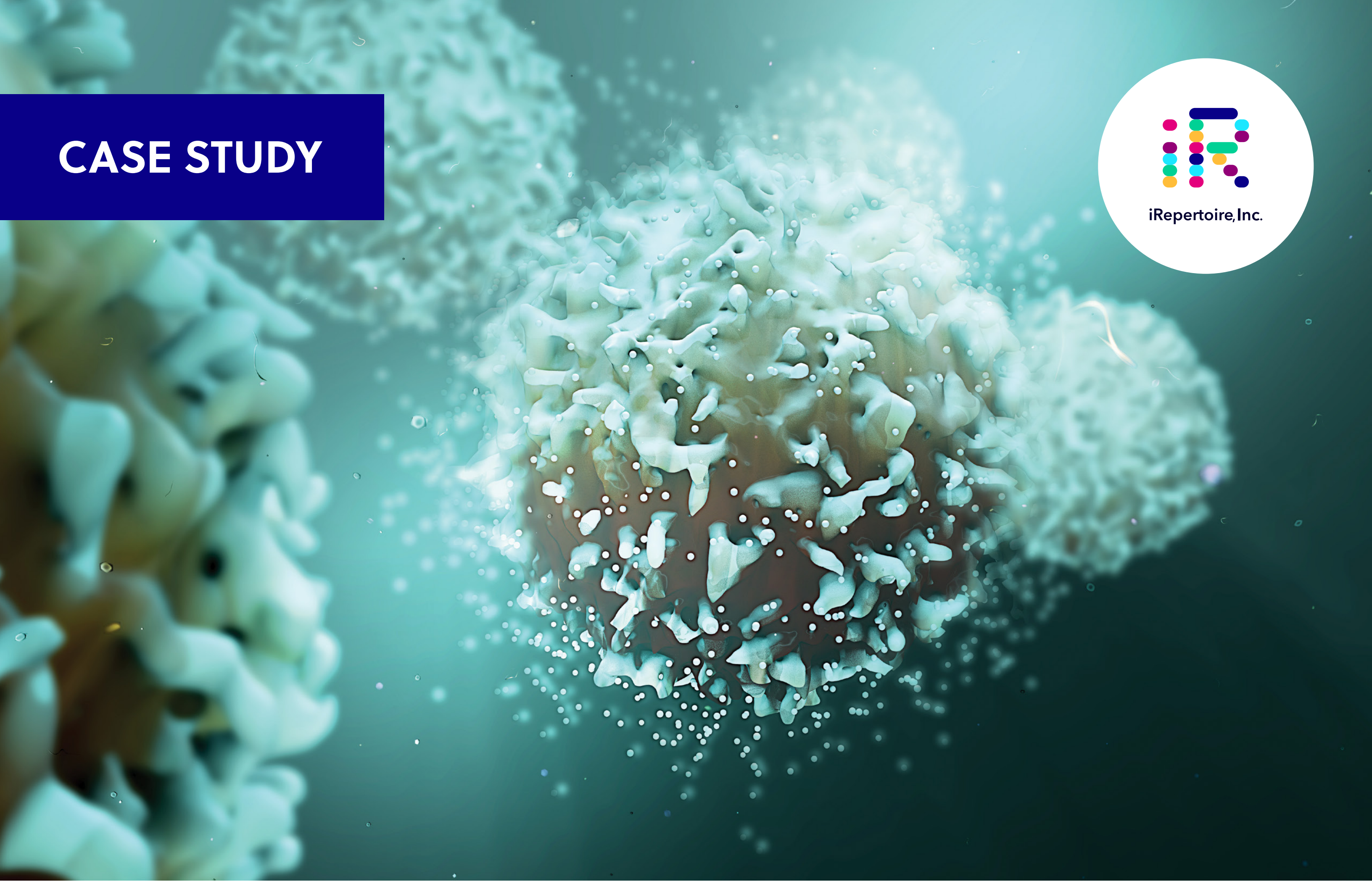

Leveraging Adaptive Immune Repertoire Profiling for Immunotherapy Biomarker Discovery

KEY TAKEAWAYS:

- Adaptive immune repertoire profiling and advanced analytics are fundamental for immunotherapy biomarker discovery. Biomarkers may include receptor diversity, clonal expansion, identification of specific clonotypes, and antigen mapping.
- iRepertoire's technology enables precision medicine through highly accurate TCR/BCR sequencing and comprehensive, quantitative data analysis tailored for biomarker discovery.
- iRepertoire's RepSeq+ and suite of advanced bioinformatics tools enabled the detection and characterization of TCR repertoire remodeling following infusion of an autologous TIL cell therapy in a Phase 2 clinical trial.

