Characterization of the Transcriptomic and T-Cell Receptor Clonal Heterogeneity of Tumor-Infiltrating Lymphocyte Therapy Infusion Products by Single-Cell Sequencing and Correlative Analyses With Clinical Efficacy in Patients With Advanced Cutaneous Melanoma

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Disclosures

- Employment with and stock or other ownership in Instil Bio, Inc.

Background

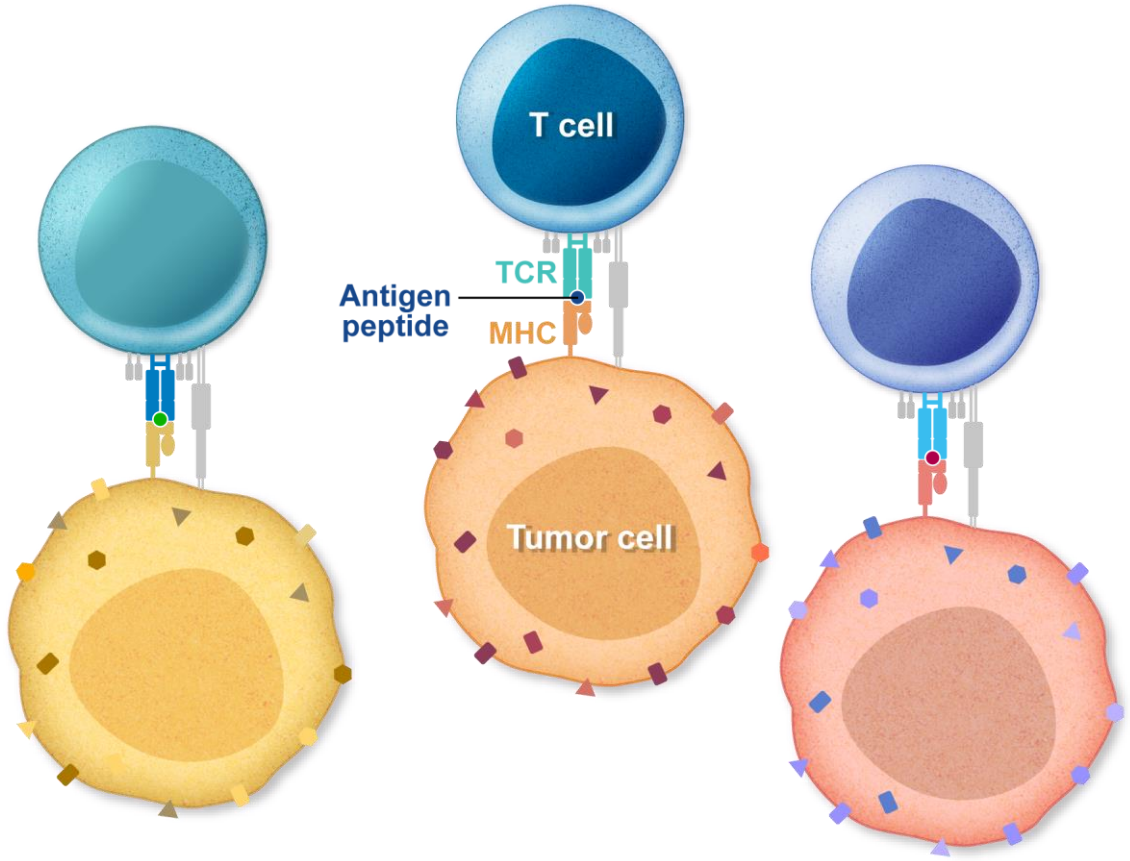
- Autologous TIL products are composed of a nonselected TCR repertoire and can recognize a broad set of tumor-associated antigens, including neoantigens specific to each patient's tumor[1]

- A compassionate use clinical series of TILs for the treatment of advanced cutaneous melanoma in 21 patients demonstrated high clinical response rates, with an ORR of 67% and a CRR of 19%, and a safety profile consistent with lymphodepletion and high-dose IL-2[2]

- The TIL products used in the compassionate use program were made with a prior version of the ITIL-168 manufacturing process and showed a high ORR (58%) among a subset of patients (n=12) who received previous PD-1 inhibitor therapy.[3] The DELTA-1 trial is currently evaluating this patient population[4]

1. Rohaan MW, et al. *J Immunother Cancer*. 2018;6:102. 2. Hawkins RE, et al. *Cancer Res.* 2021;81(13 suppl):LB150. 3. Pillai M, et al. *Ann Oncol.* 2021;32:S867-905. 4. Gastman B, et al. *J Immunother Cancer*. 2021;9(2 suppl):544.
CRR, complete response rate; IL, interleukin; TCR, T-cell receptor; ORR, objective response rate; TIL, tumor-infiltrating lymphocyte.

