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Academic point-of-care anti-CD19 CAR T-cell therapy induces durable remission and transient paroxysmal nocturnal hemoglobinuria-type cell expansion in a patient with multi-refractory immune thrombocytopenia

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Author Contributions:

BvT conceptualized the treatment concept. All authors participated in the treatment. KH led the CAR-T cell manufacturing process. KAP, KH and BvT wrote the first draft. All authors made substantial contributions to the acquisition or interpretation of data for the work, reviewed the manuscript critically for important intellectual content, approved the final version to be published and agreed to be accountable for all aspects of the work.

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The dataset analyzed during the current study is available from the corresponding author on reasonable request

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Immune thrombocytopenia (ITP) is a disorder characterized by immune-mediated platelet destruction and impaired thrombopoiesis. While many patients achieve durable responses with established immunomodulatory and thrombopoietic therapies, a small subset develops multi-refractory disease associated with increased morbidity and mortality (1). Given the central role of autoreactive B cells in ITP pathogenesis, B-cell–directed therapies have emerged as a rational therapeutic strategy. Recently anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has shown encouraging efficacy in selected autoimmune diseases, raising interest in its potential for patients with ITP (2, 3).

We report the clinical course of a 68-year-old woman with multi-refractory ITP who achieved a durable remission following academic point-of-care anti-CD19 CAR T-cell therapy. Platelet counts normalized by day 31 after infusion and remained stable during follow-up. Treatment was well tolerated, with manageable cytokine release syndrome (CRS) and transient immune effector cell–associated hematotoxicity (ICAHT). Notably, flow cytometry demonstrated the transient emergence of paroxysmal nocturnal hemoglobinuria (PNH)-type cells in the absence of clinical manifestations.

The patient had chronic primary ITP for 16 months, complicated by WHO grade IV gastrointestinal bleeding. Previous therapies, including corticosteroids, intravenous immunoglobulin, thrombopoietin receptor agonists (eltrombopag, romiplostim, avatrombopag), fostamatinib, rituximab, azathioprine, cyclosporine, mycophenolate mofetil, plasmapheresis, and splenectomy, failed to induce a sustained remission. Given the pivotal role of B cells in ITP and encouraging data from autoimmune disease studies, anti-CD19 CAR T-cell therapy was considered as an individualized therapeutic approach (2, 3). Written informed consent and cost coverage were obtained in accordance with national regulations. The study was based on an individual treatment attempt performed outside of a clinical trial and conducted in accordance with national ethical standards for individual therapeutic attempts according to the principles of the declaration of Helsinki.

Leukapheresis and manufacturing were performed on-site at an academic institution. Processing of a total blood volume of 10 000mL over a total apheresis time of 189 minutes using a shaldon catheter in this case led to 2,27 x 10^9 $CD3^+$ cells in the apheresis material, thereby exceeding the required minimal threshold of 0.3 x 10^9 $CD3^+$ cells needed to initiate manufacturing. The CliniMACS Prodigy® platform was used to transduce T-cells with the lentiviral construct MB-CART19.1 (Miltenyi Biotec, Bergisch Gladbach, Germany), encoding a second-generation CAR vector containing a 4-1BB co-stimulatory domain. Lymphodepleting conditioning consisted of fludarabine (25 mg/m² on days –5 to –3) and cyclophosphamide (1,000 mg/m² on day –3). From 1 × 10^8 T cells at culture initiation, a total of 1.34 × 10^9 CAR^+ cells were generated, of which 1 × 10^6 CAR^+ cells/kg (5.4% of the generated drug substance) were formulated into the final product. The interval from leukapheresis to reinfusion was 13 days. In vivo CAR^+ cell expansion and T cell differentiation was evaluated by multicolor flow cytometry measuring $CD3^+$ T cells in peripheral blood and CAR^+ T cell frequency separately. $CD3^+$ T cell concentration in peripheral blood was determined either via clinical routine flow cytometry or using panels validated for cellular therapy manufacturing. For the detection of CAR^+ cells within the $CD3^+$ T-cell compartment, commercially available CD19 antigen-based, biotin-conjugated CAR detection reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) was used.

