

Enrichment of putative tumor-reactive clones in NSCLC patients responding to IOV-LUN-202 TIL cell therapy: temporal single-cell analysis from a clinical trial



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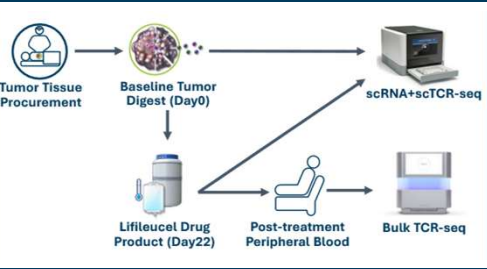
BACKGROUND

- Lifileucel is an FDA-approved autologous tumor-infiltrating lymphocyte (TIL) cell therapy for patients with advanced melanoma and has shown encouraging clinical activity in non-small cell lung cancer (NSCLC).
- Clinical efficacy of lifileucel monotherapy in previously treated advanced nonsequamous NSCLC is currently being tested in the registrational IOV-LUN-202 trial (NCT04614103).
- The cellular determinants of response to TIL therapy in NSCLC remain incompletely defined.
- Putative tumor-reactive (pTR) T cells can be identified from single-cell transcriptomes using the NeoTCR4 and NeoTCR8 signatures¹.
- Here we longitudinally track pTR T cells across baseline tumor, infused lifileucel, and post-treatment peripheral blood in chemotherapy- and immune checkpoint inhibitor (ICI)-refractory NSCLC patients to identify translational features associated with clinical response.

METHODS

- Based on available study patient samples, eight chemo- and ICI-refractory NSCLC patients from the registrational IOV-LUN-202 trial were selected for analysis.
- Lifileucel and matched baseline tumor digests were analyzed by single-cell RNA sequencing (scRNA-seq) and single-cell T-cell receptor sequencing (scTCR-seq).
- Baseline tumor T cell clusters were annotated manually based on marker genes, published cell-type specific signatures, and automated annotation results.
- The pTR T-cell cluster was identified using the published NeoTCR8 method, and clone trajectories were tracked across sample types.
- Bulk TCR-seq of post-treatment peripheral blood was analyzed for persistence of pTR clonotypes.
- Responders (R, n=4) were compared with non-responders (NR, n=4). Statistical comparisons used the Mann-Whitney U test unless noted otherwise.
- Single-cell analysis tool stack (e.g., Seurat², scran³, Harmony⁴, scATOMIC⁵, scRepertoire⁶, ggaluvial⁷, GSA⁸, UCell⁹) as applied in related Iovance single-cell analyses^{10,11}.

Figure 1. Study design and longitudinal sampling schema



Legend. Baseline tumor digests (Day 0) from IOV-LUN-202 (chemo- and ICI-refractory NSCLC patients) and paired infused lifileucel drug product (Day 22) were analyzed by scRNA- and scTCR-seq. Post-treatment peripheral blood was analyzed by bulk TCR-seq.

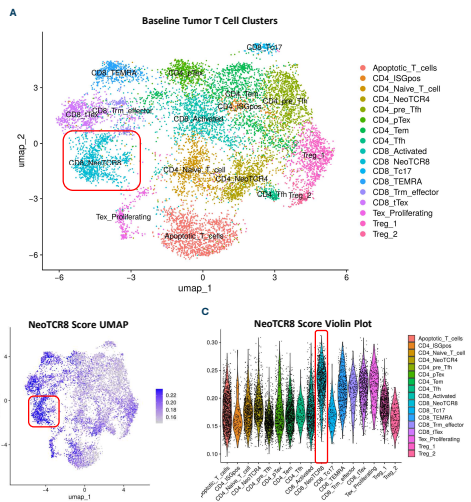
RESULTS

Table 1. Baseline and cell-product characteristics, responders vs non-responders

Characteristic	Responders (n=4)	Non-responders (n=4)	p-value
Age (median), years	65.5 (39-68)	59.5 (26-63)	0.2000
Female, %	50	0	n/a
Adenocarcinoma, %	75	75	n/a
KRAS mutated, %	0	50	n/a
Bulk cell dose (median, ×10 ⁶)	31.4 (16.6-47.0)	28.0 (17.0-31.8)	0.6857
IL-2 doses (median)	5.5 (1-6)	6.0 (3-6)	0.7143
CDB in resected tumor (median, %)	30.6 (24.8-39.0)	30.9 (22.1-37.9)	0.6857
pTR CDB in resected tumor (median, %)	8.4 (5.5-16.6)	8.0 (4.1-12.6)	0.6857
pTR CDB in infused TIL (median, ×10 ⁶)	2.902 (1.7-8.2)	0.276 (0-3.7)	0.1143
Tregs in resected tumor (median, %)	9.7 (6.1-11.0)	6.85 (3.5-8.3)	0.2000
Tregs in infused TIL (median, ×10 ⁶)	0 (0-0.02)	0 (0-0.02)	0.4533

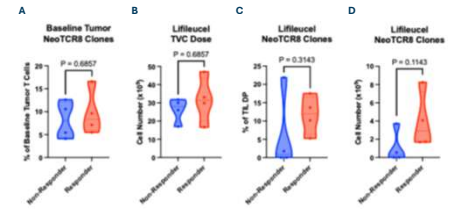
Legend. Values are medians (range) unless noted. p-values by Mann-Whitney U test; "n/a" indicates comparison not calculated. Single-cell quantification was updated after abstract submission using a revised analysis pipeline; clinical parameters and all statistical comparisons are unchanged.