Cellular and Molecular Complexity of TIL Therapy



- Intratumor genetic heterogeneity
- T-cell transcriptional heterogeneity
- TCR receptor diversity
  - There are billions of potential TCR-β clonotypes, most of which are not well characterized by function or previously annotated[1]
- While only a subset of clones from TILs will demonstrate antitumor activity[2], a nonselected approach to manufacturing TIL products may maximize the potential for clones with antitumor activity to reach the final TIL product

Objective: To characterize the transcriptomic and TCR clonal heterogeneity of TIL therapy infusion products and its correlation to clinical efficacy

1. Freeman JD, et al. *Genome Res*. 2009;19:1817-1824. 2. Mora T, et al. *Curr Opin Syst Biol*. 2019;18:104-110.
IL, interleukin; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

Methods

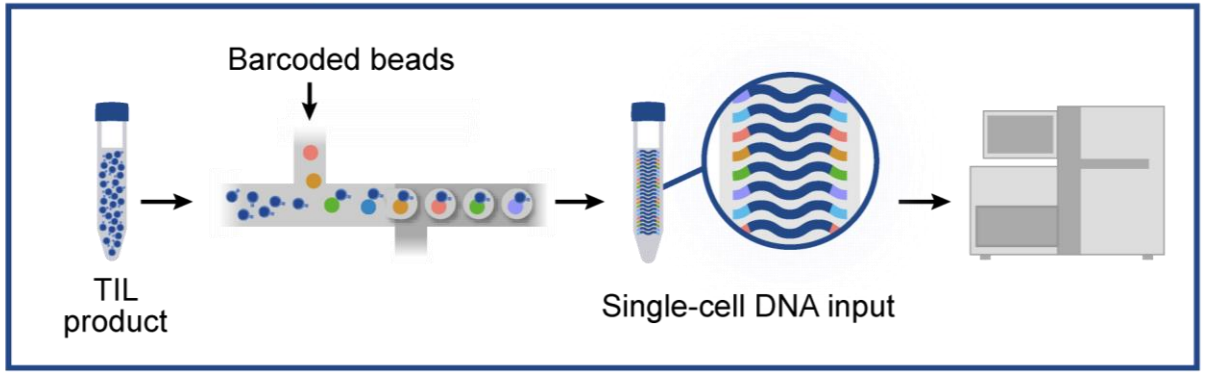
TIL Products

Compassionate Use Program

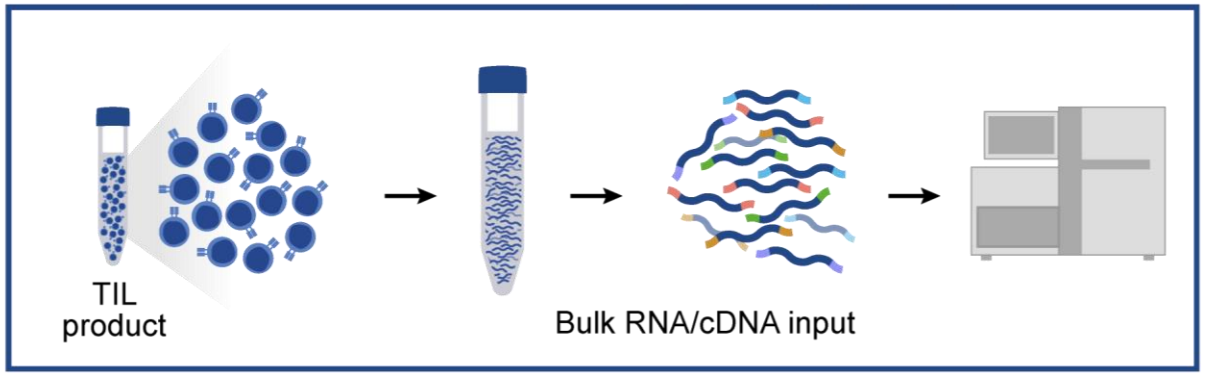
- 21 metastatic melanoma patients with no standard of care treatment options were treated with TIL between 2011 and 2019 at the Christie Hospital in Manchester, England
- All TIL products were manufactured by Instil Bio UK using a prior version of the autologous TIL therapy, ITIL-168, manufacturing process
- Disease responses were characterized retrospectively and were guided by RECIST 1.1

Analytical Assessments

Paired single-cell RNA and TCR sequencing (*n*=18)



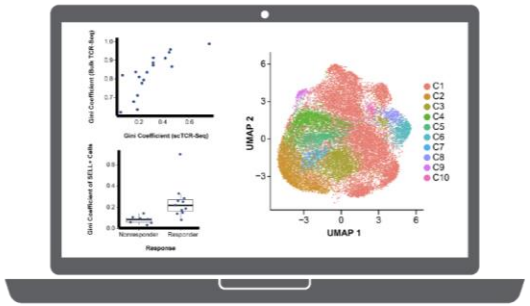
RNA-based bulk TCR sequencing (*n*=20)



Characterizations

- Gini coefficient[1]
- Inference of putative antigen-reactive clones[2]
- Unsupervised clustering of cells and differential gene expression analysis[3]
- Gene ontology analysis[4]
- Gene regulatory network analysis[5]