During conditioning, the patient developed urosepsis requiring brief intensive care unit admission but recovered fully prior to infusion. Grade 1 CRS occurred on day 3, progressed to grade 2 on day 4, and resolved after a single dose of tocilizumab (8 mg/kg; Fig 1a). No neurotoxicity or ≥ grade 3 non-hematologic adverse events were observed. CAR T-cell expansion peaked on day 11 (166 250 $CAR^+/CD3^+$ cells/mL; Fig 1b). $CD19^+$ B cells were undetectable before infusion due to previous rituximab exposure and remained absent through day 429. Platelet counts began to rise on day 11 and normalized by day 31, remaining stable at last follow-up 429 days after CAR-T-infusion (Fig 1a). Avatrombopag was discontinued after platelet normalization. Neutropenia (CTCAE grade 3) corresponding to early ICAHT (immune effector cell associated hematotoxicity) grade 1 first occurred on day

16 and was managed successfully with intermittent G-CSF on days 18, 23 and 28 (Fig. 1a). The patient was discharged on day 28 in good condition.

Unexpectedly, flow cytometric analysis on day 38 as part of the routine diagnostic workup revealed a population of partially glycosylphosphatidylinositol (GPI)-deficient granulocytes (max. 56%) and monocytes (max. 3%) detected by FLAER and CD157 staining, in the following referred to as PNH-like cells (Fig. 1b, 2). No clinical or laboratory evidence of hemolysis was present; moreover, no abnormal or prolonged neutropenia was observed following CAR T-cell therapy. A targeted myeloid next-generation-sequencing panel in pre-apheresis peripheral blood including *PIGA* sequencing revealed no pathogenic variants, however, variants of unknown significance (VUS; Tier 3) were identified in *BCORL1* and *SAMD9*. In addition, whole-genome sequencing was performed with analysis of current relevant genomic alterations, including small nucleotide variants (SNVs), copy number variants (CNVs) and structural Variants (SVs). Again, no pathogenic variants and the VUS in *BCORL1* and *SAMD9* were identified (data not shown). The PNH-like cells started to decline by day 100 and have no longer been detected at day 345. The decline coincided with the contraction of the CAR^+ cells after the initial expansion phase. Yet, CAR^+ T cells remained at levels above 1000 cells/mL up to day 233 and were still detectable at day 429, being in line with an ongoing B-cell aplasia. The CAR construct with a 4-1BB costimulatory domain used in this case is associated with prolonged CAR T-cell persistence and differentiation towards a central memory phenotype.

Thus far, only a few cases of successful CAR T-cell therapy for primary or secondary ITP have been reported (4 - 6). In addition to CD19 as a CAR T-cell target, as used in our case, an increased number of successful cases using BCMA (7) or dual target CD19/BCMA (8) CAR T-cells have been published. To our knowledge, there is only one other documented case using a point-of-care, academically manufactured CAR T-cell product for the treatment

of ITP in combination with autoimmune hemolytic anemia and antiphospholipid syndrome (9). Point-of-care CAR T-cell manufacturing requires approx. 2-3 × 10^8 T-cells in the apheresis product. Even with less starting material, a product can be successfully manufactured (data not shown). This is only a fraction compared to the requirements for commercial manufacturing starting material, ranging from approximately 1 x 10^9 to 3 x 10^9 T-cells depending on the product/manufacturer. Low lymphocyte or T-cell counts in peripheral blood below the recommended 200 $CD3^+$/µL might especially occur in patients after immunosuppressive treatment and would require the processing of up to 15-17 liters (depending on the product) of total blood volume (TBV) (10). Processing of such volumes leads to long apheresis times and easily exceeds the medically recommended processing volume in case of low-weight or frail patients. Based on collection data from our CAR T patient collective and a minimum of 3 × 10^8 T-cells in the starting material, apheresis time and processed TBV for point-of-care manufacturing can be reduced by approximately 60-80%. This would significantly reduce side effects and the need for shaldon catheters for large volume or long processing times. This is an advantage especially in patients potentially at risk for bleeding due to low platelet counts. Additionally, excessive T-cells can be cryopreserved and stored on-site. This enables re-manufacturing on a short notice in case of a process failure without the need for a second apheresis, increasing patient safety. Despite the low T-cell input due to a reduced peripheral blood T-cell count in this case (254/µl $CD3^+$ lymphocytes at the day before apheresis), with 2.27 x 10^9 $CD3^+$ cells in the apheresis material and 1.34 × 10^9 $CD3^+CAR^+$ in the end product the process still yielded sufficient drug substance for the formulation of a fresh product and multiple cryopreserved backup doses. Reduced requirements for the starting material, simplified logistics and shortened vein-to-vein time of academic CAR T-cell manufacturing allow access to CAR T-cell therapy for patients with compromised T-cell counts in peripheral blood and/ or poorly controlled disease (e.g. aggressive neoplasms with limited effective bridging therapy options or refractory autoimmune diseases not responding to current immunosuppression).

