



## Investigating Recipient Immune and CAR T-cell

## Populations during CAR-T Therapy with Multi-omics

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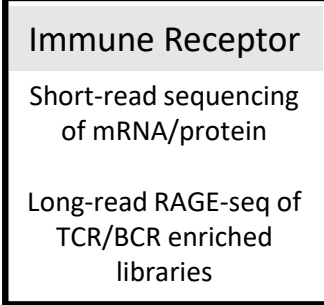
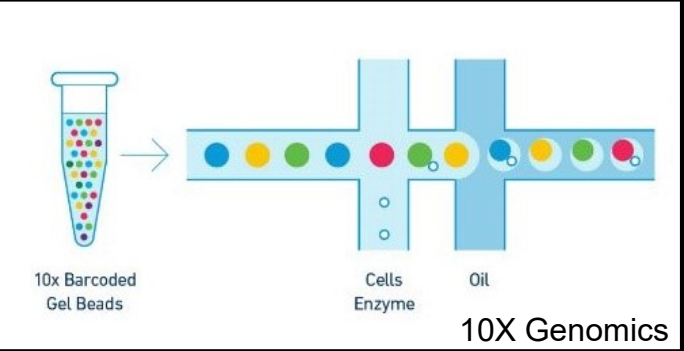
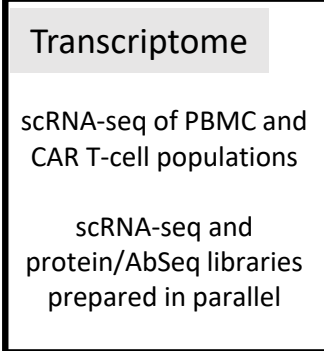
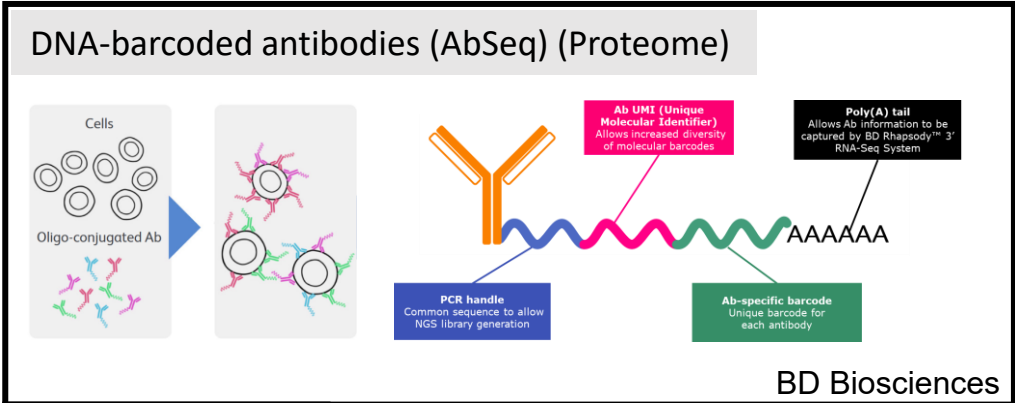
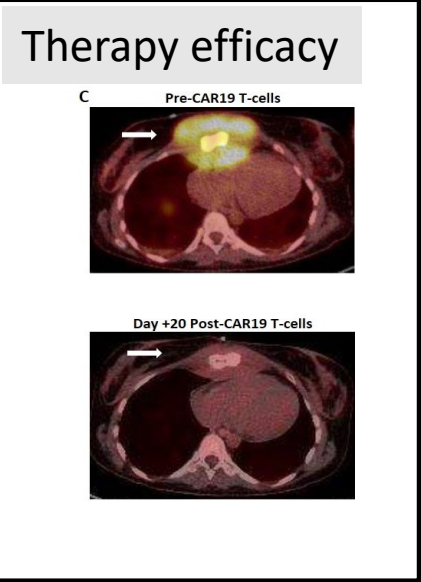
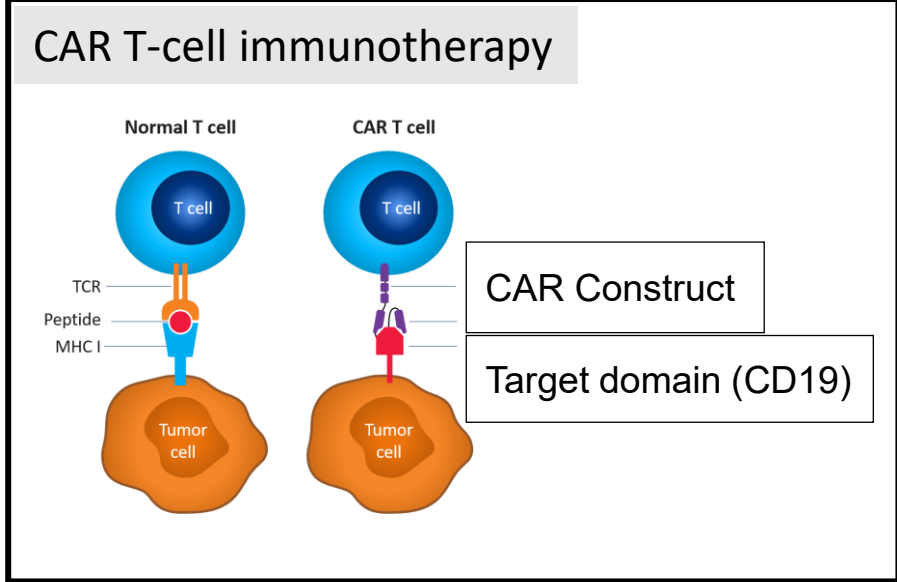
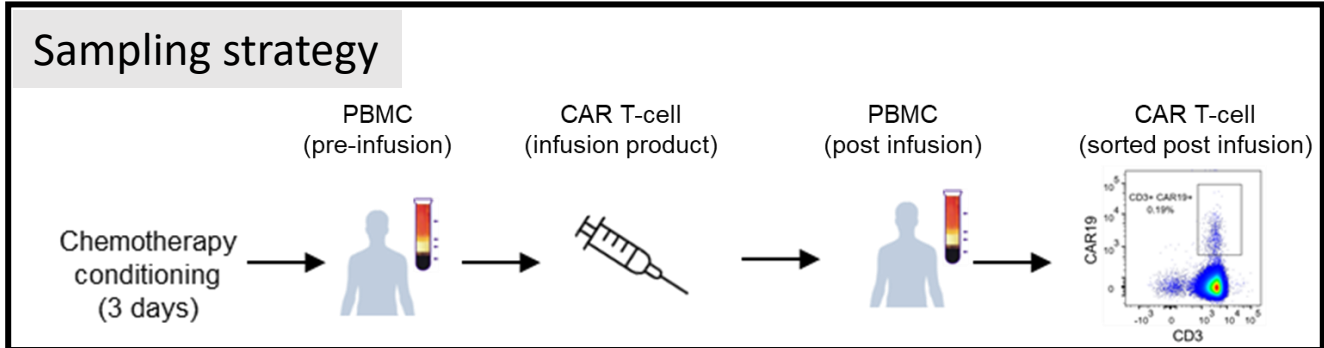
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\* Contributed equally