Readout

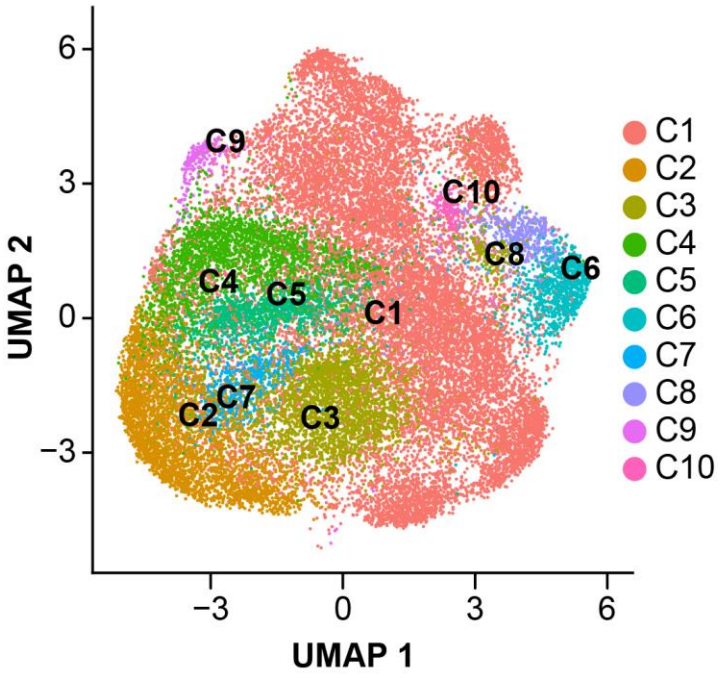


1. Nazarov V, et al. Immunomind/immunarch: 0.6.5: Basic single-cell support (0.6.5). Zenodo. 2020. Available at: https://doi.org/10.5281/zenodo.3893991. Accessed March 22, 2022. 2. Huang H, et al. *Nat Biotechnol*. 2020;38:1194-1202. 3. Hao Y, et al. *Cell*. 2021;184:3573-3587. 4. Yu G, et al. *OMICS*. 2012;16:284-287. 5. Aibar S, et al. *Nat Methods*. 2017;14:1083-1086.

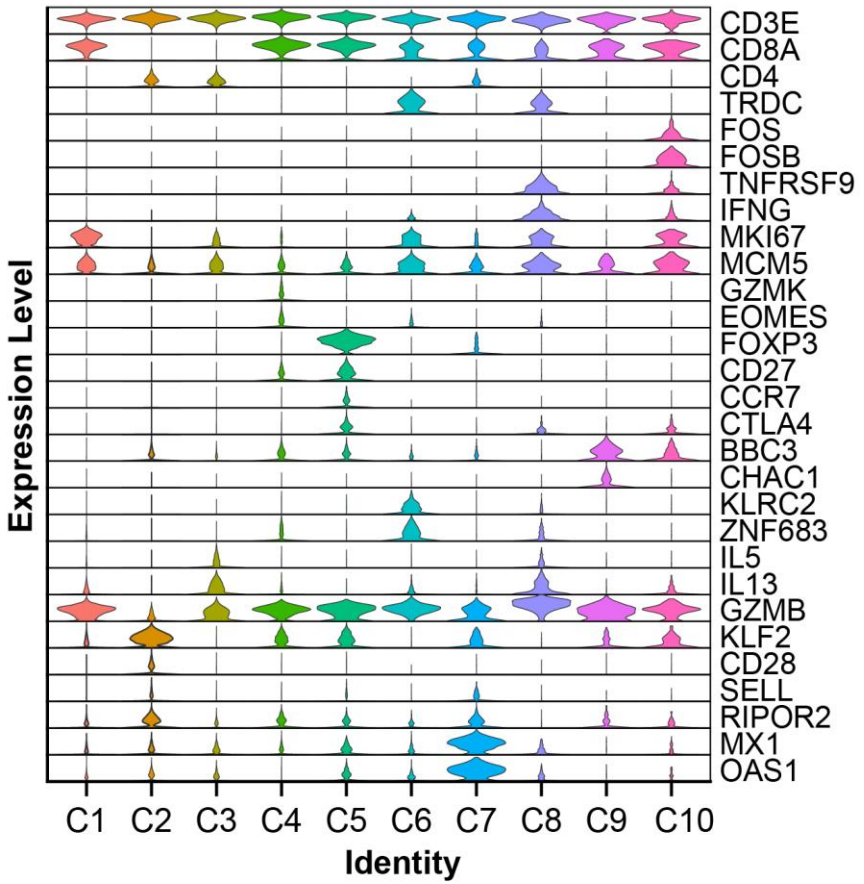
RECIST, Response Evaluation Criteria in Solid Tumours; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; UK, United Kingdom.