In addition, we report for the first time the occurrence of a large, yet transient, number of PNH-like leukocytes emerging after CAR T-cell therapy. Low-level PNH-type cells have previously been observed following CAR T-cell therapy in patients with B-cell malignancies and late ICAHT (11). The growth advantage of PNH-like hematopoietic stem and progenitor cells (HSPCs) in PNH has been linked to the reduced susceptibility to T-cell cytotoxicity of GPI-deficient cells (12). The absence of a PNH-typical *PIGA* mutation in the ITP patients cells is in line with the mere partial loss of GPI expression, reminiscent of type II PNH-type cells, and the absence of complete GPI-deficient PNH-like cells that are typically observed in PNH patients (Fig 2e). Interestingly, the patient carries a *BCORL1* mutation, which has been detected in acquired aplastic anemia (AA) and myelodysplastic syndrome (MDS) (13, 14). Also, an increased type II PNH cell population has been observed in the setting of immunologically mediated disorders such as AA and MDS and is less frequently associated with hemolytic manifestations (15, 16). The inflammatory stress associated with CAR T-cell therapy may transiently favor the selection of HSPCs or leukocytes with reduced GPI expression along the lines of the described elevated type II PNH-like cells in bone marrow failure (12). After all, the link between interferon (IFN)γ and acquired aplastic anemia is well established in both animal models and clinical analyses (17). The *BCORL1* mutation might confer susceptibility to immune-mediated bone marrow suppression and, as an escape mechanism, lead to the production of the type II PNH-like cells we have observed in the patient following CAR T-cell therapy. Yet, the exact mechanism of IFNγ induced bone marrow failure syndromes in humans is not completely understood. Also unknown is the mechanism of the observed reduced GPI expression. Due to clonal mosaicism, the presence of structural variants affecting *PIGA* and the limited sensitivity of bulk NGS and WGS approaches, the causative *PIGA* alteration may have remained undetected. Alternatively, if no *PIGA* mutation is truly present in this case, a post translational mechanism could be considered. For instance, endoplasmatic reticulum stress has been shown to impair the trafficking of misfolded GPI-anchored proteins and to result in their aberrant surface expression (17, 18).

In conclusion, academically manufactured anti-CD19 CAR T-cell therapy induced rapid and durable remission in a patient with multi-refractory ITP. The transient expansion of PNH-type cells suggests that CAR T-cell–induced immune pressure can affect hematopoietic clonal dynamics. Even though the clinical significance remains unclear so far, monitoring for PNH-type cells and underlying mutations of clonal hematopoiesis should be considered in patients receiving CAR T-cell therapy for non-malignant diseases.

