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

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Over the last decade, the use of chimeric antigen receptor (CAR) T cells has emerged as a new strategy in the treatment of relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). The immune activation plays a pivotal role, both in the 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]. We here report 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. After the diagnosis, she received treatment according to the FRALL-POST-2004 protocol with the addition of imatinib. Prior to the CAR-T cell infusion, a lymphodepleting regimen consisting of fludarabine and cyclophosphamide was administered. Subsequently, in May 2023 the patient received an infusion of anti-CD19 CAR-T cells (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 the CAR-T cell infusion and required treatment with tocilizumab and dexamethasone. After a temporary improvement, on day +7 after the infusion, fever and neurological symptoms were observed. The child 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 the CAR-T cell infusion.

The patient was switched to the imatinib-based EsPHALL-2017 protocol. After a temporary improvement, on day +7 after the infusion, fever and neurological symptoms were observed. The child was diagnosed with primary refractory ALL and qualified for CAR-T cell therapy.

The bridging therapy was based on the FRALL-POST-2004 protocol with the addition of imatinib. Prior to the CAR-T cell infusion, a lymphodepleting regimen consisting of fludarabine and cyclophosphamide was administered. Subsequently, in May 2023 the patient received an infusion of anti-CD19 CAR-T cells (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 the CAR-T cell infusion and required treatment with tocilizumab and dexamethasone. After a temporary improvement, on day +7 after the infusion, fever and neurological symptoms were observed. The child 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 the CAR-T cell infusion.

Flow cytometry of CAR-T cells was performed on specific days (days 0, +1, +2, +3, +5, +6, +7, +10, and +14). The changes in the cytokine profiles and proinflammatory mediators are set out in Figure 1. Despite the observed toxicities, C-reactive protein (CRP) was repeated, revealing 29.5% blast cells. She was subsequently...
Figure 1. Results of laboratory tests, cytokine profiles, and flow cytometry assessed during 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.<5 mg/L during the entire observation period. The girl was discharged on day +17 after the infusion in good general condition, with scheduled follow-up appointments in the outpatient clinic.

The in vivo kinetics of CAR-T cells have provided crucial insights into the therapeutic response and its associated side effects [3]. Although the CAR-T cell count was initially low in the first few days after infusion in our described case, a similar trend has been observed in other studies, with an exponential increase in CAR-T cells levels being observed between days +7 and +11 [4, 5]. Furthermore, the expansion of CAR-T cells happened at the same time as 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]. Incidences of those toxicities were associated also with an increase in both proinflammatory mediators (IL-6 and 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 (with tocilizumab) and ICANS (with steroids) was successfully applied [8]. However, there is a subset of patients who experience therapy-resistant CRS/ICANS, highlighting the need to identify new targets for toxicity treatment [2]. In our patient, the second episode of CRS and ICANS coincided 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 to 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 conclusion, the monitoring of kinetics of CAR-T cells and cytokine profile provided a valuable evaluation of the therapeutic response and its associated adverse effects. Understanding the underlying mechanisms of CAR-T cell-related immune responses is crucial for improving therapy outcomes, and for the early detection of toxicities and their better management. The presence of CAR-T cells might be a good prognostic factor for continuous remission in ALL.

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Authors’ contributions

Conflict of interest
The authors declare no conflict of interest.

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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 and uniform requirements for manuscripts submitted to biomedical journals.

References