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Kinetics of CAR-T cells and the immunological profile after tisagenlecleucel therapy

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Over the last decade, the use of chimeric antigen receptor (CAR) T cells has emerged a new strategy in treatment of relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). The immune activation plays a pivotal role both in therapeutic effect of CAR-T cells and the side effects of the therapy. The most common toxicities related to CAR-T cell treatment, which are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), are caused by the excessive activation of effector cells and the release of high levels of cytokines [1, 2]. In this report, we analyzed the profile of immunological response in a patient treated with CAR-T cells due to primary refractory ALL.

The patient, a 5-year-old girl was diagnosed with B-common ALL with co-expression of CD36 in December 2022. Since the diagnosis, she has been receiving treatment according to the AIEOP-BFM-2017-Poland therapeutic protocol. On the 15th day of treatment, the therapy response was unsatisfactory, with 49.5% blast cells in the bone marrow. On the 33rd day, minimal residual disease (MRD) was measured at 3×10^{-1}. Due to the identification of activating aberrations of the ABL-kinase family in blast cells, the therapy was switched to imatinib-based EsPHALL-2017 protocol. At that point a bone marrow aspirate biopsy was

repeated, revealing 29.5% blast cells. She was subsequently diagnosed with primary refractory ALL and qualified for CAR-T cell therapy.

The bridging therapy was based on FRALLPOST-2004 protocol with addition of imatinib. Prior to CAR-T cell infusion, a lymphodepleting regimen consisted of fludarabine and cyclophosphamide was administered. Subsequently, on 9-May-2023 the patient received an infusion of anti-CD19 CAR-T cell (tisagenlecleucel, Novartis). No immediate infusion-related toxic effects were observed. The post CAR-T cell infusion course was complicated by grade I CRS and grade II ICANS which occurred at day +4 after CAR-T cell infusion and required treatment with tocilizumab and dexamethasone. After temporary improvement, on day +7 after infusion, fever and neurological symptoms were observed. The girl was diagnosed with grade I CRS and grade III ICANS with complete remission after treatment with four doses of tocilizumab and dexamethasone. Laboratory test results, including complete blood morphology, C-reactive protein, ferritin, cytokine profiles and flow cytometry of lymphocyte subpopulation were monitored daily from day -1 to day +14 after CAR-T cell infusion. Flow cytometry of CAR-T cells was performed on specific days (days 0, +1, +2, +3, +6, +10, +14). The changes in the cytokine profiles and proinflammatory mediators are presented in Figure 1. Despite the observed toxicities, C-reactive protein (CRP) was <5 mg/L during the entire observation period. The girl was discharged on the day +17 after infusion in good general condition, with scheduled follow-up appointments in the outpatient clinic.

