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T cell clone abundance-weighted precursor mapping identifies pre-infusion tumor T cell states associated with response to lifileucel

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Background

- Lifileucel is an FDA-approved autologous tumor-infiltrating lymphocyte (TIL) cell therapy for advanced melanoma.[1] Clinical efficacy has been reported in advanced melanoma and is being tested in previously treated advanced non-small cell lung cancer (NSCLC).[2-4]
- The cellular determinants of response to lifileucel remain incompletely defined. Our prior single-cell analyses identified putative tumor-reactive (pTR) T cells in lifileucel products as a candidate correlate of response.[5-7]
- It is not known which baseline tumor T cells give rise to the dominant clones in the infused product, or whether that origin matters for response. Knowing this could guide manufacturing toward the cells that drive efficacy.
- Individual TCR clones can span more than one baseline transcriptional state, so single-label clone assignment discards information.
- We developed a clone abundance-weighted precursor-state model that backtracks infused lifileucel clones to their full baseline state distribution, weighted by abundance in the product.
- Here we apply this model to paired baseline tumor and lifileucel single-cell data from patients with melanoma or NSCLC, including both immune checkpoint inhibitor (ICI)-naive and ICI-refractory patients, to identify baseline T cell states associated with clinical response. These analyses are exploratory and hypothesis-generating.

Methods

- Twenty-two patients from four Iovance clinical trials were analyzed (Table 1). Baseline (Day 0) tumor digest and matched Day 22 lifileucel drug product were profiled by single-cell RNA sequencing (scRNA-seq) and single-cell T-cell receptor sequencing (scTCR-seq).
- Clinical responders were defined as best overall response (BOR) of complete response (CR) or partial response (PR) per RECIST v1.1.
- Day 0 T cells were integrated and clustered, and T cell subsets were annotated manually using marker genes, published cell-type signatures, and automated annotation. Analysis stack: Seurat v5,[9] sctransform,[10] Harmony,[11] and scATOMIC.[12] pTR clusters were identified using the NeoTCR signatures.[8] Gene set scoring was performed with UCell.[13]
- **Weighted precursor-state model:** each Day 22 lifileucel clonotype was weighted by its abundance in the drug product and back-mapped to the Day 0 tumor T cells carrying it, and the weighted Day 0 state distributions were summed within patient to give a patient-level precursor profile (Figure 1).
- **Statistics:** per-cluster weighted precursor-state scores were compared between responders (n = 12) and non-responders (n = 10) by Wilcoxon rank-sum test with Benjamini-Hochberg (BH) adjustment. Matched comparisons across baseline precursor groups (all 22 patients) used the Friedman test with Dunn's multiple comparison correction.

Results

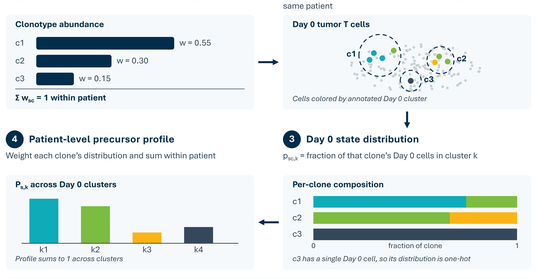
Paired Day 0 tumor and Day 22 lifileucel single-cell data were analyzed from 22 patients across four trials spanning melanoma and NSCLC (Table 1). Clones originating from baseline pTR CD8 T cells dominated the infused product, and the clone abundance-weighted precursor-state model identified baseline T cell states that differed between responders and non-responders (Figures 2 to 4).

Table 1. Patients analyzed, by trial

Trial (registration)	Indication	Prior ICI status	n
C-144-01 (NCT02360579)	Melanoma	ICI-refractory	7
IOV-COM-202 cohort 1A (NCT03645928)	Melanoma	ICI-naive	3
IOV-COM-202 cohort 3A (NCT03645928)	NSCLC	ICI-naive	2
IOV-LUN-202 (NCT04614103)	NSCLC	ICI-refractory	10
All trials	**Melanoma 10 (45%)** **NSCLC 12 (55%)**	**ICI-refractory 17 (77%)** **ICI-naive 5 (23%)**	**22**

Translational analysis cohort, descriptive (no statistical test). Clinical response: responders (BOR of CR or PR) 12 (55%) and non-responders 10 (45%) per RECIST v1.1. The cohort includes two patients added after abstract submission (submitted abstract, n = 20).