T-Cell Subpopulations With Distinct Gene Expression Profiles in TIL Products Revealed by Single-Cell RNA Sequencing Analysis

T-Cell Subpopulations



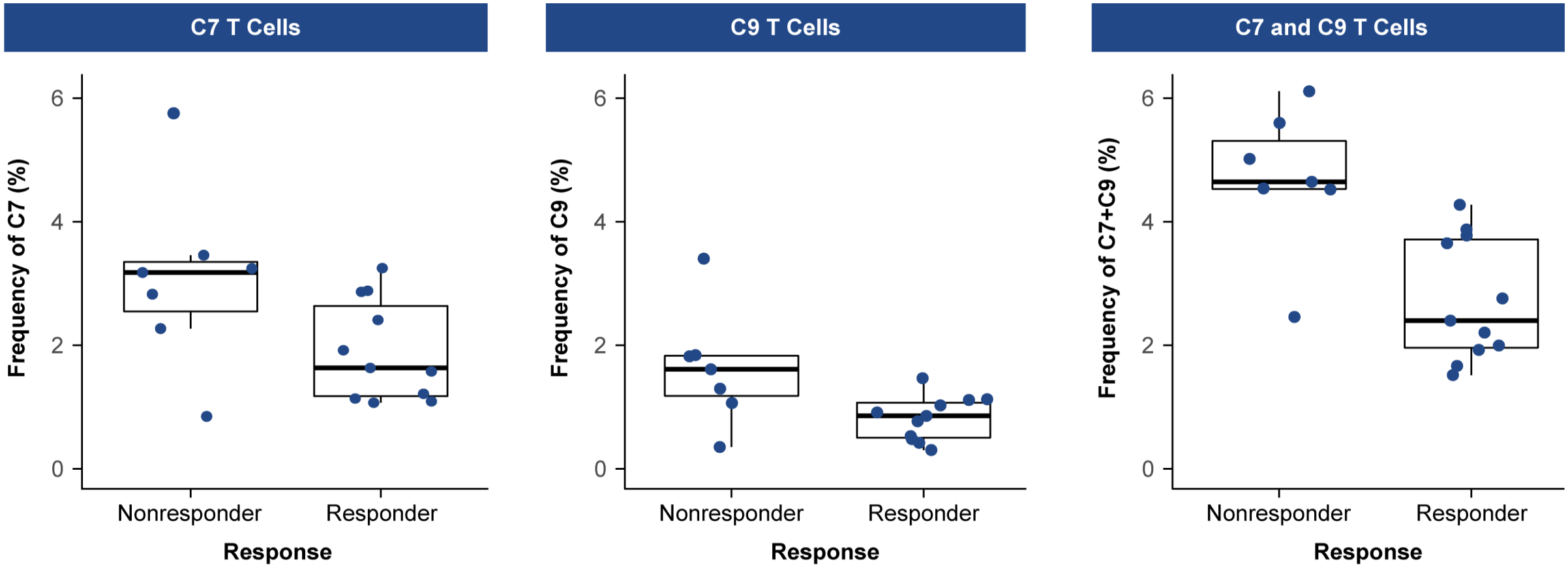
Gene Expression Profile of T-Cell Subpopulations



- Single-cell RNA sequencing analysis of TIL product samples identified several T-cell subpopulations with distinct gene expression profiles
- Multiple subpopulations were previously undescribed in TIL product samples

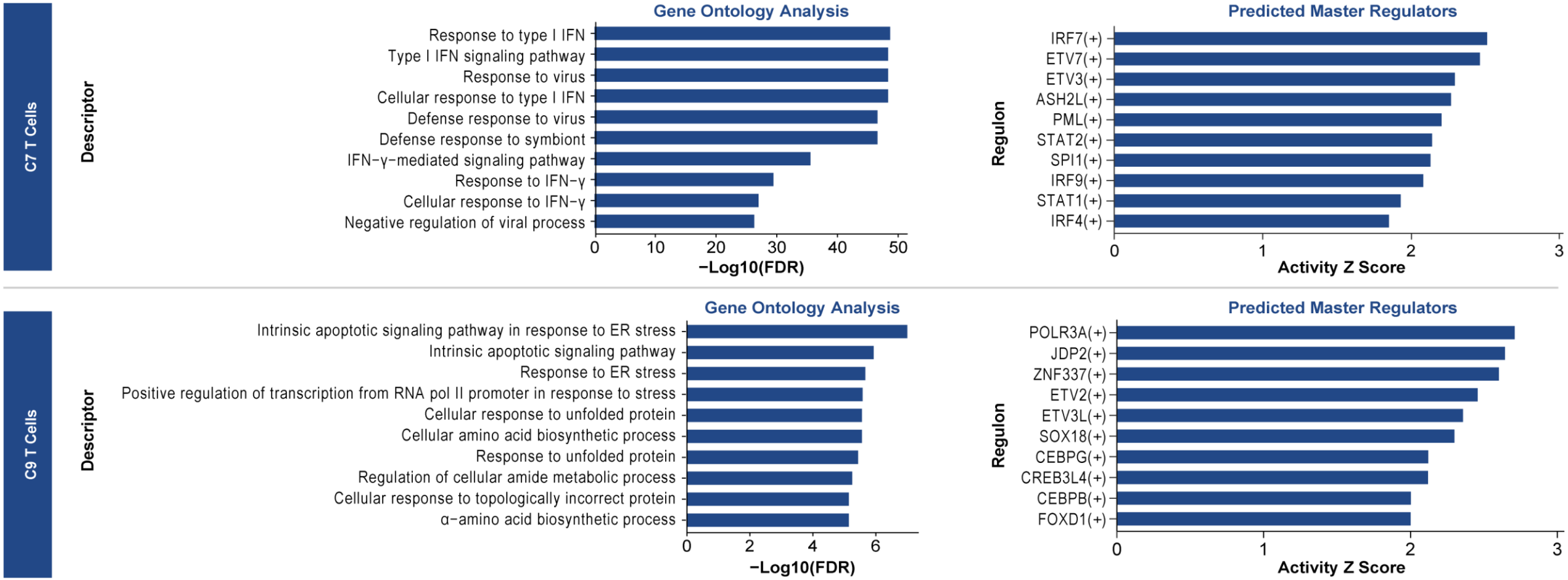
BBC, B cell lymphoma 2 binding component; CCR, chemokine receptor; CD, cluster of differentiation; CHAC, ChaC glutathione-specific gamma-glutamylcyclotransferase; C, cluster; CTLA, cytotoxic T lymphocyte associated antigen; EOMES, eomesodermin; FOS, family transcription factors; FOXP, forkhead box; GZM, granule enzyme; IFN, interferon; IL, interleukin; KLF, krüppel-like factor; KLRC, killer cell lectin-like receptor; MCM, minichromosome maintenance complex component; MKI67, marker of proliferation Ki-67; MX, MX dynamin like GTPase; OAS, oligoadenylate synthetase; RIPOR, ras homologous family interacting cell polarization regulator; SELL, selectin L; TIL, tumor-infiltrating lymphocyte; TNFRSF, tumor necrosis factor receptor superfamily; TRDC, T-cell receptor delta constant; UMAP, uniform manifold approximation and projection; ZNF, zinc finger protein.

