Multimodal single-cell sequencing analysis reveals putative tumor-reactive population in lifileucel TIL products

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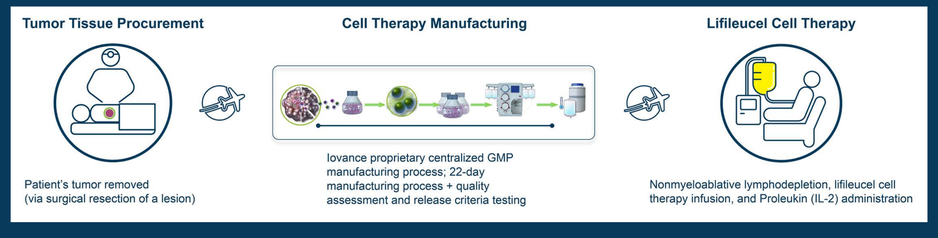
Background

- Lifileucel is an FDA-approved autologous TIL cell therapy for the treatment of advanced melanoma[1]
- We previously presented assessment of TIL drug product (DP) at a single-cell (sc) level from 20 patients

Figure 1. Lifileucel Tumor-infiltrating Lymphocyte (TIL) Cell Therapy



- What are the potential cellular determinants of response to TIL cell therapy?

Objectives

- To further characterize lifileucel at a sc level using TIL DP from 34 patients and analyze baseline tumor digest T cells from a subset of these patients using high-dimension multimodal sequencing
- To characterize and compare putative tumor-reactive T cells in baseline tumor and TIL DP

Methods

- Cell barcode-matched scRNA-sequencing and scTCR-sequencing
  - 10x genomics 5' immune profiling
- C-144-01 metastatic melanoma dataset (NCT02360579)
  - Lifileucel, N=27
  - Matched tumor digest (CD45 enriched) and lifileucel, N=7
- Baseline tumor digest single-cell analysis
  - After data quality check and subsetting on T cells, sctransform[2,3] was used for normalization and feature selection, followed by PCA and Harmony[4] integration, clustering, and PaCMAP[5] dimensionality reduction visualization
  - Clusters were annotated manually based on marker genes, cell-type–specific gene signatures using VISION,[6] and automated annotation using scATOMIC[7]
  - Putative tumor-reactive T-cell clusters were identified based on expression level of the NeoTCR8 gene signature[8]
  - scTCR-Seq visualizations were generated using scRepertoire[9] and ggalluvial[10]
- Lifileucel single-cell analysis
  - To aid with cell-type annotation, CITE-Seq was performed on a subset of 4 patients (2 R, 1 SD, 1 PD)
  - Multimodal weighted-nearest neighbor analysis and manual cluster annotation were used to develop a single-cell TIL reference map
  - Gene set quantification was performed after sample level pseudo-bulking of NeoTCR8 clusters using GSVA[11]
- GraphPad Prism was used for summary data visualizations and statistics

Results

Figure 2. Profiles of Tumor T-Cell Subsets

- Putative tumor-reactive T-cell proportion in tumor appears to be associated with response (**Figure 2** and **Figure 3**)

