment Analyzer (Agilent) and concentration (~10 ng/μL) measured with the Infinite 200Pro (Tecan) and Quant-iT HS Assay Kit (Thermo Fisher Scientific). Libraries were diluted to 162 pM and sequenced as paired-end 150 bp reads on an Illumina NovaSeq6000 (Illumina; San Diego, CA, USA) with a 35x covarage in control and 70x in CART. Data were processed using the in-house megSAP pipeline (https://github.com/imgag/megSAP, 2023_11) and the ngs-bits package (https://github.com/imgag/ngs-bits, version 2024_06). Alignment to the GRCh38 human reference genome was conducted using bwa-mem2 (version 2.2.1), and variant calling was performed using freebayes (version 1.3.6), VEP (version 110), and strelka (version 2.9.10) in paired-sample mode. WGS

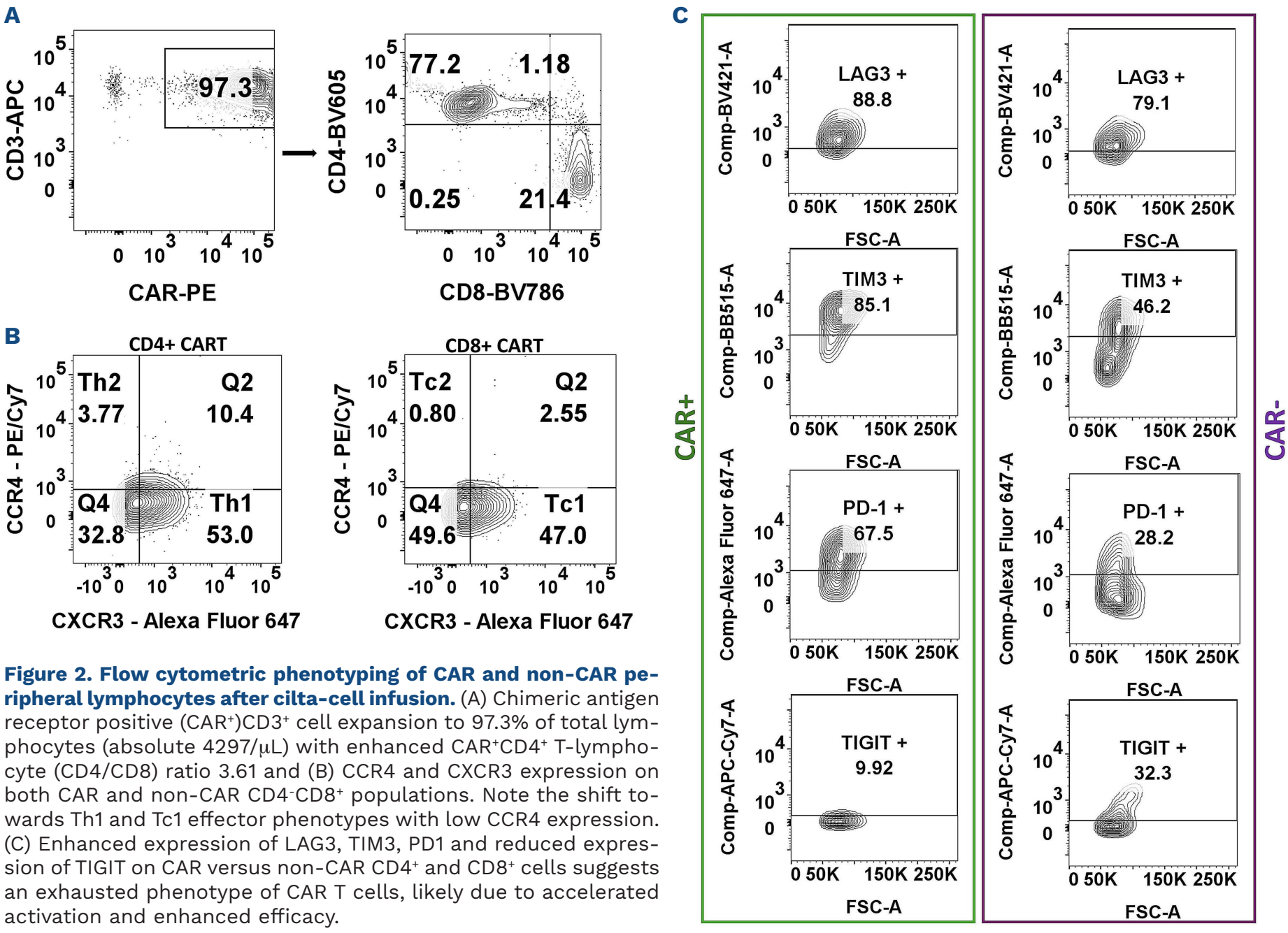

Figure 2. Flow cytometric phenotyping of CAR and non-CAR peripheral lymphocytes after cilta-cell infusion. (A) Chimeric antigen receptor positive (CAR^{+})$CD3^{+}$ cell expansion to 97.3% of total lymphocytes (absolute 4297/μL) with enhanced $CAR^{+}CD4^{+}$ T-lymphocyte (CD4/CD8) ratio 3.61 and (B) CCR4 and CXCR3 expression on both