INTRODUCTION

The adaptive immune system can determine the success or failure of immunotherapy. T cells and B cells mount antigen-specific responses through their surface receptors—the T-cell receptor (TCR) and B-cell receptor (BCR). The diversity and dynamics of these receptors reflect the immune system's ability to recognize and eliminate tumor cells and respond to therapeutic intervention.

As immuno-oncology enters a precision era, the demand for biomarkers that explain response, resistance, and durability has grown rapidly. Yet commonly used markers such as PD-L1 expression or tumor mutational burden often fail to fully predict clinical outcomes because they do not directly measure the adaptive immune response itself.

TCR and BCR repertoire sequencing fills this gap by providing a quantitative view of immune diversity, clonal expansion, and persistence. By tracking how the adaptive immune system evolves during treatment, repertoire analysis can reveal biomarkers that inform patient selection, predict treatment response, uncover mechanisms of action, and identify potential adverse events—ultimately supporting the development of more effective immunotherapies.

THE CHALLENGES OF ADAPTIVE IMMUNE REPERTOIRE PROFILING

Immunotherapy biomarker discovery is both technically and analytically demanding. Realizing its full clinical utility requires overcoming a series of interconnected challenges that—without the right technology and expertise—can compromise data quality, reproducibility, and interpretability.

Challenges to immunotherapy biomarker discovery include:

- Inconsistent collection methods that increase natural inter-patient sample variability
- Accurately capturing the extreme biological diversity of the adaptome—the complete, functional, and diverse collection of unique TCR and BCR sequences that enable the immune system to recognize and respond to foreign and self antigens.
- Eliminating technical bias and sequencing artifacts
- Connecting TCR and BCR sequences to cellular phenotype, antigen specificity, and functional states
- Linking biomarkers to mechanistic insights

Addressing these challenges demands not only advanced sequencing chemistry, but also purpose-built bioinformatics with validated analytical frameworks aligned with regulatory expectations.

TECHNICAL AND ANALYTICAL SOLUTIONS FOR IMMUNOTHERAPY BIOMARKER DISCOVERY

iRepertoire's comprehensive immune repertoire profiling platform—built on proprietary multiplex PCR technology, quantitative sequencing workflows, and validation plans aligned with FDA expectations—is engineered to overcome these obstacles and deliver the precise, reproducible, and clinically actionable biomarker data that immunotherapy development requires.

SEQUENCING SOLUTIONS

Our RepSeq+ service is designed for comprehensive analysis of the adaptome, repertoire diversity, and clonal discovery from a wide range of samples (DNA, RNA, FFPE, whole blood, sorted cells, bone marrow, and tissues). Our sequencing technology delivers quantitative analysis of any combination of all seven chains of the adaptive immune system in a single reaction, enhanced with unique molecular identifier (UMI)-based error correction and customizable for sequencing depth. Simultaneous multi-chain amplification enables the calculation of over 200 immune repertoire metrics as potential biomarkers.

ANALYTICAL SOLUTIONS

To extract meaningful insights from complex TCR and BCR sequencing data, we have built an automated software pipeline that filters data to remove errors that may have occurred during the amplification and sequencing process. Data incorporating UMIs is processed to detect errors and determine sequence frequency. Once the data is filtered, several different types of analyses are performed.

For bulk VDJ analysis we provide a standard data analysis report and offer customizable advanced data analysis services tailored to your specific biomarker studies. Our advanced data analytics tools provide valuable insights for biomarker discovery that may include:

Diversity and Clonality Metrics

- Shannon entropy for overall repertoire diversity
- D50 analysis: percentage of unique vs. dominant clones
- Clonality scores and their interpretation in therapeutic context
- Comparing diversity across timepoints and patient cohorts

Clonal Dynamics and Expansion

- Identifying expanding vs. contracting clones
- Quantifying clonal replacement and turnover
- Distinguishing treatment-induced from bystander responses
- Tracking tumor-associated clonotypes

V/J Gene Usage and CDR3 Features

- V/J gene segment enrichment in responders
- CDR3 length distribution and amino acid composition
- Public vs. private TCR/BCR repertoires
- Convergent recombination patterns

Comparative and Correlative Analysis

- Repertoire similarity metrics between patients and timepoints
- Integration with clinical outcomes (survival, response rates)
- Correlation with other biomarkers (PD-L1, TMB, gene expression)
- Machine learning approaches for predictive signatures

Beyond adaptive immune repertoire profiling, we offer ImmunoSight™, a customizable targeted NGS assay that quantifies the expression of 150+ immunophenotype genes. This expanded data can reveal biomarkers associated with therapeutic safety and efficacy, adding additional insights that drive immunotherapy development.

