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

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 the pathogenesis of ITP, 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 the follow-up. The treatment was well tolerated, with manageable cytokine release syndrome and transient immune effector cell-associated hematotoxicity. 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 World Health Organization (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 studies in autoimmune disease, 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 product manufacturing were performed on-site at an academic institution. Processing of a total blood volume of 10,000 mL over a total apheresis time of 189 minutes using a Shaldon catheter in this case led to 2.27x10^9 $CD3^+$ cells in the apheresis material, thereby exceeding the required minimal threshold of 0.3x10^9 $CD3^+$ 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/m^2 on days –5 to –3) and cyclophosphamide (1,000 mg/m^2 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 routine clinical flow cytometry or using panels validated for manufacturing cellular therapy. For the detection of CAR^+ cells within the $CD3^+$ T-cell compartment, a commercially available CD19 antigen-based, biotin-conjugated CAR detection reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) was used.

During conditioning, the patient developed urosepsis requiring a brief admission to an intensive care unit, but recovered fully prior to infusion. Grade 1 cytokine release syndrome occurred on day 3, progressed to grade 2 on day 4, and resolved after a single dose of tocilizumab (8 mg/kg) (Figure 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) (Figure 1B). $CD19^+$ B cells were undetectable before infusion due to previous exposure to rituximab and remained absent through day 429. Platelet counts began to rise on day 11 and normalized by day 31, remaining stable at the last follow-up 429 days after the CAR-T infusion (Figure 1A). Avatrombopag was discontinued after normalization of the platelet count. Neutropenia (grade 3 according to Common Terminology Criteria for Adverse Events), corresponding to early immune effector cell associated hematotoxicity grade 1, first occurred on day 16 and was

