Multimodal single-cell analysis of lifileucel tumor-infiltrating lymphocyte (TIL) product

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Introduction

- Autologous TIL cell therapies such as lifileucel have demonstrated durable responses in patients with various solid tumors including melanoma and lung cancer[1,2]
- TIL drug phenotypes were previously assessed[3-5] and extensively characterized based on the bulk population and the CD8$^+$ subset of TIL analyzed using single-cell RNA sequencing[6]
- The current research further characterizes lifileucel TIL drug product in its entirety at a single-cell level using high-dimension multimodal sequencing and explores features that have a potential to inform on future development of TIL cell therapies

Methods

- Manufactured lifileucel TIL drug product from 20 patients (BOR: 6 CR, 4 PR, 5 SD, 5 PD) from the registrational melanoma C-144-01 trial were analyzed using single-cell RNA and TCR sequencing, resulting in 153K single-cell transcriptomes
  - To aid with cell-type annotation, 130 surface proteins on TIL drug product samples from a subset of 4 patients (BOR: 2 CR, 1 SD, 1 PD) were analyzed via CITE-seq
- Using Seurat v5 R package,[7] multimodal weighted-nearest neighbor analysis combined with manual cluster annotation (via known marker genes and proteins), cell-type-specific gene signatures, and automated annotation were used to develop a single-cell TIL reference map, resulting in high-resolution subsets of cells
- TCR clonotypes from lifileucel TIL drug product were compared with those from respective pretreatment tumors previously identified by bulk TCR sequencing
  - Single-cell TCR sequence visualizations were generated using scRepertoire R package[8]
- CD4$^+$ and CD8$^+$ TIL subsets were analyzed using Seurat v5 with single-cell Transform regularization and cell-cycle regression followed by PCA, knn, clustering, and UMAP
- Pseudotime analysis was performed using Monocle3 R package,[9-11] with *SELL* defining the root cluster in CD8$^+$ TIL and *IL7R* defining the root cluster in CD4$^+$ TIL
  - Gene set quantification was performed using Escape and UCell R packages[12,13]
- GraphPad Prism and ggplot2 R package[14] were used for summary data visualizations and statistics

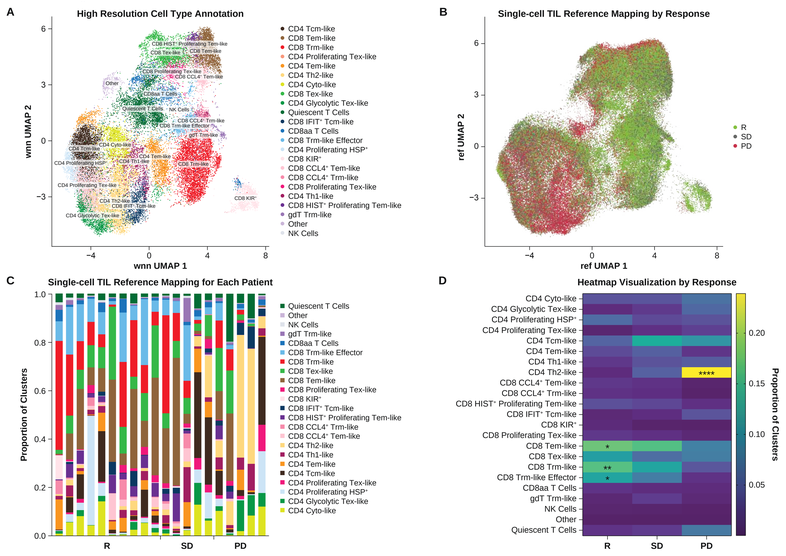
Key Findings

- Compared with the TIL drug products from responders, TIL drug products from patients with PD exhibited:
  - Lower proportions of CD8$^+$ Tem- and Trm-like subsets
  - Higher proportions of CD4$^+$ Th2-like subset
  - Lower capture of the tumor-resident expanded TCR clones
  - Lower cytotoxicity, TCR signaling, and stemness
  - Higher terminal differentiation
- These results suggest that the capture, expansion, and reinvigoration of CD8$^+$ TIL expressing tumor-resident expanded TCR clones is important for clinical response to TIL cell therapy