FROM DATA TO BIOMARKERS

The analysis of highly accurate, unbiased immune sequencing data with iRepertoire's purpose-built solutions for immune repertoire profiling enables researchers to develop clinically impactful biomarkers with greater efficiency.

Our bioinformatics suite provides unique insights at every stage of immunotherapy biomarker development. As a result, researchers can analyze population-level repertoire diversity and clonality metrics to establish biomarkers for disease detection or therapeutic selection. Tracking dominant clones across timepoints may reveal biomarkers associated with therapeutic response. Discovery of TCR/BCR sequences associated with therapeutic response can enable the development of biomarkers that support cost-effective screening of large cohorts and accelerate clinical studies.

Case study: Monitoring T Cell Repertoire Dynamics in Response to an Autologous Tumor-Infiltrating Lymphocyte (TIL) Cell Therapy

A Phase 2 multicenter clinical study1 was conducted to evaluate lifileucel, an autologous tumor-infiltrating lymphocyte (TIL) therapy, as a potential treatment for metastatic non-small cell lung cancer (mNSCLC) resistant to prior immune checkpoint inhibitor (ICI) therapy. iRepertoire's technology enabled quality control of the TIL product and molecular characterization of pre- and post-infusion blood samples, supporting regulatory requirements and mechanistic correlates in the clinical trial.

Specifically, the team profiled TCR ß chain CDR3 sequences (i.e., clonotypes) using iRepertoire's RepSeq+ with the addition of unique molecular identifiers (UMIs) in baseline tumor tissues (FFPE), in the TIL product, in pre-infusion blood samples (day 7), and in post-infusion blood samples (day 42) to assess TCR repertoire dynamics and *in vivo* persistence of T cell clonotypes over time that may contribute to observed clinical response, Unique CDR3 sequences were identified and quantified using iRepertoire's proprietary algorithms. Additional analyses included normalization and filtering of clonotypes for limit of detection, followed by analysis of clonality, diversity, and samples' TCR repertoire overlaps.

Data analysis showed that the TCR repertoire of all sample types was highly polyclonal and the clonality of the post-infusion blood more closely resembled that of the TIL product rather than pre-infusion blood samples (**Figure 1A**). Infusion of lifileucel clearly led to peripheral TCR repertoire changes with notable expansion and persistence of clonotypes present in the TIL infusion product. Data showed a mean of 3,090 unique CDR3 clones present in baseline tumor samples, a mean of 4,076 unique CDR3 clones present in TIL infusion products, and a mean of 417 shared clones between baseline tumors and TIL products (5.5%) (**Figure 1B**). Shared clones persisted in post-infusion blood through month 6 in both responders and non-responders (**Figure 1C**).
TIL clones were also measured in both pre- and post-infusion blood and their percentage of the TCR repertoire at the post-infusion timepoint showed an expansion of TIL product clones in both responders and non-responders (**Figure 1D**).

