301 SINGLE-CELL RNA STUDY OF TRANSCRIPTIONAL ACTIVITIES ASSOCIATED WITH ANAKINRA PROPHYLAXIS AND AXICABTAGENE CILOLEUCEL IN PATIENTS WITH RELAPSED OR REFRACTORY LARGE B-CELL LYMPHOMA

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Background Chimeric antigen receptor T-cell (CAR-T) therapy has shown unprecedented treatment outcomes for B-cell malignancies. The wider utilization of CAR-T, however, is limited by CAR-T-induced adverse events including cytokine release syndrome (CRS). Tocilizumab, a monoclonal antibody blocking Interleukin (IL)-6 receptor, is the only approved treatment for CRS. Recent animal studies suggest that anakinra, a recombinant form of IL-1 receptor antagonist, could have potential benefit in managing toxicity, with or without tocilizumab. We have opened a clinical trial (NCT04150913) testing the use of prophylactic anakinra in patients with relapsed or refractory large B-cell lymphoma eligible to receive axicabtagene ciloleucel (axi-cel) as per the registration study ZUMA-1. Here, we used single-cell RNA sequencing (scRNA-seq) on clinical samples of the first ten subjects on the study[1] to probe the molecular pathways altered by anakinra and to discover potential mediators of breakthrough cases of CRS.

Methods We conducted scRNA-seq on the peripheral blood mononuclear cells (PBMCs) of 10 patients 7 days post infusion (D7) and the infusion products (IP). We compared data from the trial subjects to 4 control samples from the same institution along with our previously generated scRNA-seq IPs and D7 PBMCs from 19 subjects that were treated with axi-cel without prophylaxis for toxicity.[2] We utilized pseudo-bulk differential expression analysis of major immune cell types to reveal transcriptional signals associated with CRS and anakinra treatment.

Results Our study revealed that IL-4 and IL-10 anti-inflammatory pathways in IPs of both anakinra and non-anakinra cohorts were negatively associated with breakthrough CRS. We further observed that the same pathways were enriched at D7 in anakinra-treated CAR+ CD4+ T cells populations. Anakinra prophylaxis had little effect on the overall CAR+ T-cell compositions from IPs to D7, but was associated with an increased proportion of CAR+ regulatory T cells (Tregs). Expression of interferon gamma (IFN□) pathways, cytokine levels of IFNγ and IFNγ-induced protein 10 (CXCL10) in CD14+ monocytes were significantly enriched in patients with breakthrough CRS treated with tocilizumab in the anakinra cohort. Differential cell–cell interaction analysis further showed the association of IFNγ ligand receptor activities with breakthrough toxicity uniquely among anakinra- and tocilizumab-treated patients.

Conclusions We identified key molecular pathways and immune cell populations that were possibly modulated by anakinra, including the upregulation of IL-10 signaling pathway in CAR+ CD4+ T cells and increased abundance of Tregs. IFNγ enrichment in patients with breakthrough CRS further suggests that this pathway is also targetable and not inhibited by anakinra alone.

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Trial Registration NCT04150913 (A Phase 2 Trial of Anakinra for the Prevention of CAR-T Cell Mediated Neurotoxicity)

REFERENCES

1. Frigault, M. J. *et al*. A Phase II Trial of Anakinra for the Prevention of CAR-T Cell Mediated Neurotoxicity. *Blood*. 2021;**138**:2814.
2. Haradhvala, N. J. *et al*. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B-cell lymphoma. *Nature Medicine*. 2022; in press. http://medrxiv.org/lookup/doi/10.1101/2022.04.04.22273422. doi:10.1101/2022.04.04.22273422.

Ethics Approval The clinical study complies with IRB-approved protocol (19-348) at the Dana-Farber/Harvard Cancer Center.

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