Certain T-Cell Subpopulations Were More Frequently Observed in TIL Products Given To Nonresponders Than Responders



P values were not shown due to the retrospective nature of this analysis of data from a compassionate program. Trends observed were based on univariate analyses not adjusted for clinical factors that may have prognostic implications and thus should be interpreted with caution. Data are from univariate analyses that were not adjusted for clinical variances that may have prognostic implications.
C, cluster; TIL, tumor-infiltrating lymphocyte.

Gene Regulatory Network Analysis of TIL Products Identified Predicted Master Regulators of C7 and C9 T-Cell Subpopulations

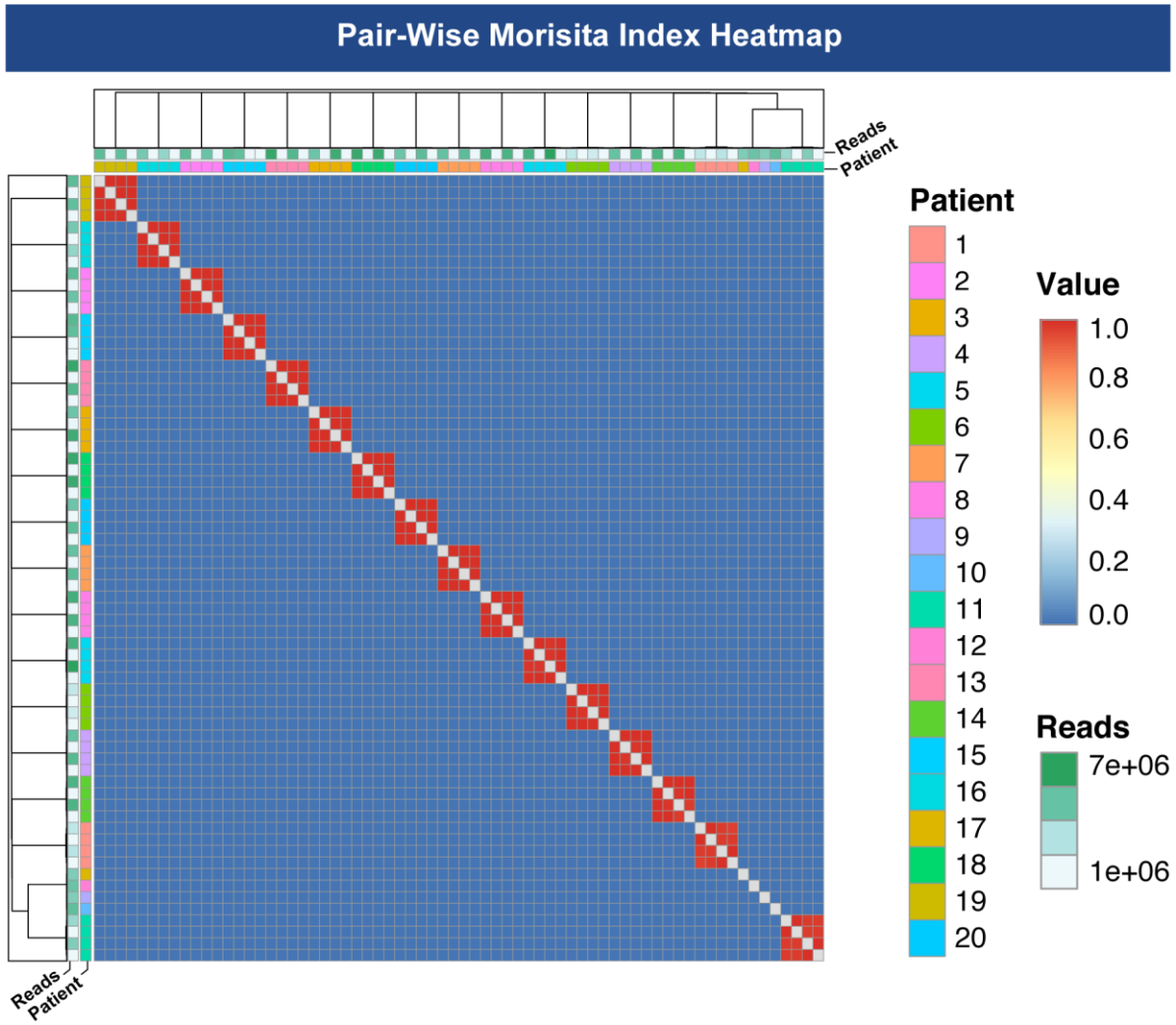


- Upregulation of type I IFN signaling pathway has been associated with shorter T-cell persistence in other cellular therapies[1]

1. Chen et al. *Cancer Discov*. 2021;11:2186–2199.