Results

- Mapping of the full dataset to the novel single-cell TIL reference revealed a CD8$^+$ TIL-dominant composition of responder TIL drug product (**Figure 1A**)
  - Compared with patients with CR/PR and to a lesser extent patients with SD, TIL from patients with PD exhibited reduced proportion of CD8$^+$ Tem-like and Trm-like T cells
  - In both patients with CR/PR and most patients with SD, CD4$^+$ Th2-like subset was rare or virtually absent; however, TIL from patients with PD exhibited an increased proportion of this subset (**Figure 1B-D**)

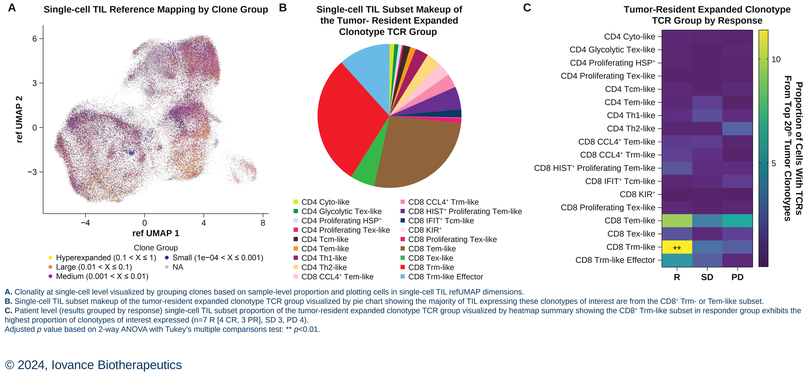
Figure 1: Multimodal Characterization of Lifileucel Identifies Specific CD8$^+$ TIL Subsets Associated With Response and CD4$^+$ TIL Subsets Associated With Disease Progression



A. Weighted-nearest neighbor clustering and UMAP visualization of single-cell CITE-seq dataset (n=2 CR, 1 SD, 1 PD).
B. Single-cell TIL reference mapping results visualized in refUMAP dimensions colored by response.
C. Stacked bar plot of single-cell TIL reference mapping results visualized for each patient.
D. Single-cell TIL reference mapping results grouped by response visualized by heatmap summary (n=10 R [6 CR, 4 PR], 5 SD, 5 PD).
Adjusted *p* value based on 2-way ANOVA with Tukey's multiple comparisons test: **** *p*<0.0001, ** *p*<0.005, * *p*<0.05.

- Expanded clonotypes (TCRb CDR3s with a frequency in the top 20th quantile) in pretreatment tumor tissues were more prevalent in TIL TCR repertoires specifically of responders (compared with patients with SD and PD) and were more likely to be expressed by CD8$^+$ (specifically CD8$^+$ Tem- and Trm-like TIL) than CD4$^+$ T cells (**Figure 2A-C**)