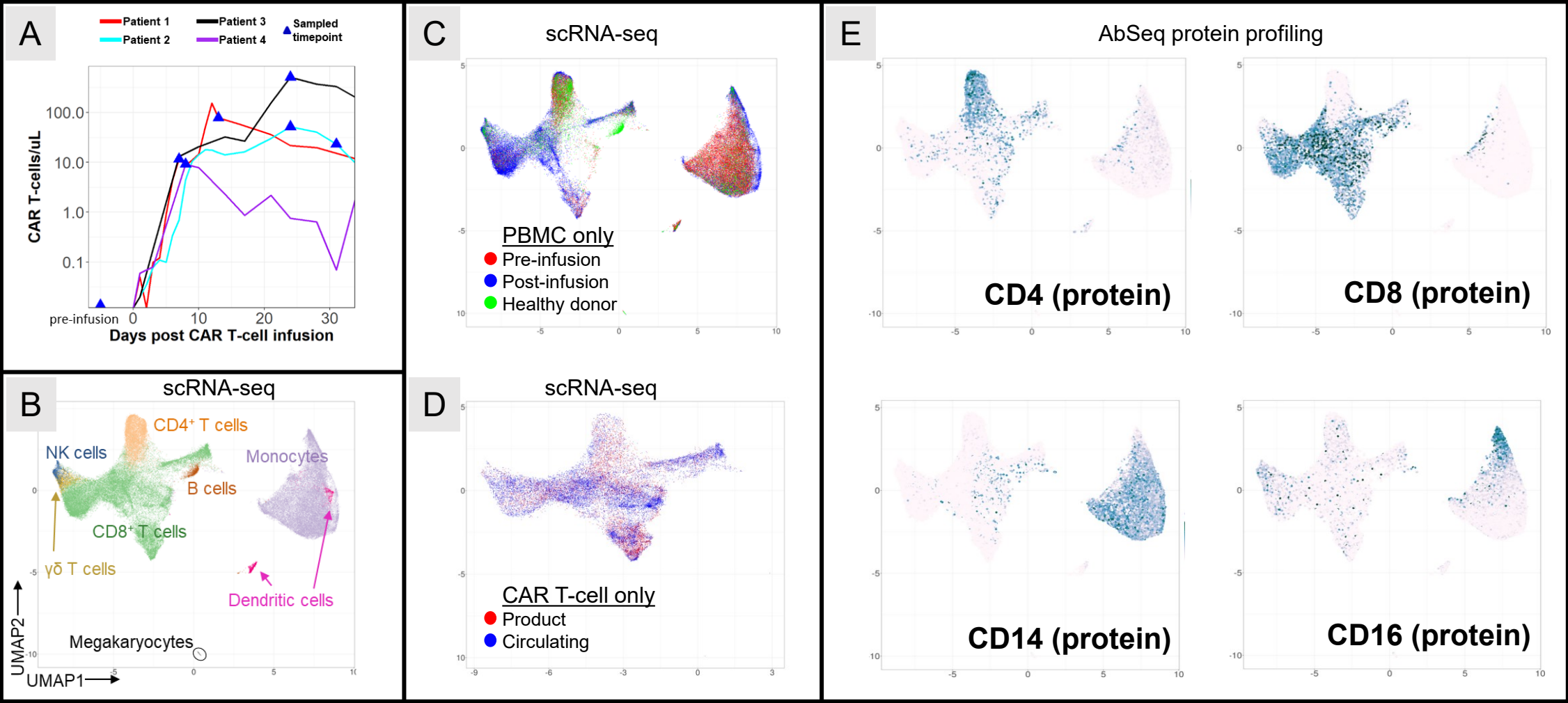
**Emerging Technologies in Single Cell Research (November 2020)**