ASH2L, absent, small, or homeotic-like histone lysine methyltransferase complex subunit; CEBPB, CCAAT Enhancer Binding Protein Beta; CEBPG, CCAAT Enhancer Binding Protein Gamma; CREB3L4, cathelicidin antimicrobial peptide responsive element binding protein 3 like 4; ETV, ETS variant transcription factor; ETV3L, ETS variant transcription factor 3 like; FOX, forkhead box; IRF, interferon regulatory factor; JDP, jun dimerization protein; PML, promyelocytic leukemia protein; POLR3A, RNA polymerase III subunit A; SOX, SRY box transcription factor; SPI1, spi-1 proto-oncogene; STAT, signal transducer and activator of transcription; ZNF, zinc finger protein.

Polyclonal TIL Products With Little Overall TCR Repertoire Overlap

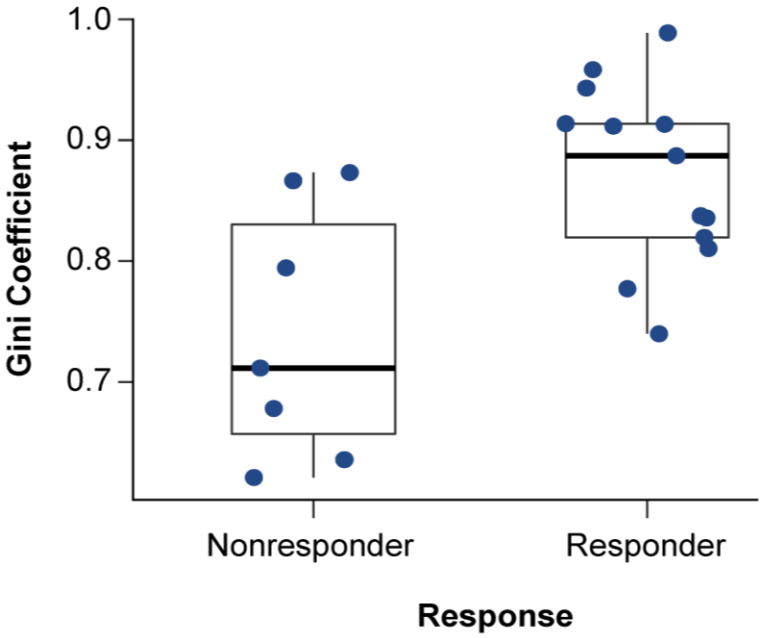


- A median of 39,597 (range, 3742-130,236) unique TCR β-chains were detected across 20 evaluable patients
- A low Morisita index[1] was observed across patients, suggesting a minimal overlap in TCR repertoire and distinct TCR β-chain repertoires between TIL samples
- The top 10 most abundant clonotypes were unique for all TIL product samples, and none have been previously annotated in the VDJdb public database[2]