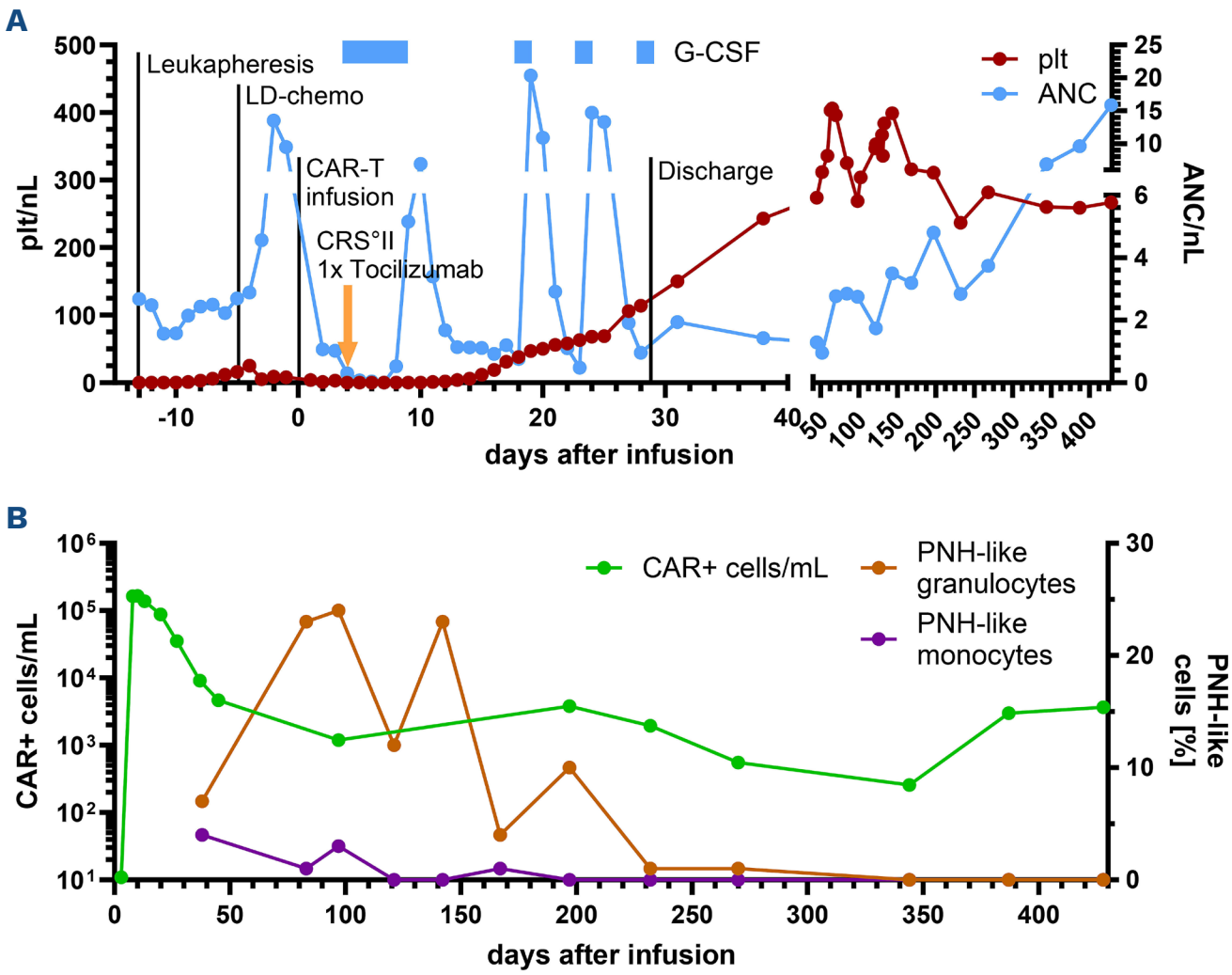


Figure 1. Clinical course, hematologic recovery and cellular dynamics following chimeric antigen receptor T-cell therapy. (A) Platelet counts and absolute neutrophil counts after chimeric antigen receptor (CAR) T-cell reinfusion. Tocilizumab was administered once for grade 2 cytokine release syndrome (day 4). Granulocyte colony-stimulating factor 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) Dynamics of $CD3^+CAR^+$ T-cell expansion and paroxysmal nocturnal hemoglobinuria-type cells (FLAER/CD157-deficient granulocytes and monocytes). ANC: absolute neutrophil count; CRS: cytokine release syndrome; G-CSF: granulocyte colony-stimulating factor; LD chemo: lymphodepleting chemotherapy; plt: platelets; PNH: paroxysmal nocturnal hemoglobinuria.

managed successfully with intermittent administration of granulocyte colony-stimulating factor on days 18, 23 and 28 (Figure 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 (maximum 56%) and monocytes (maximum 3%) detected by fluorescein-labeled proaerolysin (FLAER) and CD157 staining, in the following referred to as PNH-like cells (Figures 1B and 2). No clinical or laboratory evidence of hemolysis was present; moreover, abnormal or prolonged neutropenia was not 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, copy number variants, and structural variants. 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 were no longer detected at day 345. The decline coincided with the contraction of the CAR^+ cells after the initial phase of expansion. Yet, CAR^+ T cells remained at levels above 1,000 cells/mL up to day 233 and were still detectable at day 429, in line with an ongoing B-cell aplasia. The CAR construct with a 4-1BB co-stimulatory 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 increasing number of successful cases using B-cell maturation antigen (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 approximately $2\text{-}3\times10^8$ T cells in the apheresis

product and even with less starting material, a product can be successfully manufactured (*data not shown*). This is only a fraction compared to the requirements for starting material for commercial manufacturing, which range from approximately 1x10^9 to 3x10^9 T cells depending on the product/manufacturer. Low lymphocyte or T-cell counts in the peripheral blood, below the recommended 200 CD3$^+$/μL, might occur particularly in patients after immunosuppressive treatment and would require the processing of up to 15-17 liters (depending on the product) of total blood.[10] Processing such large 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 patients and a minimum of 3×10^8 T cells in the starting material, apheresis time and processed total blood volume 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 volumes or long processing times. This is an advantage, especially in patients potentially at risk of bleeding because of low platelet counts. Additionally, excessive T cells can be cryopreserved and stored on-site. This enables re-manufacturing at short notice in the case of a process failure, without the need for a second apheresis, increasing patients' safety. Despite the low T-cell input due to a reduced peripheral blood T-cell count in this case (254/μL CD3$^+$ lymphocytes on the day before apheresis), with 2.27x10^9 CD3$^+$ cells in the apheresis material and 1.34×10^9 CD3$^+$CAR$^+$ cells 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 levels of PNH-type cells have previously been observed following CAR T-cell therapy in patients with B-cell malignancies and late immune effector cell-associated hematotoxicity.[11] The growth advantage of PNH-like hematopoietic stem and progenitor cells 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 patient's ITP 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 (Figure 2E). Interestingly, the patient carries a *BCORL1* mutation, which has been detected in acquired aplastic anemia and myelodysplastic syndrome.[13,14] An increase of a type II PNH cell population has been observed in

