Profiling the peripheral blood immune cell repertoire in large-B cell lymphoma patients treated with CD19 CAR-T

Giulia Cheloni, PhD[1], Eleni Kanata[1,2], Dina Stroopinsky, PhD[1], Dimitra Karagkouni, PhD[1,2], Jessica J. Liegel, MD[1], Daniela Torres[1], Joshua Hauser[1], Poorva Bindal, MD[1], Anita G. Koshy, MD[1], Yered Pita-Juarez, PhD[1,2], Adrian Bot, MD[3], PhD , Mike Mattie, PhD[3], Justin Chou, PhD[3], Jacalyn Rosenblatt, MD[1], David Avigan, MD[1], Ioannis S Vlachos, PhD[1,2,4] .

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Broad Institute of MIT and Harvard, Cambridge, MA; [3]Kite, a Gilead Company, Santa Monica, CA; [4]Spatial Technologies Unit, Harvard Medical School Initiative for RNA Medicine, Boston, MA.

Beth Israel Lahey Health
Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL

BACKGROUND

In Zuma-1 study, approximately 40% of patients with refractory or relapsed large B cell lymphoma (LBCL) showed durable response to Axi-cel, an autologous anti-CD19 chimeric antigen receptor (CAR)-T cell therapy. The identification of immunologic factors predictive of therapeutic efficacy and tumor escape is a critical area of investigation. The impact of CAR T cell activation on the native T cell repertoire and lymphoma specific immunity has not yet been elucidated.

AIM

We sought to determine the role of host immune activation in response to tumor associated antigens and the impact of consequent epitope spreading on CAR-T mediated therapeutic efficacy. To this end, we performed longitudinal single cell immunoprofiling of peripheral blood samples from 32 ZUMA-1 patients to evaluate differences in the immune cell subsets and native T cell repertoire based on response to axi-cel (non-responder, relapsed, long-term responder). For sample preparation optimization and protocol evaluation, a pilot analysis was performed on 3 patients, across 4 timepoints.

METHODS

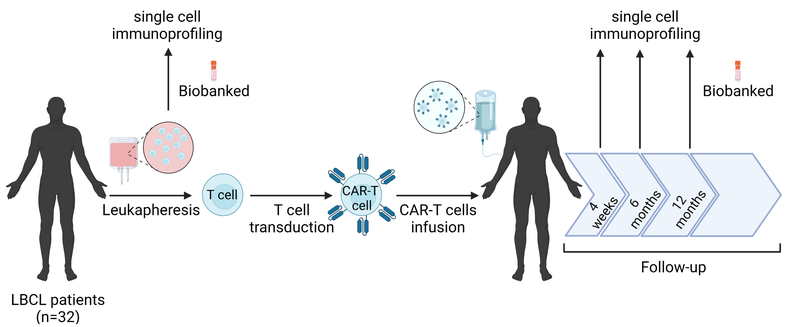


Figure 1: Sample processing pipeline depicting sample acquisition and preparation for scRNA-seq/scTCR-seq/scBCR-seq datasets.

Single cell immunoprofiling was performed on PBMC samples from three ZUMA-1 patients as a pilot implementation, collected at leukapheresis, 4 weeks, and 6 months post CAR-T cell infusion, scRNA-seq was performed using 10x Genomics Chromium Next GEM Single Cell 5' Kit v1.1. Full-length paired α/β TCR and BCR libraries were obtained using the Chromium Single Cell V(D)J Enrichment, Human T Cell/B cell kits following manufacturer instructions, while γ/δ TCR libraries were generated using custom primers.

Bioinformatics

Cell Ranger v.4.0.0 was used for cell assignment and gene expression quantification. Gene expression libraries were loaded as Seurat objects (v3.2.3) in R v3.6.0. A total of 22,403 cells were kept after QC filtering (mitochondrial content < 20%, number of genes (features) in each cell 400 < n < 4000, total number of molecules within a cell 400 < n < 20,000). Clustering and two-dimensional representations were performed with Seurat (v3.2.3).g

RESULTS

Single-Cell transcriptomic analysis on PBMC samples of the 3 patients included in the pilot implementation.

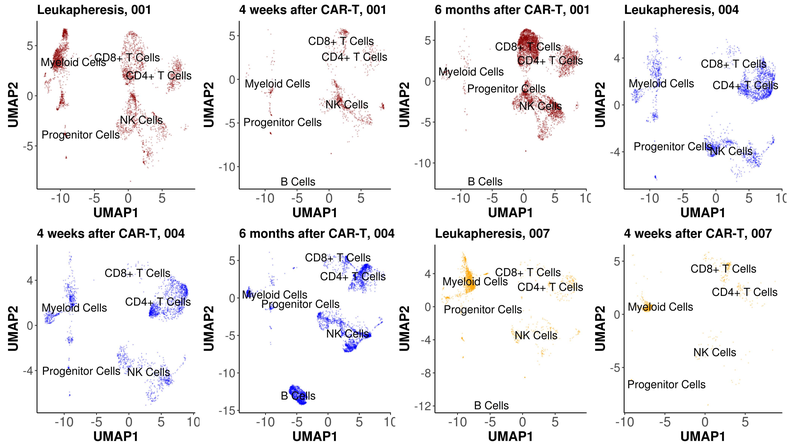


Figure 2: Two-dimensional uniform manifold approximation and projection (UMAP) of all cells passing quality-check separated per patient and timepoint. A total of 22,403 cells passed QC capturing 6 major compartments, corresponding to *31 cellular populations.*