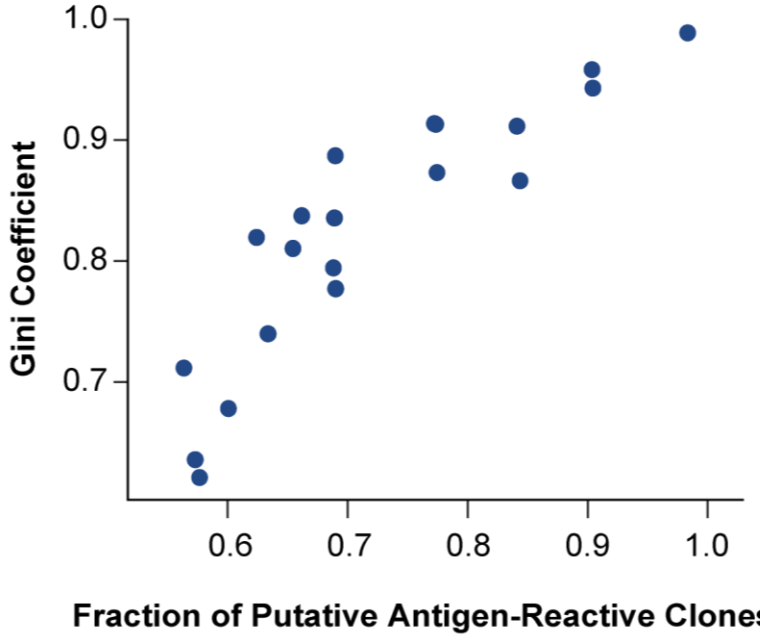
TIL product samples from all patients were tracked. Each color represents a uniquely identified clonotype.
1. Yang H, et al. *Front Oncol.* 2021;11:537735. 2. Shugay M, et al. *Nucleic Acids Res*. 2018;46(D1):D419-D427.
TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

Lower TCR β-Chain Repertoire Clonality Was More Frequently Observed in TIL Products Given To Nonresponders Than Responders

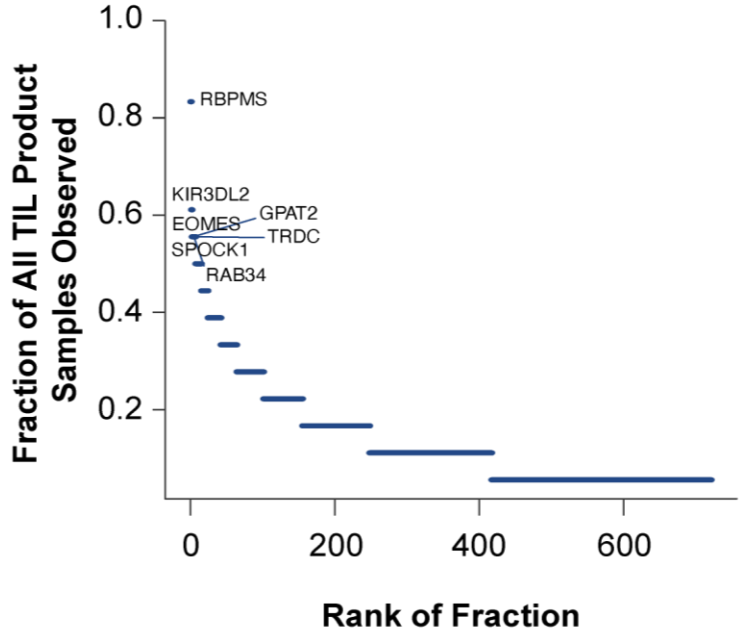
Gini Coefficient



Putative Antigen-Reactive Clones



Genes Associated With Higher Clonality



- Higher TCR β-chain repertoire clonality correlated with a higher fraction of in silico putative antigen-reactive T cells based on GLIPH2
- Higher clonality was frequently observed in more differentiated (EOMES+) T-cell subpopulations in TIL products

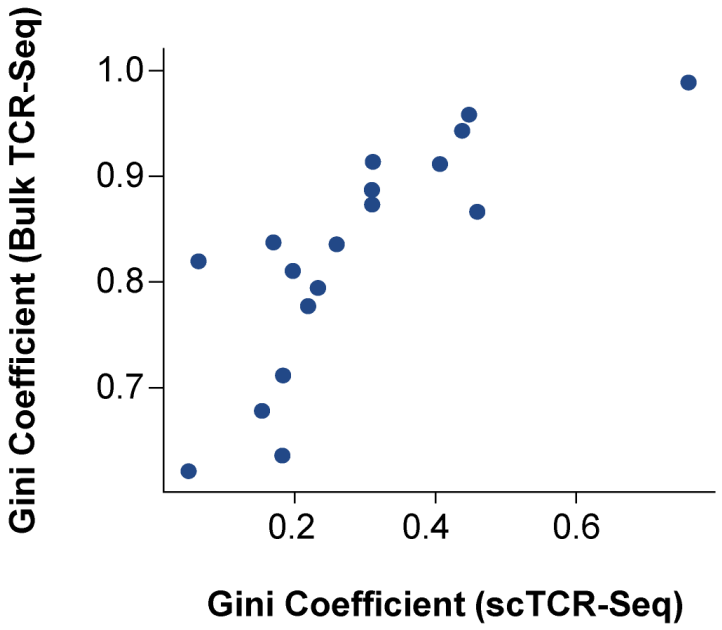
Clonality was measured by Gini coefficient on a scale of 0 (even distribution) to 1 (uneven distribution).[1] Antigen-reactive T cells were inferred by the GLIPH2 algorithm.[2] *P* values were not shown due to the retrospective nature of this analysis of data from a compassionate program. Trends observed were based on univariate analyses not adjusted for clinical factors that may have prognostic implications and thus should be interpreted with caution.

1. Thomas PG, et al. *Proc Natl Acad Sci USA*. 2013;110:1839-1844. 2. Huang H, et al. *Nat Biotechnol*. 2020;38:1194-1202.