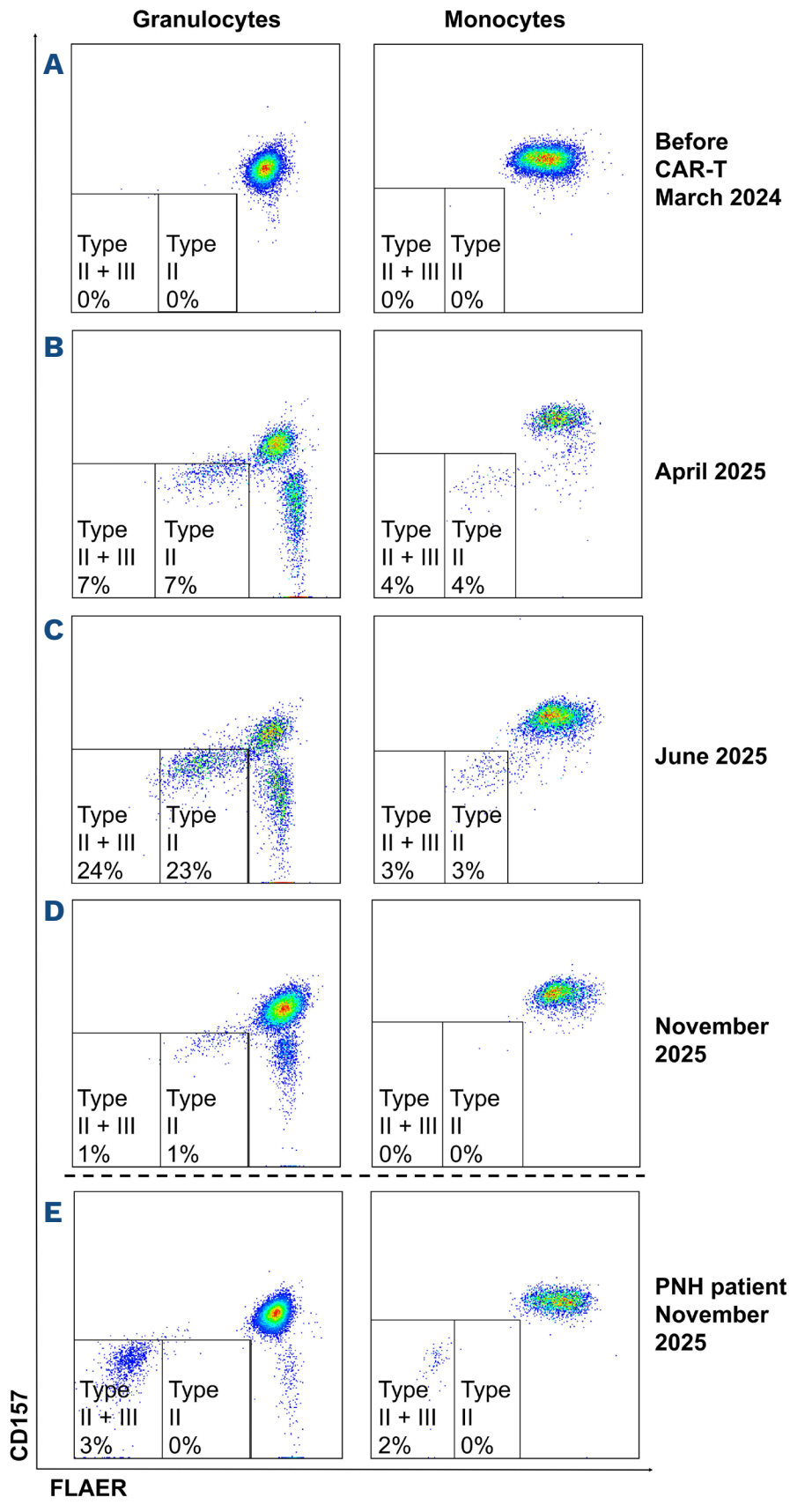


Figure 2. Flow cytometric analysis of glycosylphosphatidylinositol-expression in granulocytes (CD15$^+$) and monocytes (CD64$^+$) using FLAER/CD157 at serial time points. The patient presented showed exclusively the partially glycosylphosphatidylinositol (GPI)-deficient type II populations. (A) No GPI-deficient/reduced cells were detectable 11 months prior to chimeric antigen receptor (CAR) T-cell therapy. (B) Type II paroxysmal nocturnal hemoglobinuria (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.

the setting of immunologically mediated disorders such as aplastic anemia and myelodysplastic syndrome 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 hematopoietic stem and progenitor cells or leukocytes with reduced GPI expression along the lines of the described elevated levels of type II PNH-like cells in bone marrow failure.[12] After all, the link between interferon-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 observed in the patient following CAR T-cell therapy. Nevertheless, the exact mechanism of interferon-γ-induced bone marrow failure syndromes in humans is not completely understood. The mechanism of the observed reduced GPI expression is also unknown. Due to clonal mosaicism, the presence of structural variants affecting *PIGA* and the limited sensitivity of bulk next-generation sequencing and whole-genome sequencing 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, endoplasmic 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.