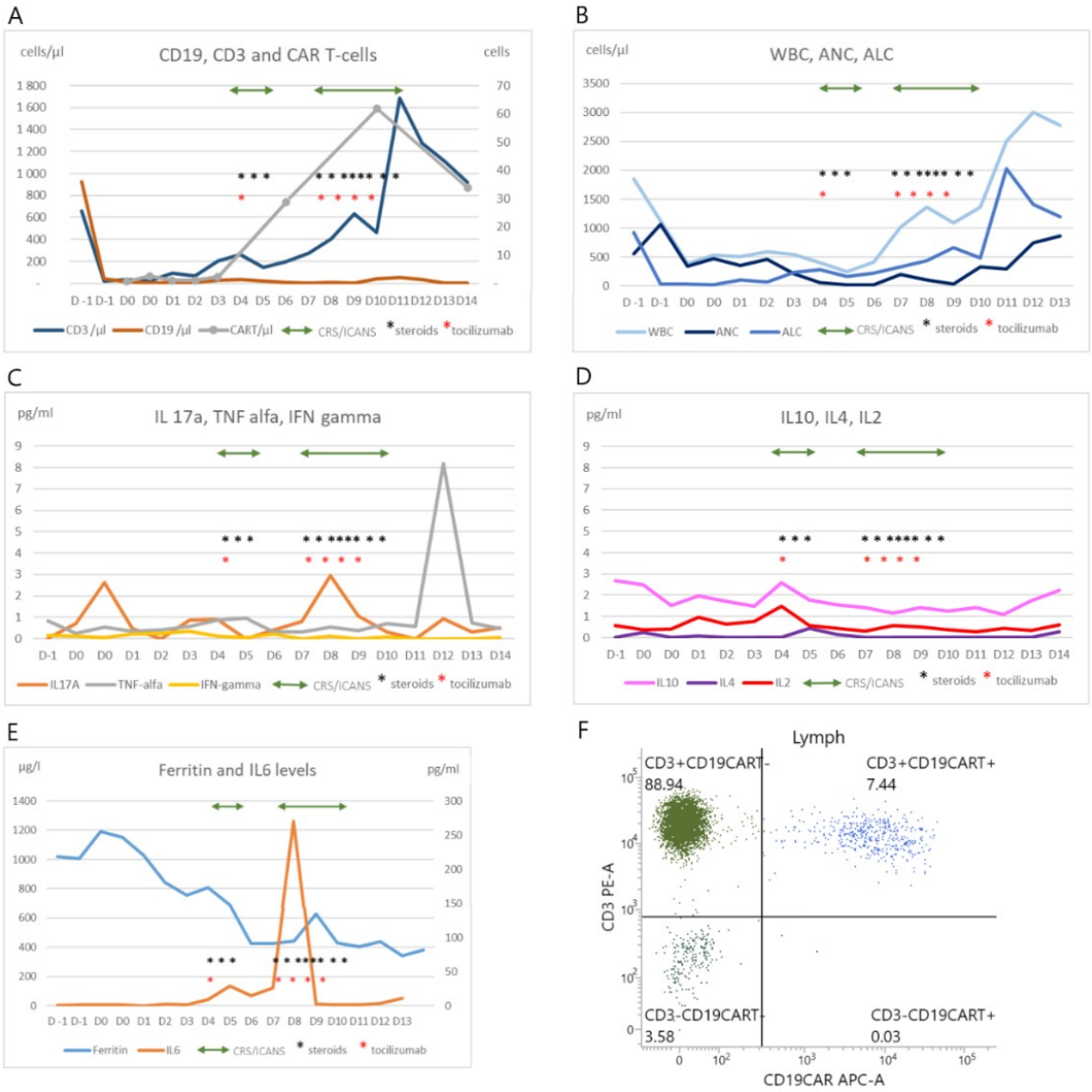

Figure 1. The results of laboratory tests, cytokine profiles, and flow cytometry assessed during the observational period, along with their relationship to cytokine release syndrome (CRS)/immune effector cell-associated neurotoxicity syndrome (ICANS) episodes and administered anti-inflammatory treatment: **A.** CD19, CD3 and chimeric antigen receptor (CAR) T cells count; **B.** White blood cells (WBC) count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC); **C.** Interleukin (IL)-17a, tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma levels; **D.** IL-10, IL-4, IL-2 levels; **E.** IL-6 and ferritin levels; **F.** CAR-T cells in flow cytometry, day +14

The *in vivo* kinetics of CAR-T cells provided crucial insights into therapeutic response and associated side effects [3]. Although CAR-T cell count was initially low in the first few

days after infusion in the described case, a similar trend was observed in other studies, with an exponential increase in CAR-T cells levels observed between days +7 and +11 [4, 5]. Furthermore, the expansion of CAR-T cells was timely related with the occurrence of CRS and ICANS. It is still not fully understood whether the peak of CAR-T cells is the cause of the toxicities itself or an effect of immune-related CAR-T cell expansion [4, 6, 7]. Incidence of those toxicities were associated also with an increase in both proinflammatory mediators (IL-6, ferritin) and a slight increase in anti-inflammatory cytokines (IL-10). After anti-inflammatory therapy with tocilizumab and steroids, a rapid decrease in cytokine levels, but not CAR-T cells occurred.

Treatment of CRS (tocilizumab) and ICANS (steroids) was successfully applied [8]. However, there is a subset of patients who experience therapy-resistant CRS/ICANS, highlighting the necessity of identifying new targets for toxicity treatment [2]. In our patient, the second episode of CRS and ICANS was timely related with a significant peak in tumor necrosis factor alpha (TNF-α) levels accompanied by a peak in CAR-T cell count. This finding is in line with the results of early studies of CAR-T cell therapy, where toxicities were related with a notable increase in TNF-α level, making TNF-α a potential target for CRS and ICANS therapy [1, 9]. In some severe cases, TNF-α blockade in combination with tocilizumab could effectively reverse CRS [10].

In summary, the monitoring of kinetics of CAR-T cells and cytokine profile provided valuable evaluation of therapeutic response and associated adverse effects. Understanding the underlying mechanisms of CAR-T cell-related immune responses is crucial for improving therapy outcomes, early detection of toxicities, and their better management. Presence of CAR-T cells might be a good prognostic factor for continuous remission in ALL.

Authors' declarations

JaS, MRP — design of the study. MRP, KC, RD, AM, ED — clinical data. JoS, JaS — writing manuscript. MK, BKR, RD — laboratory analysis. EM, KG, MRP, ŁL — CAR-T. JaS, MRP, KC — critical review. All authors — final approval.

Conflict of interest

The authors declare no conflict of interest.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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