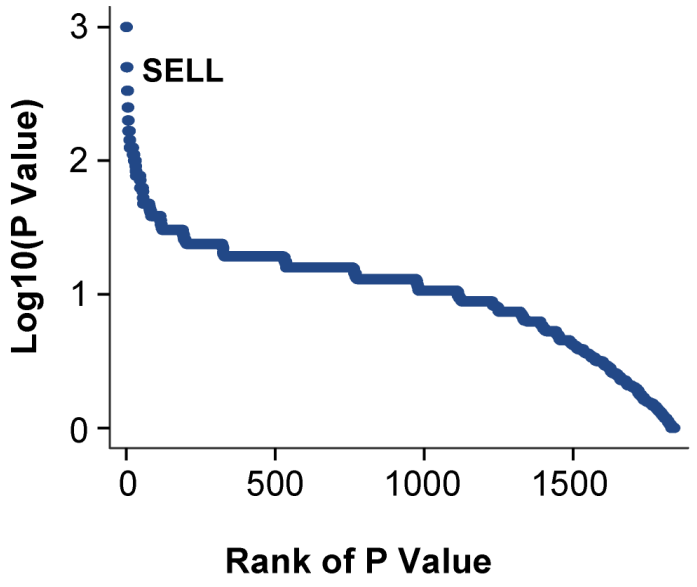
EOMES, eomesodermin; GLIPH2, grouping of lymphocyte interactions by paratope hotspots 2; GPAT2, glycerol-3-phosphate acyltransferase 2, mitochondrial; KIR3DL2, killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 2; RAB34, member rat sarcoma virus oncogene family; RBPMS, RNA binding protein, MRNA processing factor; SPOCK1, secreted protein acidic and cysteine rich, cwcv and kazal like domains proteoglycan 1; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TRDC, T-cell receptor delta constant.

Joint Analysis of Paired Single-Cell RNA and TCR Sequencing Data May Uncover a Novel Class of Biomarkers for Response

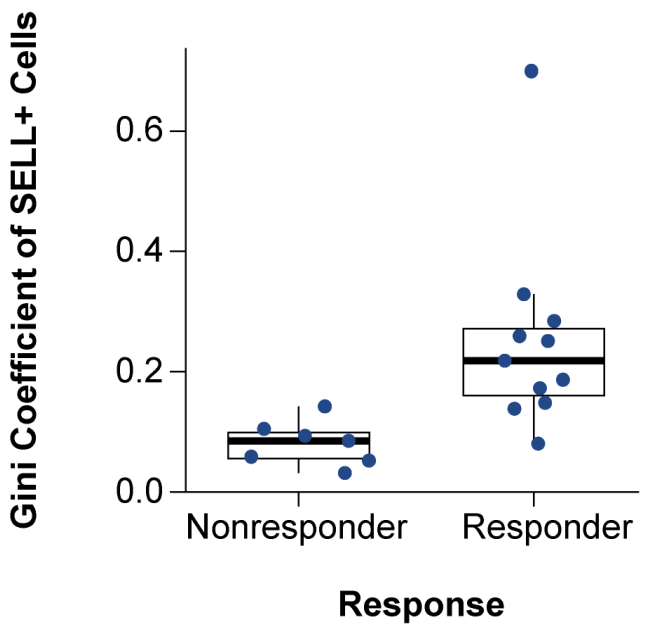
Concordance Between Bulk and Single-Cell TCR Sequencing



Top Single-Gene-Defined Subpopulations Whose Clonality Most Significantly Correlated with Response



Gini Coefficient of SELL+ T-Cell Subpopulation



- TCR repertoire clonality assessed by bulk and single-cell TCR sequencing demonstrated concordance
- TIL products with higher paired TCRαβ-chain repertoire clonality among SELL+ (CD62L+) cells were more frequently given to responders than nonresponders

[a]The SELL gene identified corresponds to the protein CD62L. Clonality was measured by Gini coefficient on a scale of 0 (even distribution) to 1 (uneven distribution).[1]
P values were not shown due to the retrospective nature of this analysis of data from a program. Trends observed were based on univariate analyses not adjusted for clinical factors that may have prognostic implications and thus should be interpreted with caution.

1. Thomas PG, et al. *Proc Natl Acad Sci USA*. 2013;110:1839-1844.

SELL, L-selectin; Seq, sequence; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

Conclusions

- Retrospective subanalysis of TIL therapy products utilized bulk and single-cell sequencing techniques
- TIL products showed high diversity both transcriptionally and with respect to TCR β-chain repertoire
- Recipients of TIL products with higher TCR β-chain repertoire clonality and lower frequencies of certain T-cell subpopulations were more frequently observed to develop a response to therapy
  - The role of the starting material and TIL manufacturing process in creating, maintaining, or altering the diversity of final products remains an open area of investigation
- These preliminary findings are based on a retrospective clinical and translational review of a compassionate use program performed at a single clinical center and require validation in prospective clinical studies such as the ongoing DELTA-1 trial (NCT05050006)

TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

Acknowledgments

- Thank you to the patients with metastatic melanoma who provided samples to inform this work

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