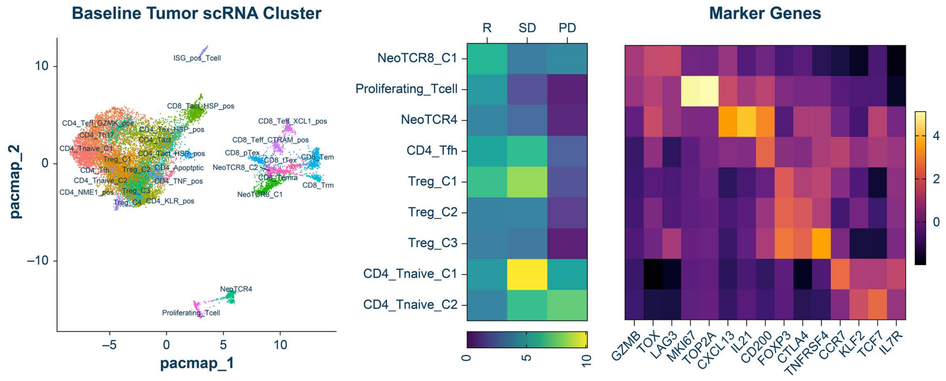


Figure 3. Profiles of scTCR Clones

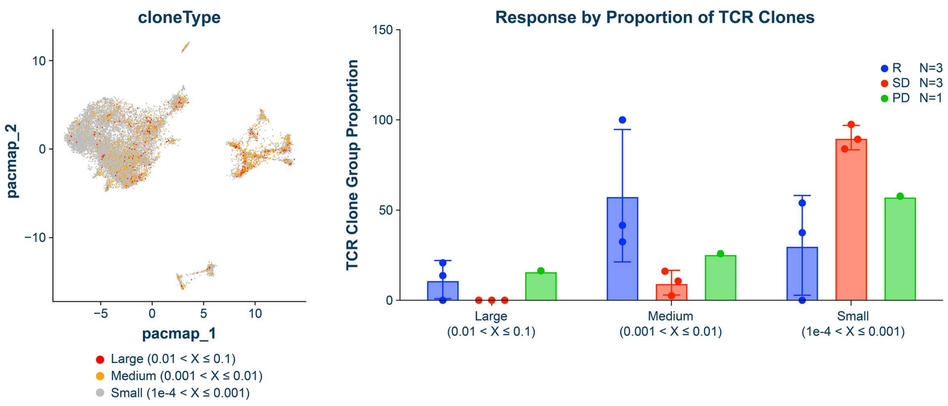
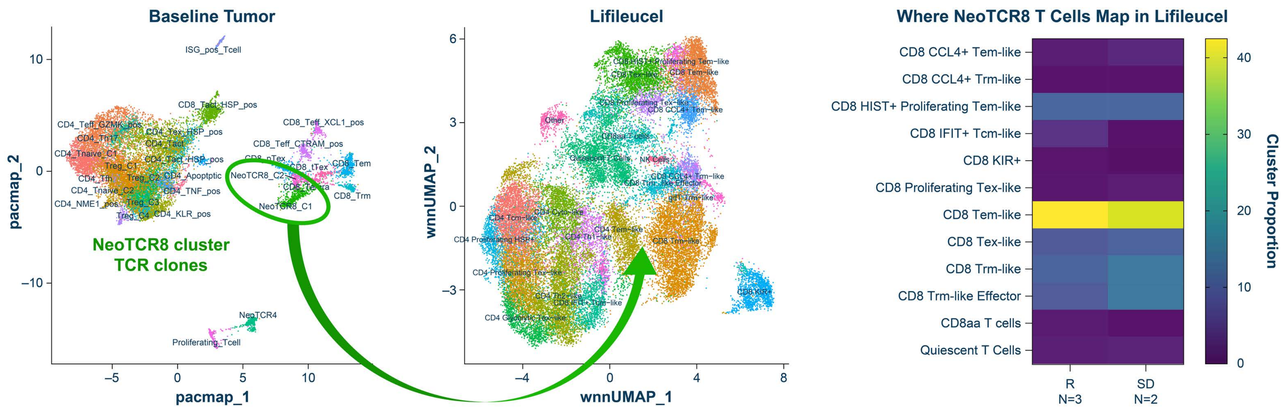
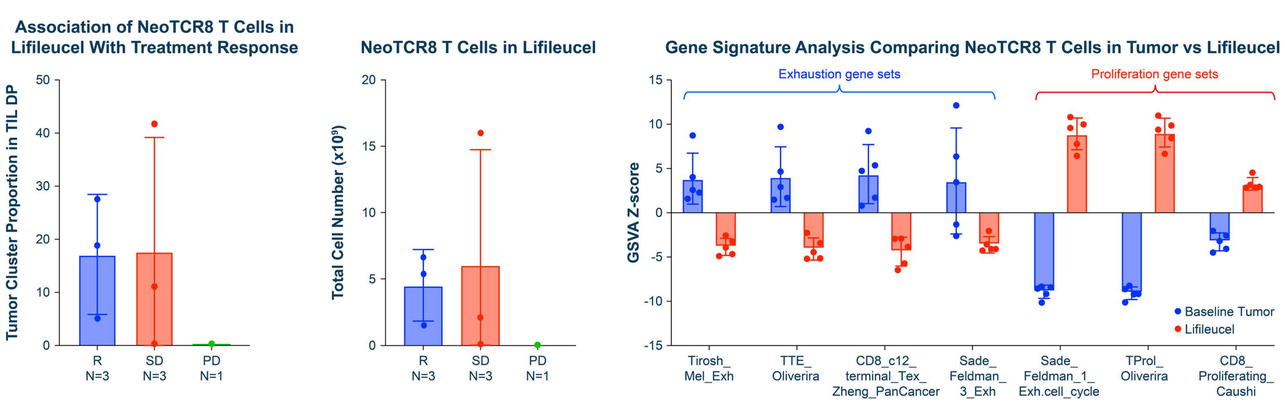


Figure 4. NeoTCR T-Cell Proportion in Lifileucel Appears to be Associated with Response

- The TIL DP received by the patient with best overall response of PD lacks this putative tumor-reactive T-cell population (**Figure 4**)
- NeoTCR8 T cells map to CD8 effector memory T-cell (T_{EM})-like cluster in TIL DP
- The lifileucel manufacturing process shifts phenotype of NeoTCR8 T cells
  - NeoTCR8 T cells expand into the billions in lifileucel
  - Upon expansion, NeoTCR8 T cells downregulate exhaustion and upregulate proliferation





Conclusions

- **The capture, reinvigoration, and expansion of CD8+ TIL-expressing tumor-reactive TCR clones appear to be important for clinical response to TIL cell therapy**
- **Preliminary results suggest the tumor immune infiltrate from patients with stable disease and progressive disease contains more naive CD4 T cells, while responders have more putative tumor-reactive TIL**
- **The lifileucel manufacturing process shifts the phenotype of putative tumor-reactive TIL from exhausted to proliferating T cells**
- **Putative tumor-reactive TIL, post rapid expansion, map to the CD8 T_{EM}-like cluster in the final DP**
- **The proportion and total number of cells in the CD8 T_{EM}-like cluster appears to be associated with response to TIL cell therapy**

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Abbreviations

CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; DP, drug product; FDA, Food and Drug Administration; GMP, Good Manufacturing Practice; IL-2, interleukin-2; PD, progressive disease; R, responder; scRNA, single-cell RNA; scTCR, single-cell T-cell receptor; SD, stable disease; T_{CM}, central memory T cell; TCR, T-cell receptor; T_{EM}, effector memory T cell; Tex, exhausted T cell; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cell; T_{RM}, tissue resident memory T cell.

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Disclosures

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