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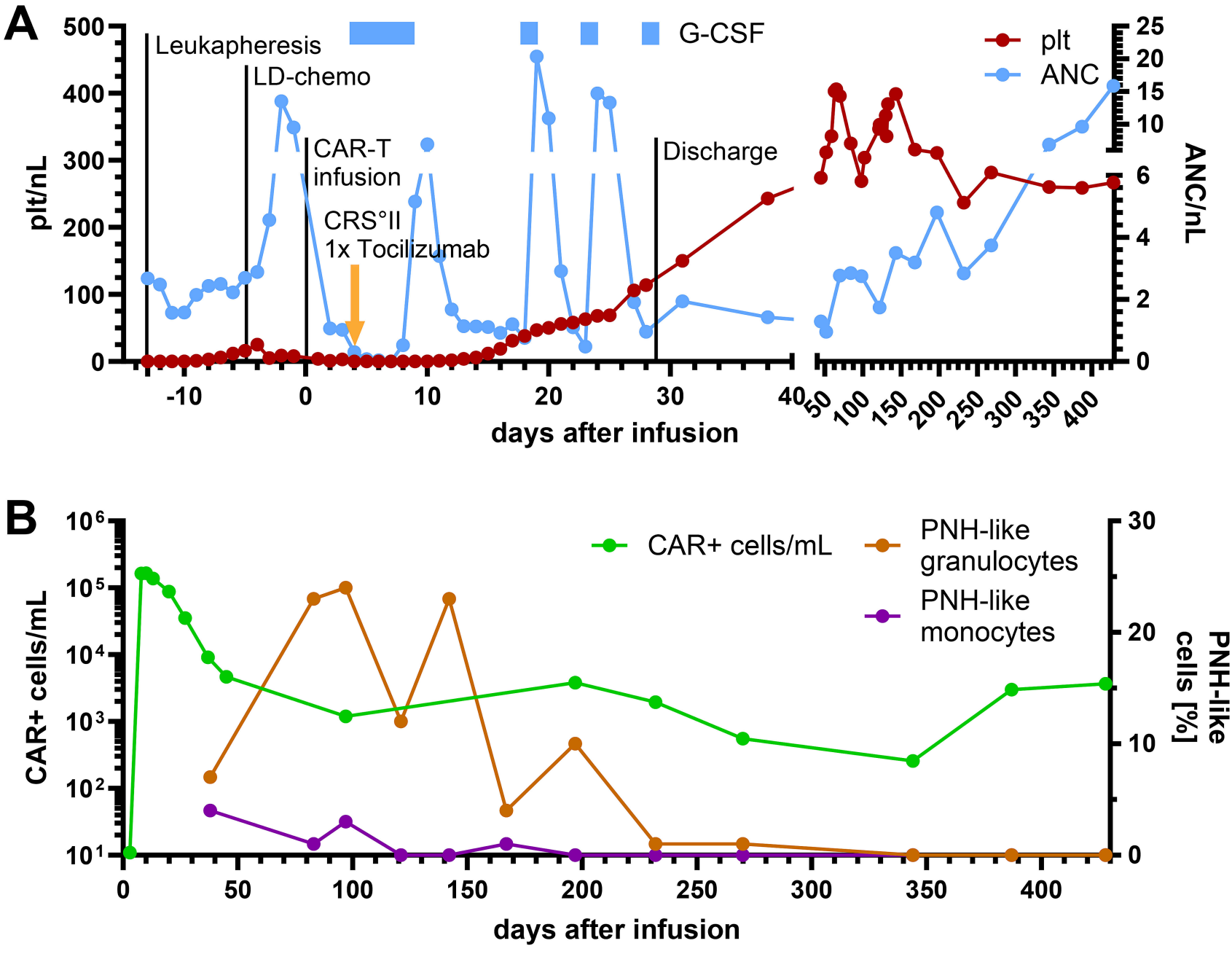
Figure legends:

Fig.1: Clinical course, hematologic recovery and cellular dynamics following CAR T-cell therapy. (a) Platelet counts (PLT) and absolute neutrophil count (ANC) after CAR T-cell reinfusion. Tocilizumab was administered once for CRS grade 2 (day 4). Granulocyte colony-stimulating factor (G-CSF) was administered for neutropenia after lymphodepleting chemotherapy in the first 7 days after reinfusion and in three single doses for immune effector cell–associated hematotoxicity; (b) $CD3^{+}CAR^{+}$ T-cell expansion and PNH-type cells (FLAER/CD157-deficient granulocytes and monocytes) dynamics.

Fig. 2: Flow cytometric analysis of GPI-expression in granulocytes (CD15+) and monocytes (CD64+) using FLAER/CD157 at serial time points. The presented patient showed exclusively the partially GPI-deficient type II populations. (a) No GPI-deficient/reduced cells were detectable 11 month prior to CAR T-cell therapy. (b) Type II PNH-like granulocytes and monocytes were first identified in April 2025, day 38 after CAR T-cell reinfusion, (c) peaked in June 2025 and (d) markedly declined by November 2025. (e) A representative patient with a clinical PNH manifestation displaying exclusively type III GPI-deficient granulocytes and monocytes is shown for comparison.