Figure 2. Single-cell transcriptomic landscape of tumor identifies pTR T cells



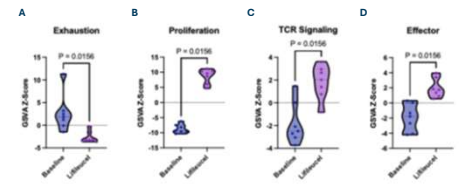
Legend. Integration of tumor single-cell data and clustering with manual cell-type annotation. (A) Harmony-integrated UMAP with manually labeled T-cell clusters. (B) Harmony-integrated UMAP with individual-cell NeoTCR8 scores calculated by UCell. (C) Violin plots of individual-cell NeoTCR8 scores calculated by UCell for each cluster annotated in (A). The NeoTCR8 cluster is indicated by a red box.

Figure 3. pTR T-cell content in lifileucel trends lower in non-responders



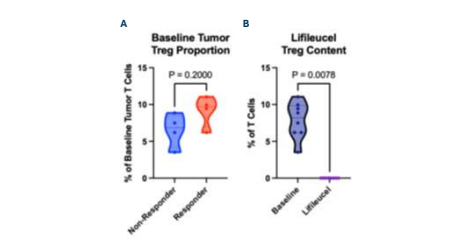
Legend. Proportion and/or absolute number of pTR CDB T cells in baseline tumor and infused lifileucel, compared between responders and non-responders. (A) Baseline tumor pTR content was similar between groups. (B-C) Analysis of the infusion product: (B) total viable cell number and (C) proportion of NeoTCR8 clones in the infused lifileucel product were similar between groups; (D) non-responders tended to contain fewer pTR T cells in their infusion product.

Figure 4. Manufacturing process reinvigorates pTR T cells relative to baseline tumor



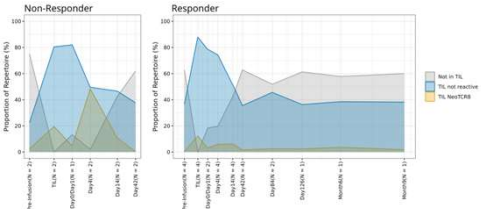
Legend. Gene-signature analysis comparing pTR (NeoTCR8) T cells in baseline tumor versus lifileucel. Relative to baseline, pTR T cells in lifileucel showed (A) lower exhausted¹² and (B) higher proliferative¹³, (C) TCR signaling¹⁴, and (D) effector¹⁵ cell state. Patient-level pseudobulk GSEA score (z-score method), baseline tumor vs paired lifileucel, n=7; two-sided Wilcoxon signed-rank test (p = 0.0156 for all comparisons).

Figure 5. Tumor Treg content is higher in responders but absent in lifileucel



Legend. Regulatory T-cell (Treg) content in baseline resected tumor and in infused lifileucel. (A) Baseline tumors from responders showed a trend toward a higher proportion of Tregs than non-responders. (B) TCR clones originating from baseline tumor Tregs were undetectable in lifileucel in all patients, n=6; two-sided Wilcoxon signed-rank test. This baseline association and the lack of Treg in lifileucel contrast with reports linking Tregs to ICI therapy resistance^{16,17}.

Figure 6. pTR clonotypes persist in post-treatment peripheral blood



Legend. Persistence of NeoTCR8 clonotypes in peripheral blood by bulk TCR-seq. Persistence was observed in both responders and non-responders after lifileucel infusion.

CONCLUSIONS

- Baseline tumor samples, irrespective of response to lifileucel, may possess a particularly high level of exhausted T cells owing to the resection of chemotherapy- and ICI-refractory tumors.
- Baseline tumor pTR content was comparable between responders and non-responders.
- Non-responders tended to have fewer pTR T cells in the infused lifileucel product, suggesting that the amount of pTR in lifileucel may influence response.
- pTR T cells appear to be reinvigorated during manufacturing, shifting from an exhausted T cell phenotype toward an effector and proliferative state.
- The observed trend toward higher baseline-tumor Treg content in responders, combined with the absence of Tregs in lifileucel, may indicate a therapeutic opportunity for patients with prior Treg-mediated resistance to checkpoint blockade.

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ABBREVIATIONS

ICI, immune checkpoint inhibitor; IL-2, interleukin-2; NR, non-responder; NSCLC, non-small cell lung cancer; NeoTCR, neoantigen-reactive TCR; pTR, putative tumor-reactive; R, responder; scRNA-seq, single-cell RNA sequencing; scTCR-seq, single-cell T-cell receptor sequencing; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TIL-IP, TIL drug product; Treg, regulatory T cell; TVC, total viable cells; UMAP, Uniform Manifold Approximation and Projection.

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