1 Figure 1. Clone abundance-weighted precursor-state model



$$P_{s,k} = \Sigma_c \, w_{sc} \cdot p_{sc,k}$$

with $\Sigma_c w_{sc} = 1$ within each patient s, so weights are normalized within patient before pooling across patients

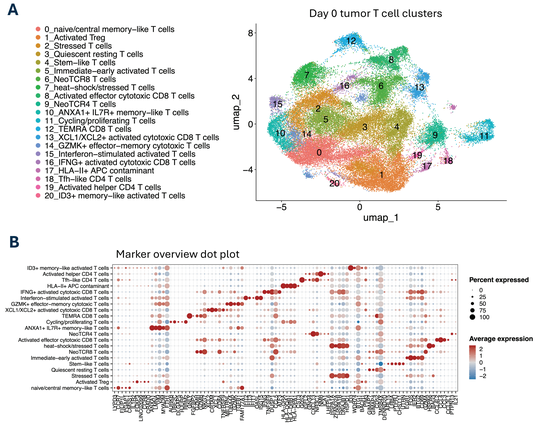
s, patient; c, sample-specific clonotype; k, Day 0 cluster; w, Day 22 abundance weight; p, Day 0 state distribution

Compare patient-level profiles between groups: responders (n = 12) versus non-responders (n = 10), BH-adjusted Wilcoxon rank-sum test

Day 0 cluster (illustrative) k1 k2 k3 k4 no matching Day 22 clonotype

Schematic of the clone abundance-weighted precursor-state model. (1) Each Day 22 lifileucel clonotype c in patient s is assigned a weight w_{sc} from its abundance in the drug product. (2) Each clonotype is back-mapped to all Day 0 tumor T cells carrying the same patient-specific clonotype. (3) For each clonotype, the Day 0 state distribution $p_{sc,k}$ is computed as the fraction of that clone's Day 0 cells in each Day 0 cluster k, so clones spanning more than one baseline state are not forced to a single label. (4) The patient-level precursor profile is the weighted sum $P_{s,k} = \Sigma_c \, w_{sc} \cdot p_{sc,k}$, with $\Sigma_c \, w_{sc} = 1$ within each patient. Clusters, clone counts and weights shown are illustrative.

2 Figure 2. Single-cell landscape of baseline tumor T cells



(A) UMAP of integrated Day 0 tumor-infiltrating T cells from 22 patients, colored by annotated T cell subset. (B) Marker gene expression dot plot supporting cluster annotation. Descriptive; no between-group comparison or statistical test.

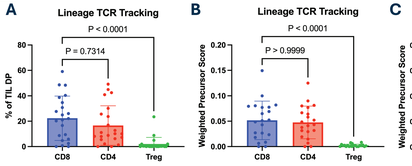
KEY FINDING

Dominant lifileucel TCR clones trace back to putative tumor-reactive NeoTCR8 T cells in the baseline tumor, and capture of this precursor state trended with clinical response.

Exploratory, hypothesis-generating analysis of paired Day 0 tumor and Day 22 lifileucel single-cell data

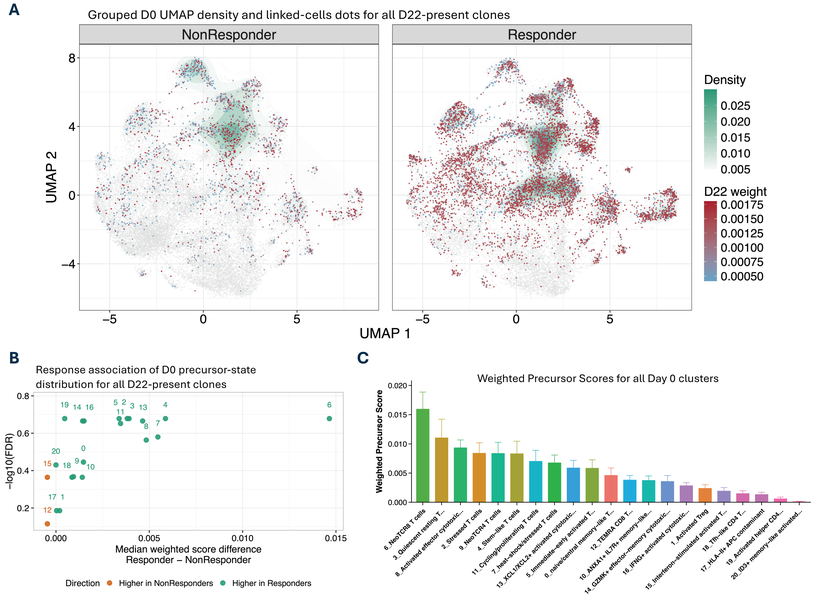
22 patients | **4** clinical trials | **2** tumor types