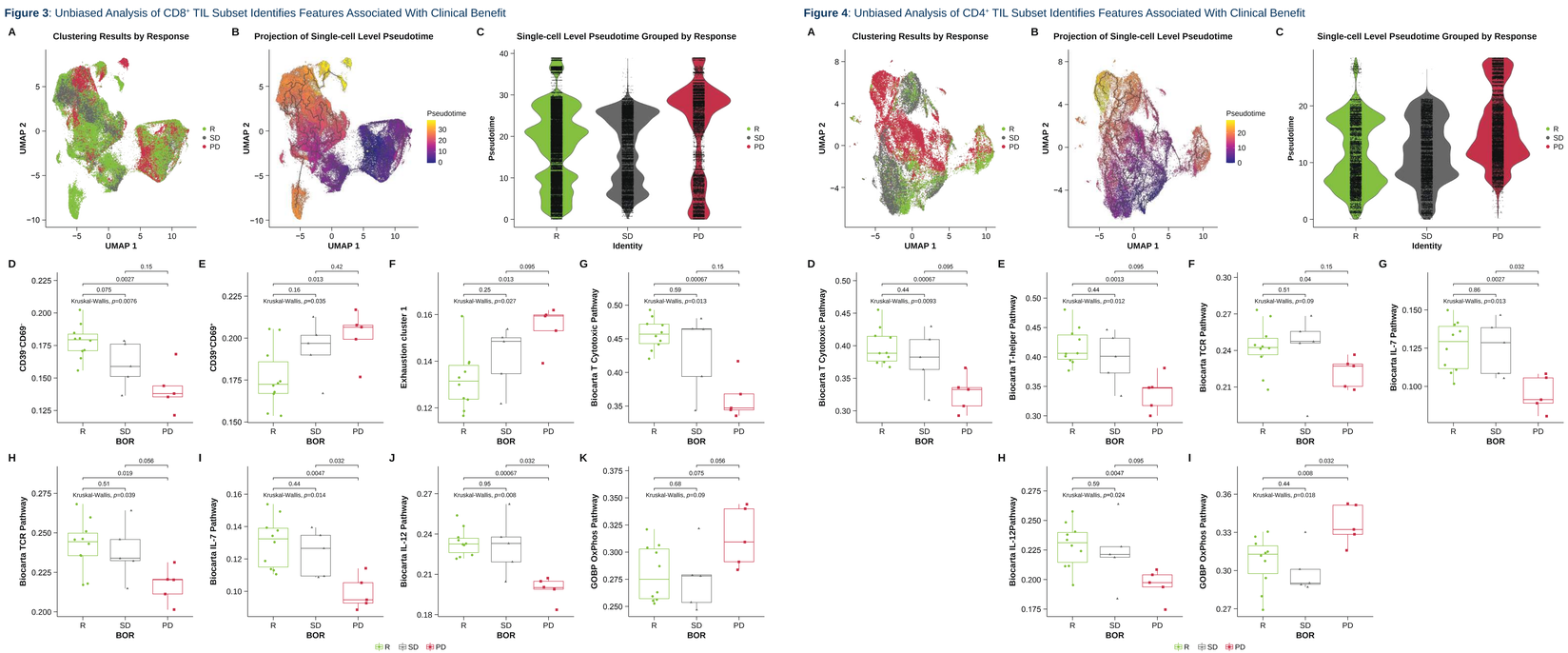
Figure 2: Single-cell TCR Sequencing Characterization of Lifileucel Further Supports an Association of CD8$^+$ Tem and Trm TIL Subsets With Response



A. Clonality at single-cell level visualized by grouping clones based on sample-level proportion and plotting cells in single-cell TIL refUMAP dimensions.
B. Single-cell TIL subset makeup of the tumor-resident expanded clonotype TCR group visualized by pie chart showing the majority of TIL expressing these clonotypes of interest are from the CD8$^+$ Trm- or Tem-like subset.
C. Patient level (results grouped by response) single-cell TIL subset proportion of the tumor-resident expanded clonotype TCR group visualized by heatmap summary showing the CD8$^+$ Trm-like subset in responder group exhibits the highest proportion of clonotypes of interest expressed (n=7 R [4 CR, 3 PR], SD 3, PD 4).
Adjusted *p* value based on 2-way ANOVA with Tukey's multiple comparisons test: ** *p*<0.01.

- Pseudotime trajectory analysis found that both CD8$^+$ (**Figure 3A-C**) and CD4$^+$ (**Figure 4A-C**) TIL of patients with PD had more terminally differentiated cells compared with TIL of responders and patients with SD
- Gene signature quantification and univariate analysis showed CD8$^+$ TIL expression of the CD39$^-$CD69$^-$ (stem-like) gene signature and CD39$^+$CD69$^+$ (terminal differentiation) gene signature exhibit a "dose curve"–like difference between responders, patients with SD, and patients with PD with responders being more stem-like, patients with PD more terminally differentiated, and patients with SD intermediate (**Figure 3D-F**)
  - For the IL-7, IL-12, cytotoxic T cell, and TCR signaling pathways, TIL of responders and patients with SD expressed these pathways at a similar level while patients with PD had significantly lower expression (**Figure 3G-J**)
  - The opposite was true for the OxPhos pathway gene signature with responders and patients with SD being similar and patients with PD exhibiting higher expression of this pathway (**Figure 3K**)
- CD4$^+$ TIL responders and patients with SD were similar while TIL of patients with PD tended to have lower expression of the TCR signaling, cytotoxic T cell, helper T cell, IL-12 pathway, and IL-7 pathway gene signatures and higher expression of OxPhos pathway gene signatures (**Figure 4D-I**)

Figure 3: Unbiased Analysis of CD8$^+$ TIL Subset Identifies Features Associated With Clinical Benefit