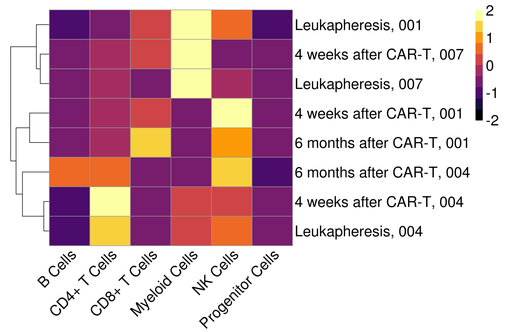


Figure 3: Cellular proportions are portrayed for each patient at the indicated time points. Lower to higher cellular proportions are marked with black to yellow color gradients, respectively.

In 2 of the 3 patients analyzed, CD8 T cells, after an initial decrease at 4 weeks post-CAR T infusion, exhibited an increase at 6 months post CAR T infusion reaching higher levels than those observed prior to CAR T treatment. The third patient presented an increase of the CD8 T cell compartment at 4 weeks compared to pretreatment. A similar trend was observed for CD4 T cell population, with an increase at 6 months post CAR-T to a level higher than prior to CAR T infusion. On the contrary, the myeloid cell compartment depicted a gradual decrease from leukapheresis to 6 months post CAR T. B cells were observed only in 1 of the 3 patients at 6 months (Figures 2,3).

RESULTS

T and B cell receptor clonality at single cell resolution.

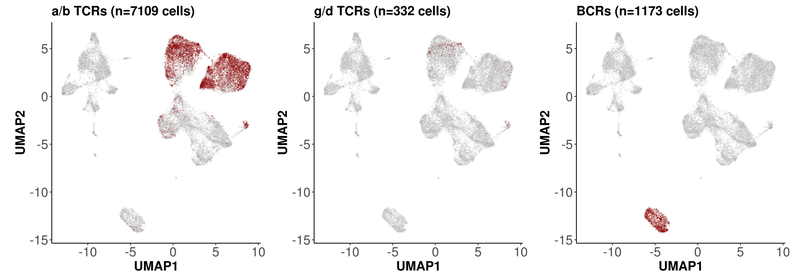


Figure 4: α/β TCR, γ/δ TCR and BCR clonotypes were identified and projected on the 2-dimensional embedding. T and B cells with one or more clones are colored.

α/β clonotype frequency per timepoint for each patient.

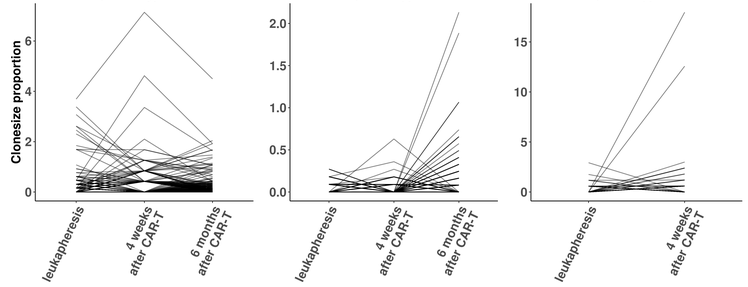
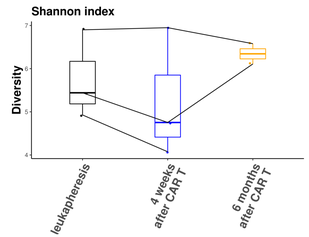


Figure 5: a/b clonotype frequency per timepoint for each patient.



Full-length paired α/β TCR at single cell level showed that some of the most abundant clonotypes at baseline continued to be prominent in post CAR T timepoints. An expansion of new clonotypes was observed after CAR T cells infusion.

Figure 6: T Cell receptor Shannon diversity index per timepoint for the 3 patients.

CONCLUSION

The pilot application of single cell immunoprofiling on longitudinal samples from Axi-cel-treated LBCL patients successfully captured changes in the cellular transcriptional landscape, cell proportions, and TCR/BCR space across the time axis in high resolution. We proceeded with the analysis of the remaining 29 patients, which is currently ongoing. We anticipate that the analysis of the complete cohort (32 patients, 95 samples) will capture the transcriptional program in response to CAR-T cell therapy in high resolution.