Authors

Kai Arne Plümer,* Keven Hörster,* Marcel Teichert, H. Christian Reinhardt, Asterios Tzalavras, Alexander Röth and Bastian von Tresckow

Department of Hematology and Stem Cell Transplantation, West German Cancer Center and German Cancer consortium (DKTK partner site Essen), University Hospital Essen, University of Duisburg-Essen, Essen, Germany

**KAP and KH contributed equally as first authors.*
Correspondence:
B. VON TRESCKOW - bastian.vontresckow@uk-essen.de

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Contributions

BvT conceptualized the treatment. 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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Data-sharing statement

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

References

1. Miltiadous O, Hou M, Bussel JB. Identifying and treating refractory ITP: difficulty in diagnosis and role of combination treatment. Blood. 2020;135(7):472-490.
2. Müller F, Taubmann J, Bucci L, et al. CD19 CAR T-cell therapy in autoimmune disease - a case series with follow-up. N Engl J Med. 2024;390(8):687-700.
3. Roeser A, Lazarus AH, Mahévas M. B cells and antibodies in refractory immune thrombocytopenia. Br J Haematol. 2023;203(1):43-53.
4. Trautmann-Grill K, von Bonin M, Georgi A, et al. Salvage treatment of multi-refractory primary immune thrombocytopenia with CD19 CAR T cells. Lancet. 2025;405(10472):25-28.
5. Mengli X, Shiqi L, Zhi Y, et al. Autologous CD19-directed CAR-T exhibit promising safety and efficacy in refractory primary immune thrombocytopenia. Blood. 2025;146(Suppl 1):4175-4176.
6. Li M, Zhao J, Lu ZJ, et al. Anti-CD19 CAR T cell therapy for refractory SLE-ITP. Med. 2026;7(4):101039.
7. Jinhui S, Min X, Wei X, et al. BCMA-targeted CAR T cells for multi-refractory primary immune thrombocytopenia: a pilot study. Blood. 2025;146(Suppl 1):1267.
8. Yang M, Liu T, Chen B, et al. P0S0061 BCMA-CD19 dual-targeting CAR-T cell for the treatment of refractory thrombocytopenia associated with autoimmune disease. Ann Rheum Dis. 2025;84(Suppl 1):368-369.
9. Korte IK, Kharboutli S, Völkl S, et al. CD19 CAR-T therapy induces remission in refractory autoimmune hemolytic anemia with ITP and antiphospholipid syndrome. Med. 2026;7(5):101075.
10. Hayden PJ, Roddie C, Bader P, et al. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). Ann Oncol. 2022;33(3):259-275.
11. Yoshihara K, Matsuda I, Utsunomiya N, et al. High prevalence of PNH-phenotype cells in patients who received CD19-targeted CAR T-cell therapy. Hemasphere. 2021;5(9):e628.
12. Luzzatto L, Nakao S. Pathogenesis of paroxysmal nocturnal hemoglobinuria. Blood. 2025;145(26):3077-3088.
13. Chang H, Zeng F, Zhang JY, et al. Association of the interferon-gamma single nucleotide polymorphism +874(T/A) with response to immunosuppressive therapy in patients with severe aplastic anemia. Blood Cells Mol Dis. 2010;45(4):313-316.
14. Fermo E, Bianchi P, Barcellini W, et al. Immunoregulatory cytokine polymorphisms in Italian patients affected by paroxysmal nocturnal haemoglobinuria and aplastic anaemia. Eur J Immunogenet. 2004;31(6):267-269.
15. Cannizzo E, Raia M, De Propris MS, et al. Features, reason for testing, and changes with time of 583 paroxysmal nocturnal hemoglobinuria clones from 529 patients: a multicenter Italian study. Ann Hematol. 2019;98(5):1083-1093.
16. Wagner-Ballon O, Raimbault A, Debord C, et al. Neutrophil PNH type II cells are associated with thrombosis and bone marrow failure without hemolysis: evidence from analysis of the 5-year French nation-wide multicenter observational study. Blood. 2023;142(Suppl 1):4083.
17. Lin FC, Karwan M, Saleh B, et al. IFN-γ causes aplastic anemia by altering hematopoietic stem/progenitor cell composition and disrupting lineage differentiation. Blood. 2014;124(25):3699-3708.
18. Satpute-Krishnan P, Ajinkya M, Bhat S, Itakura E, Hegde RS, Lippincott-Schwartz J. ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway. Cell. 2014;158(3):522-533.