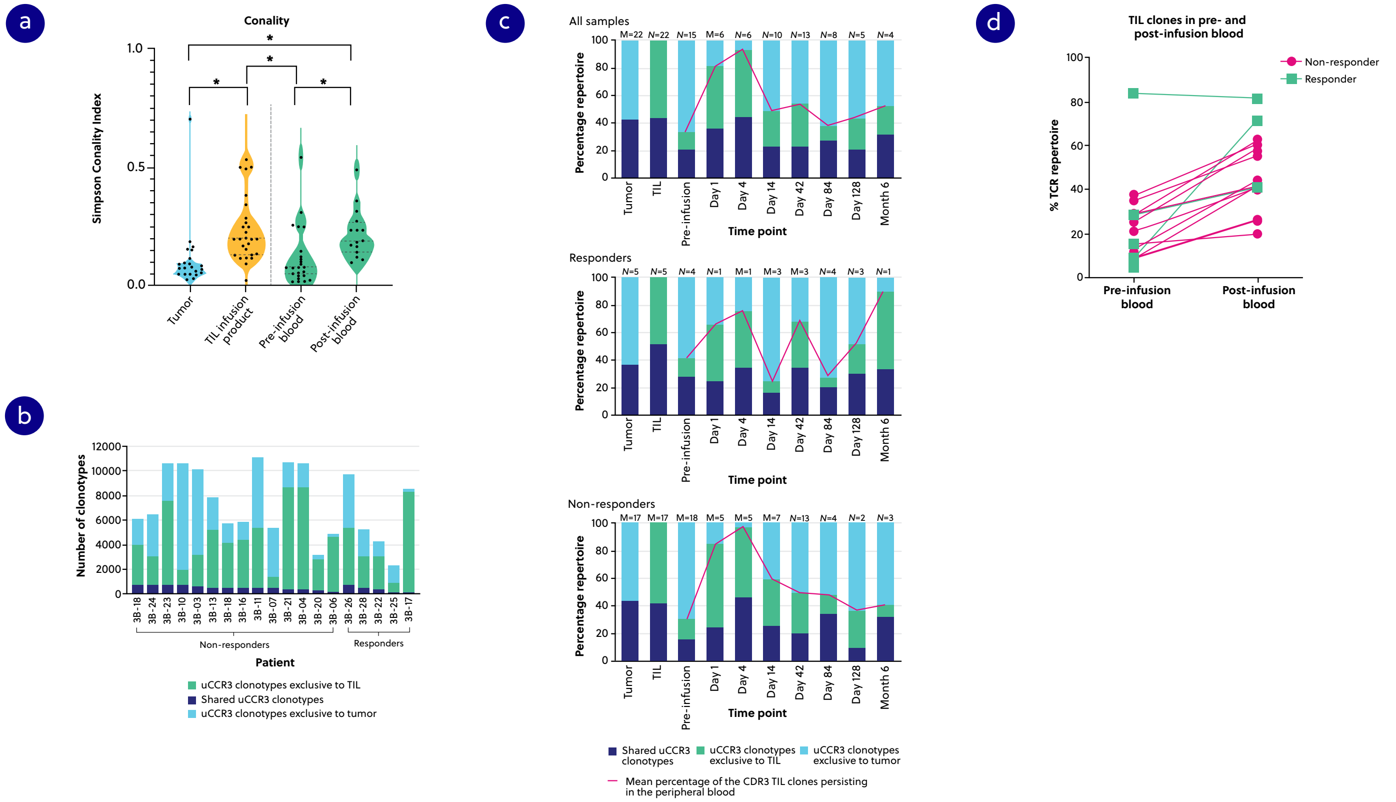

Figure 1. **(a) TCR repertoire profile across samples. (b) Data from an individual patient showing unique and shared clones between the tumor sample and TIL product. (c) TCR clonal expansion and persistence through 6 months. (d) Comparison of TIL clones in pre-infusion and post-infusion blood samples and their proportions in the total TCR repertoire in both sample types.**[1]

The small sample size in this study could not provide sufficient data to demonstrate a statistical association between TCR repertoire remodeling following lifileucel infusion and therapeutic response. These associations require further study.

iRepertoire's comprehensive immune repertoire profiling platform enabled the measurement of positive therapeutic responses to lifileucel in patients with tumor profiles typically resistant to immunotherapy, such as PD-L1-negative, low tumor mutational burden, and *STK11* and *KEAP1* mutations which play a role in ICI resistance in lung cancer, potentially addressing an unmet clinical need in patients with mNSCLC refractory to prior therapy. This same technology can be used to conduct further studies to identify and validate TCR repertoire biomarkers that may be indicative of therapeutic response.

CONCLUSION

Optimized immune repertoire sequencing paired with advanced profiling analytics provides a powerful solution for efficient immunotherapy biomarker development. iRepertoire's comprehensive services provide insights at each stage of biomarker development, and their expert team can provide assistance from study design through advanced data analysis.

Partner with iRepertoire to accelerate immunotherapy biomarker development. Contact us to get started.

REFERENCES

1. Schoenfeld, A.J., et al. Lifileucel, an Autologous Tumor-Infiltrating Lymphocyte Monotherapy, in Patients with Advanced Non-Small Cell Lung Cancer Resistant to Immune Checkpoint Inhibitors. Cancer Discov. 2024 Apr 1;14(8):1389-1402. doi: 10.1158/2159-8290 CD-23-1334