CAR and non-CAR $CD4^{-}CD8^{+}$ populations. Note the shift towards Th1 and Tc1 effector phenotypes with low CCR4 expression. (C) Enhanced expression of LAG3, TIM3, PD1 and reduced expression of TIGIT on CAR versus non-CAR $CD4^{+}$ and $CD8^{+}$ cells suggests an exhausted phenotype of CAR T cells, likely due to accelerated activation and enhanced efficacy.

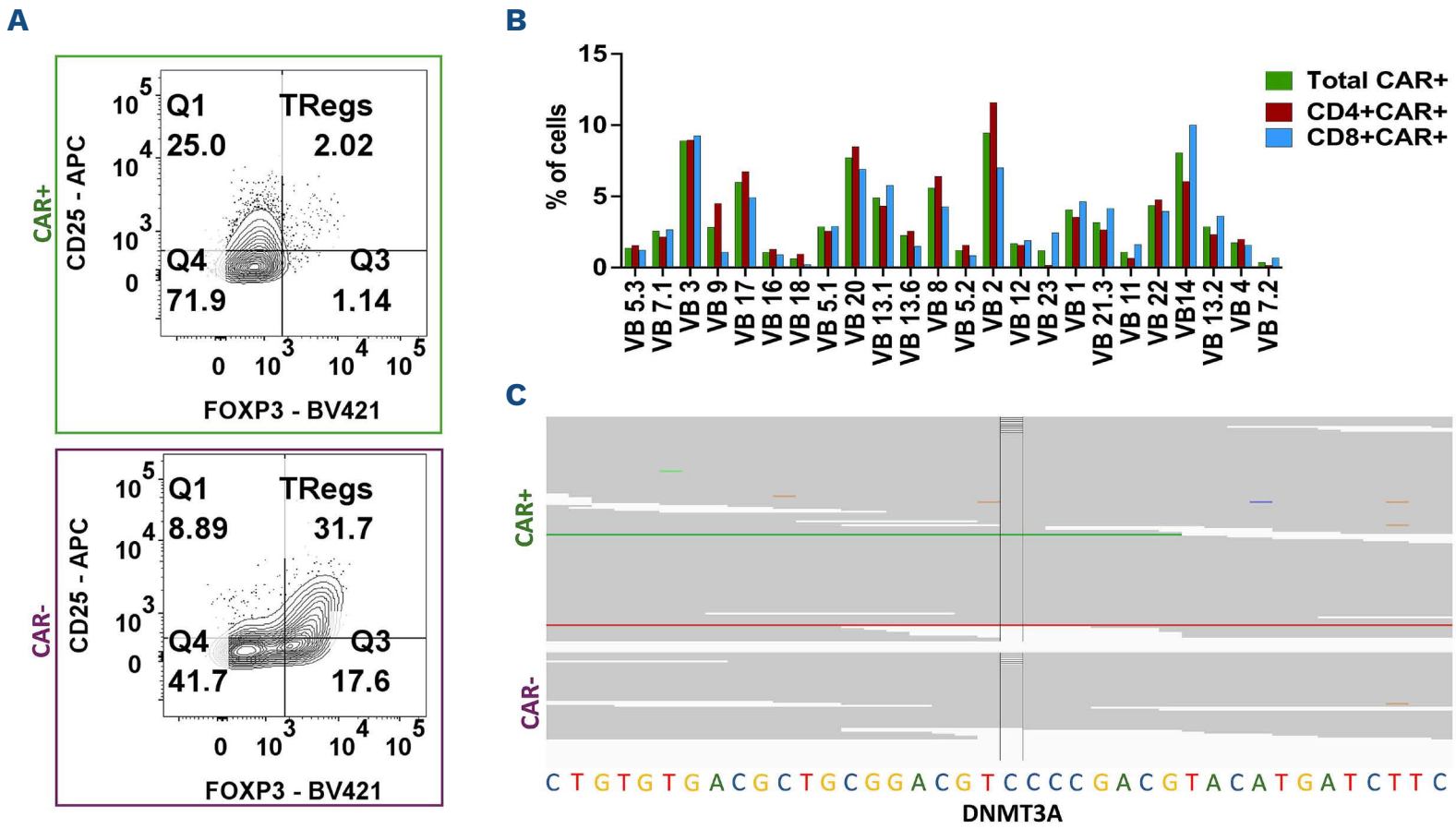

Figure 3. Chimeric antigen receptor expansion is driven by strong activation rather than a rogue clonal expansion. (A) Expression of CD25 and FOXP3 on chimeric antigen receptor (CAR) and non-CAR T cells indicates a deficiency of regulatory T cells in CAR *versus* non-CAR T cells. (B) Flow cytometry analysis of TCR Vβ usage of T-cell receptors on CAR⁺ T cells shows a robust expansion of polyclonal CAR T cells, with absence of a rogue clone. (C) Single cell whole genome sequencing reveals the presence of the same DNMT3A mutation in both CAR and non-CAR T cells. Integrative Genomics Viewer visualization of the DNMT3A frameshift mutation, detected in 6 of 39 reads (CAR⁻ T cells) and 9 of 109 reads (CAR⁺ T cells).