A
Leukapheresis
LD-chemo
CAR-T infusion
CRS°II
1x Tocilizumab
Discharge
G-CSF
plt
ANC
plt/nL
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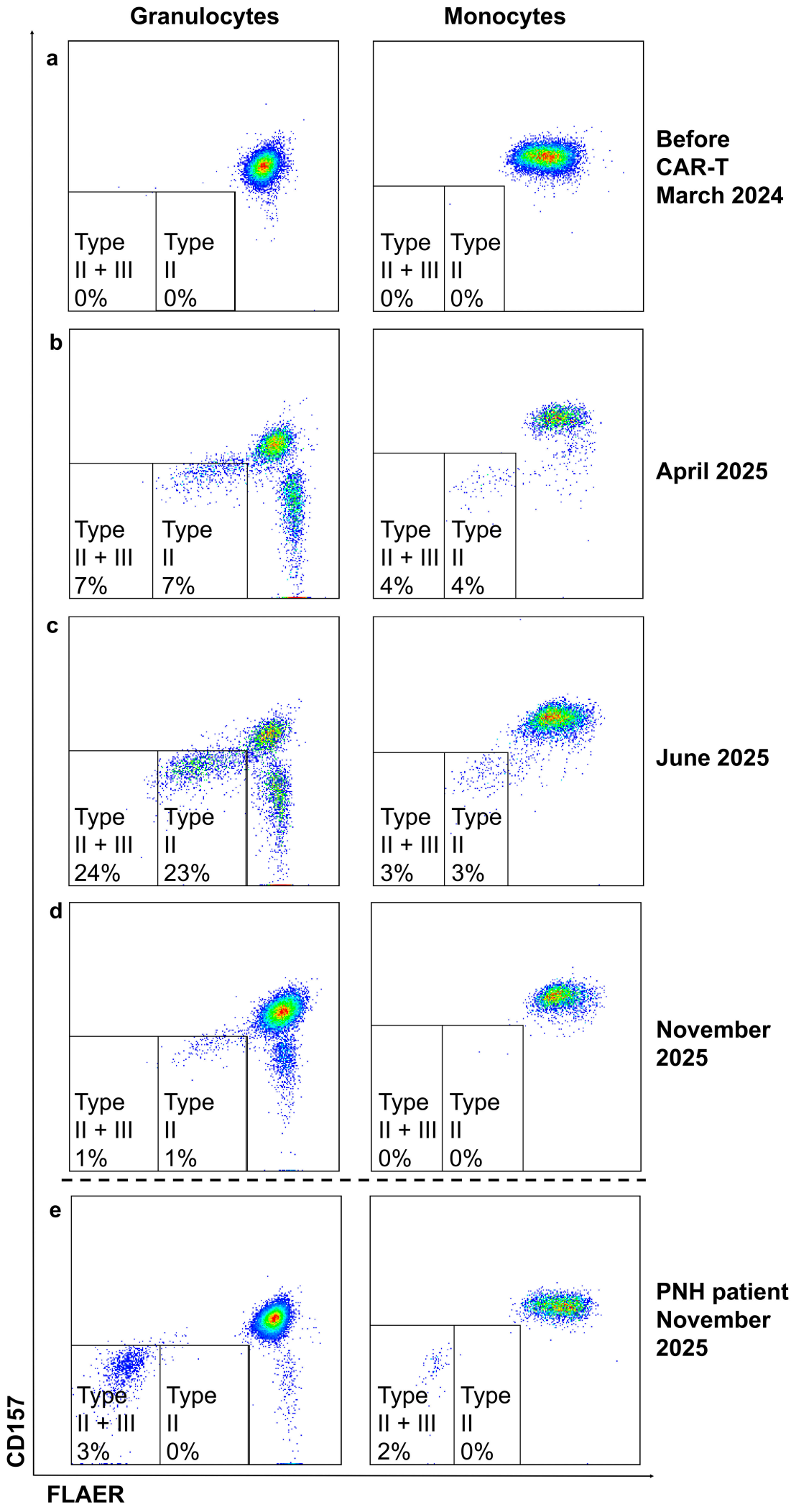
PNH-like granulocytes
PNH-like monocytes
CAR+ cells/mL
PNH-like cells [%]
days after infusion


Granulocytes
Monocytes
a
Type II + III 0%
Type II 0%
Type II + III 0%
Type II 0%
Before CAR-T March 2024
b
Type II + III 7%
Type II 7%
Type II + III 4%
Type II 4%
April 2025
c
Type II + III 24%
Type II 23%
Type II + III 3%
Type II 3%
June 2025
d
Type II + III 1%
Type II 1%
Type II + III 0%
Type II 0%
November 2025
e
Type II + III 3%
Type II 0%
Type II + III 2%
Type II 0%
PNH patient November 2025
CD157
FLAER