From the full ~150K cell single-cell TIL dataset, CD8$^+$ TIL subset was analyzed as described in methods, pseudotime calculated, gene sets of interest quantified, and response grouped; pseudobulk differential expression analysis with GSEA was performed.
A. Unbiased clustering results visualized in UMAP dimensions colored by response.
B. Single-cell level pseudotime-ordered cells projected on UMAP dimensions.
C. Single-cell level pseudotime violin plot visualization grouped by response.
D-K. Gene set quantification box plots grouped by response: **D.** CD39$^+$CD69$^+$ stemness and **E.** CD39$^+$CD69$^+$ terminal differentiation signatures;[6] **F.** Exhaustion cluster 1 signature,[15] **G.** T cytotoxic, **H.** TCR, **I.** IL-7, and **J.** IL-12 signatures from Biocarta pathway database,[16-18] and **K.** OxPhos signature from GOBP pathway database.[16-18]

Figure 4: Unbiased Analysis of CD4$^+$ TIL Subset Identifies Features Associated With Clinical Benefit

From the full ~150K cell single-cell TIL dataset, CD4$^+$ TIL subset was analyzed as described in methods, pseudotime calculated, gene sets of interest quantified, and response grouped; pseudobulk differential expression analysis with GSEA was performed.
A. Unbiased clustering results visualized in UMAP dimensions colored by response.
B. Single-cell level pseudotime-ordered cells projected on UMAP dimensions.
C. Single-cell level pseudotime violin plot visualization grouped by response.
D-I. Gene set quantification box plots grouped by response: **D.** T cytotoxic, **E.** T helper, **F.** TCR, **G.** IL-7, and **H.** IL-12 signatures from Biocarta pathway database,[16-18] and **I.** OxPhos signature from GOBP pathway database.[16-18]

Conclusions

- In this set of samples, high-parameter, single-cell level, phenotypic characteristics of TIL differentiate patients who experienced disease progression from responders and those with stable disease
  - While TIL of patients with stable disease and complete or partial responses are more similar, the presented data suggest that the capture, expansion, and reinvigoration of CD8$^+$ TIL expressing expanded TCR clones from the tumor is important for clinical response to TIL cell therapy
- Collectively, these results shed additional light on the cellular and molecular characteristics of *ex vivo* expanded lifileucel TIL drug product and suggest additional features with a potential to guide future TIL product development
  - Specifically, promotion of stemness, cytotoxicity, and metabolic fitness might be valid avenues to target for the advancement of TIL efficacy

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Acknowledgments

- The authors thank the patients who provided tumor tissue for this study
- This analysis was funded by Iovance Biotherapeutics, Inc. (San Carlos, CA)
- Editorial support was provided by Peloton Advantage, LLC, an OPEN Health company, and funded by Iovance

Disclosures

- **Joe Dean:** Employment: Iovance Biotherapeutics. Stock or Stock Options: Iovance Biotherapeutics. Travel, Accommodations, Expenses: Iovance Biotherapeutics.
- **Joe Yglesias:** Employment: Iovance Biotherapeutics. Stock or Stock Options: Iovance Biotherapeutics. Travel, Accommodations, Expenses: Iovance Biotherapeutics.
- **Hequn Yin:** Employment: Iovance Biotherapeutics. Stock or Stock Options: Iovance Biotherapeutics. Travel, Accommodations, Expenses: Iovance Biotherapeutics.
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Abbreviations

BOR, best overall response; CCL4, chemokine (C-C motif) ligand 4; CITE-seq, Cellular Indexing of Transcriptomes and Epitopes by sequencing; CR, complete response; DP, drug product; gdT, gamma delta T cell; GOBP, gene ontology biological process; GSEA, gene set enrichment analysis; HIST, histone gene family; HSP, heat shock proteins; IFIT, interferon-induced protein with tetratricopeptide repeats; IL-7, interleukin 7; IL-7R, interleukin 7 receptor; IL-12, interleukin 12; KIR, killer cell immunoglobulin-like receptors; knn, k-nearest neighbor; NK, natural killer; OxPhos, oxidative phosphorylation; PCA, principal component analysis; PD, progressive disease; PR, partial response; R, response; RNA, ribonucleic acid; sc, single-cell; SD, stable disease; TCR, T-cell receptor; Tex, exhausted CD8$^+$ T cell; Th1, t helper 1; Th2, t helper 2; Tcm, central memory T cells; Tem, effector memory T cells; TIL, tumor-infiltrating lymphocyte; Trm, tissue resident memory; UMAP, Uniform Manifold Approximation and Projection; wnn, weighted-nearest neighbor.