identified a frameshift mutation in DNMT3A. However, this mutation was not the result of insertional mutagenesis, as it was detected similarly in both CAR⁺ (9/109 reads) and CAR⁻ samples (6/39 reads) (Figure 3C). DNMT3A plays a crucial role in CART exhaustion, and preclinical studies have shown that deletion of DNMT3A may lead to enhanced CART efficacy and reduced exhaustion.[13]

In this study, we present a rare case of a hyperinflammatory overlapping syndrome associated with extensive CART activation and expansion. Beyond the clinical challenges of accurately diagnosing this rare condition and implementing re-lymphodepletion to control CART expansion, we conducted deep immunophenotyping of circulating CART and WGS. We conclude that massive CART expansion is not necessarily monoclonal or the result of lymphoma induction, but may also arise from overstimulation, e.g., by high tumor burden that is potentially further amplified by the presence of a pre-existing DNMT3A mutation in the T-cell compartment.

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WB declares consulting activities for Janssen. BB and AR declare honoraria from Janssen. All the other authors have no conflicts of interest to disclose.

Contributions

AR, LMK, SH, AEF, JCS, NC, GD, PJT and LH are responsible for data

collections. AR, LMK, NC, GD, PJT, LH and WB are responsible for data analysis. AR, CL and WB wrote the manuscript. AMPS, LMK, SH, AEF, JCS, BB, CF and LH edited the manuscript.

Data-sharing statement

Data are available on request from the corresponding author, Claudia Lengerke.

References

1. Hines MR, Knight TE, McNerney KO, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. Transplant Cell Ther. 2023;29(7):438.e1-438.e16.
2. Sidana S, Patel KK, Peres LC, et al. Safety and efficacy of standard-of-care ciltacabtagene autoleucel for relapsed/refractory multiple myeloma. Blood. 2025;145(1):85-97.
3. Ananth S, Torres NE, Sahaf B, et al. Fatal recurrence of IEC-HS following autologous stem cell boost in patients receiving BCMA CAR-T cell therapy. Blood Adv. 2025;9(15):3832-3836.
4. Fardet L, Galicier L, Lambotte O, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol. 2014;66(9):2613-2620.
5. Garfall AL, Lancaster E, Stadtmauer EA, et al. Posterior reversible encephalopathy syndrome (PRES) after infusion of anti-Bcma CAR T cells (CART-BCMA) for multiple myeloma: successful treatment with cyclophosphamide. Blood. 2016;128(22):5702-5702.
6. de Larrea CF, Harrison S, Martinez-Lopez J, et al. OA-45 Pharmacokinetic and correlative analysis of ciltacabtagene autoleucel in patients with lenalidomide-refractory multiple myeloma in the CARTITUDE-4 trial. Clin Lymphoma Myeloma Leuk. 2023;23(Suppl 2):S28.
7. Zudaire E, Madduri D, Usmani S, et al. Translational analysis from CARTITUDE-1, an ongoing phase 1b/2 study of JNJ-4528 BCMA-targeted CAR-T cell therapy in relapsed and/or refractory multiple myeloma (R/R MM), indicates preferential expansion of CD8+ T cell central memory cell subset. Blood. 219;134(Supplement_1):928.
8. Good Z, Spiegel JY, Sahaf B, et al. Post-infusion CAR TReg cells identify patients resistant to CD19-CAR therapy. Nat Med. 2022;28(9):1860-1871.
9. Knutson KL, Disis ML. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother. 2005;54(8):721-728.
10. FDA investigating CAR-related T-cell malignancies. Cancer Discov. 2024;14(1):9-10.
11. Ghilardi G, Fraietta JA, Gerson JN, et al. T cell lymphoma and secondary primary malignancy risk after commercial CAR T cell therapy. Nat Med. 2024;30(4):984-989.
12. Kobbe G, Brüggemann M, Baermann B-N, et al. Aggressive lymphoma after CD19 CAR T-cell therapy. N Engl J Med. 2024;391(13):1217-1226.
13. Prinzing B, Zebley CC, Petersen CT, et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. Sci Transl Med. 2021;13(620):eabh0272.