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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

A Multi-Omic Single-Cell Landscape of Cytokine Release Syndrome in Multiple Myeloma Patients after Anti-BCMA CAR-T Cell Therapy

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Introduction: Chimeric antigen receptor T (CAR-T) cell therapy targeting BCMA has achieved great success in the treatment of multiple myeloma, yet cytokine release syndrome (CRS) is the most severe and common complication that limits the safety and increases the expense of CAR-T cell therapy. Precise prediction of CRS severity is critical for early intervention and control of CRS, which warrants a detailed understanding of underlying cellular and molecular mechanisms. While certain cytokines have been identified as the main contributors of CRS, a comprehensive landscape of CRS unset and progression remains enigmatic.

Methods and Results: We analyzed clinical samples from patients with multiple myeloma receiving BCMA-CAR-T cell therapy, and obtained a multimodal dataset pertaining to CRS severity by multiplexed cytokine and multi-omic single-cell analyses. First, we generated a multi-plex profile to comprehensively reveal the kinetics of CRS-related cytokines. Plasma samples were collected from 6 patients with severe CRS, at serial time points: before lymphodepletion, after CAR-T infusion before CRS initiation, at CRS initiation, CRS peak and CRS recovery stage. These samples were analyzed for a 45-plex cytokine profile, and we identified 3 main cytokines (IL-2, IL-4 and IL-17A) as signatures of CRS initiation and 24 cytokines at the CRS peak stage, with the latter consisting of known CRS biomarkers (IL-1, IL-6 and CRP) and new CRS-related cytokines (IL-3, CCL20 and CXCL1). Next, we conducted single-cell multi-omic analyses on the transcriptome, immunome and epigenome of CAR-T and bystander immune cells. We collected 111 samples from 16 patients (5 with severe CRS, 6 with mild CRS and 5 non-CRS) for scRNA-seq and scTCR-seq, and 28 samples from 10 patients (3 with severe CRS, 4 with mild CRS and 3 non-CRS patients) for scATAC-seq. scRNA-seq analysis demonstrated systematic and diverse changes across cellular subpopulation

and their dynamic cytokine expression across the course of CRS. Moreover, integrated scRNA-seq and scTCR-seq analyses illustrated the developmental trajectory and clonal diversity of CAR-T and endogenous T cells. Specifically, we detected clonal expansion of a CD8+ effector memory T cell subsets upon CRS recovery, which was positively correlated with the persistence of CAR T cells *in vivo*. Meanwhile, combined analyses of scATAC-seq and scRNA-seq deciphered the chromatin accessibility and cis-regulatory network in CAR-T cells at different CRS stages. Finally, we identified a CD40LG $^{+}$ CD4 $^{+}$ subpopulation in pre-infusion CAR-T products which was associated with CRS severity and could be used as a predictive biomarker of severe CRS. These results were further validated by sorting CD40LG+ CD4+ CAR-T cell subset from the product, and co-culture with monocytes and tumor cells. This subpopulation of CAR-T cells was shown to upregulate IL-13 production and activate monocytes, resulting in the elevation of multiple cytokines associated with CRS.

Significance: Our comprehensive multi-omics analyses provide a temporal atlas of CRS at the single-cell level, delineating the critical roles of CAR-T cells, monocytes, endogenous T cells and NKT cells across the course of CRS. Further, we identified a key subpopulation in pre-infusion CAR-T products as a biomarker of CRS severity, providing clinically-relevant insights about both prediction and intervention of CRS.

Disclosures No relevant conflicts of interest to declare.

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