3 Figure 3. Clonal dominance in lifileucel by baseline precursor state



(A) Clonal abundance in the Day 22 lifileucel product, expressed as percent of the lifileucel product or (B) Weighted Precursor Score, for clones originating from CD8, CD4, or Treg lineage. (C) Clonal abundance in the Day 22 lifileucel product, expressed as Weighted Precursor Score, for clones originating from putative tumor-reactive T cells (NeoTCR8 / NeoTCR4) or putative bystander T cells (Tn/Tcm) baseline clusters. All 22 patients, with each patient contributing one value per baseline tumor cell type group shown. Statistics shown are based on Friedman test with Dunn's multiple comparison test correction.

4 Figure 4. Baseline tumor T cell landscape stratified by clinical response



(A) UMAP visualization of Day 0 tumor-infiltrating T cells from the 22 patients, faceted by response status; descriptive. Each dot is a single cell, colored by its Day 22 weighted precursor-state score; light grey indicates a clonotype not detected in the lifileucel product. Cell density is overlaid in green. (B) Weighted precursor-state score per Day 0 T cell cluster, comparing between responders (n = 12) and non-responders (n = 10) by BH-adjusted Wilcoxon rank-sum test. NeoTCR8 cluster 6 (CD8 Tpoly NCR+) stands out as a cell subset which scored higher in responders while TEMRA cluster 12 and IFN-stimulated cluster 15 were the only cell subsets more prevalent in non-responders. (C) Per-patient Day 0 cluster contribution to the Day 22 lifileucel repertoire expressed as Weighted Precursor Score, Mean and SEM; descriptive, with no statistical test applied.

Conclusions

- Clone abundance-weighted precursor mapping links each infused lifileucel clone to its full distribution of baseline tumor T cell states rather than a single label (Figure 1).
- Baseline tumor T cells resolved into 21 clusters. These include putative tumor-reactive NeoTCR8 (CD8 Tpoly NCR+) and NeoTCR4 T cells, bystander-like CD4 Tn/Tcm T cells, Treg, and TEMRA CD8 T cells (Figure 2).
- pTR NeoTCR8-derived clones outscored bystander-like naive/Tcm-derived clones (p = 0.0014), and Treg-derived clones were either not detected or did not expand (Figure 3).
- Responders' lifileucel clones traced back more heavily to the NeoTCR8 precursor state (cluster 6), a trend that was not significant after BH correction. TEMRA (cluster 12) and IFN-stimulated (cluster 15) precursors were the only numerically more prevalent precursor states in non-responders (Figure 4).
- Baseline tumor T cell state, and how well manufacturing captures and expands tumor-reactive precursors, may influence lifileucel efficacy across melanoma and NSCLC and in ICI-naive and ICI-refractory patients. If confirmed in larger cohorts, this could guide TIL manufacturing to enrich for therapeutically relevant clones.

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Abbreviations

APC, antigen-presenting cell; BH, Benjamini-Hochberg; BOR, best overall response; CR, complete response; D0/D22, Day 0/Day 22; DP, drug product; FDA, US Food and Drug Administration; FDR, false discovery rate; HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; IFN, interferon; NCR, natural cytotoxicity receptor; NeoTCR, neoantigen-reactive T-cell receptor; NSCLC, non-small cell lung cancer; PR, partial response; pTR, putative tumor-reactive; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; scRNA-seq, single-cell RNA sequencing; scTCR-seq, single-cell T-cell receptor sequencing; SEM, standard error of the mean; TCR, T-cell receptor; Tcm, central memory T cell; TEMRA, terminally differentiated effector memory T cell re-expressing CD45RA; Tfh, T follicular helper cell; TIL, tumor-infiltrating lymphocyte; Tn, naive T cell; Tpoly, polyfunctional cytotoxic T cell; Treg, regulatory T cell; UMAP, Uniform Manifold Approximation and Projection.

Disclosures, funding and notices

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