# Single-cell profiling of total immune cell (PBMC) and CAR T-cell populations during immunotherapy

- Donor-derived CAR T-cells with specificity for the CD19 (B cell) antigen were manufactured using the piggyBac transposase system
- We applied single-cell multiomics (transcriptome, proteome, immune receptor) to track immune reconstitution, CAR T-cell responses, and differentiation.



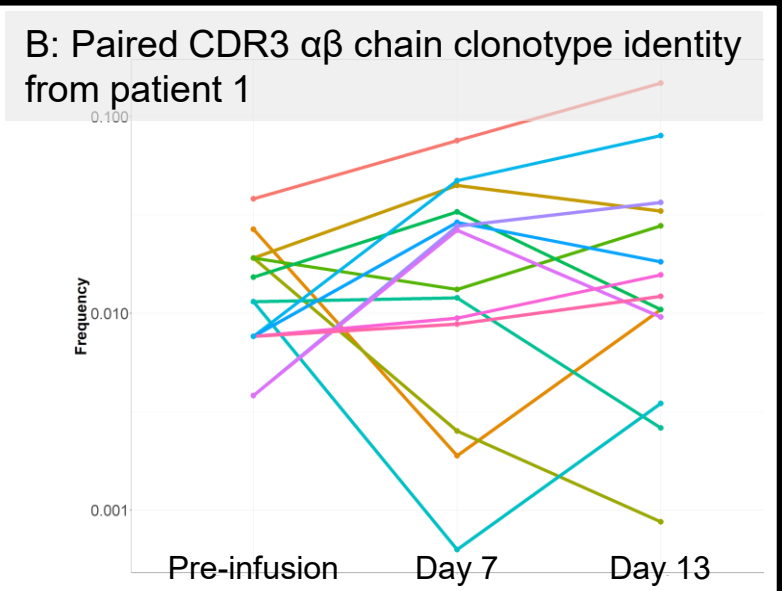
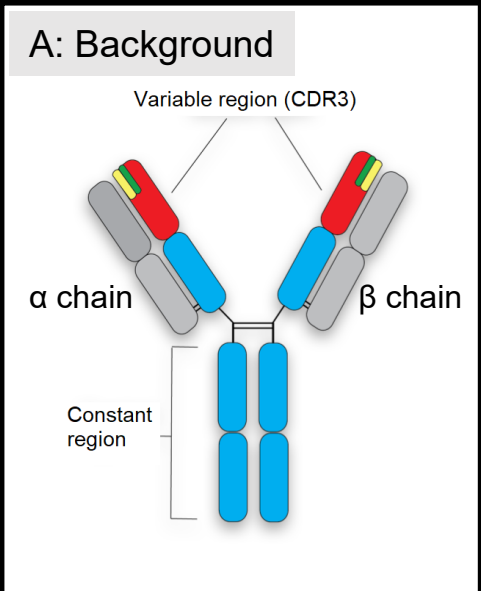
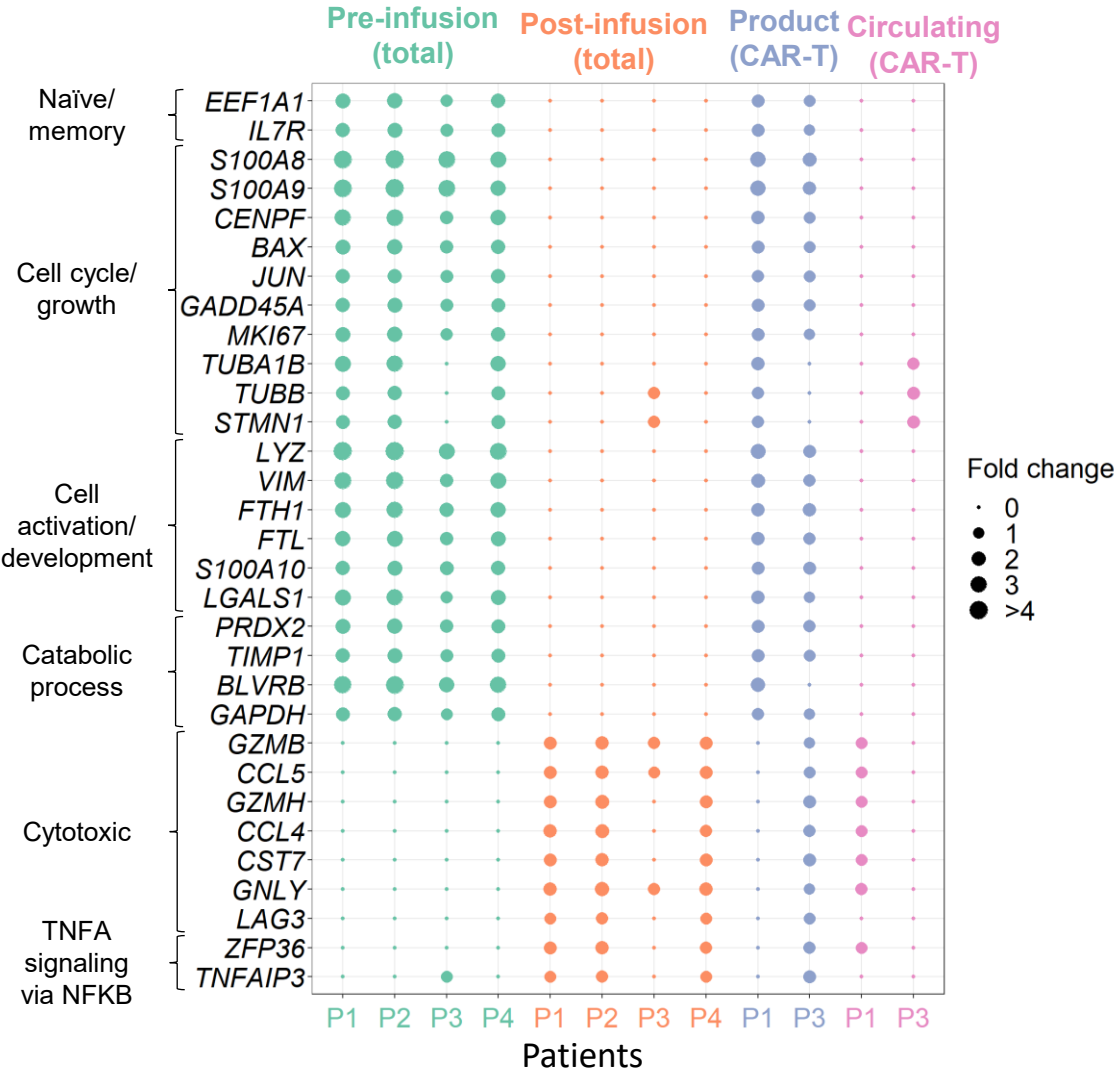
# Total immune cell population profiling reveals pre- and post-infusion reconstitution dynamics



We analysed over 50,000 single cells derived from 4 CAR-T therapy patients at pre-infusion and post-infusion timepoints and observed skewing from monocyte to T cell subsets. (A) Patient sampling timepoints. (B) UMAP of all cells. (C) UMAP portion including cells from the total immune cell population. (D) UMAP portion including only CAR T-cells sequenced following enrichment. (E) Protein expression supports assignment of canonical immune cell subsets in circulation.

# T cell evolution at the transcriptomic and clonotype levels during CAR-T immunotherapy

Dot plot of CD8<sup>+</sup> T cell differential gene expression reveals cell-cycle (pre-infusion) to a cytotoxic (post-infusion) phenotype



Evolution of total CD8<sup>+</sup> T cell populations during CAR-T therapy.

A. Structure of the TCR receptor. Each TCR is independently and randomly generated by V(D)J recombination.

B. Major T cell clonotypes from patient 1 persist and expand from pre-infusion to post-infusion.

C. Representative plot of the major clonotype plotted in UMAP space showing differentiation association with subsets over time.

