TCXpress™ and iTCXpress™: A Direct, High-throughput Platform Utilizing Parallel Processes for Efficient and Cost-Effective Discovery and Characterization of Novel TCRs

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Introduction

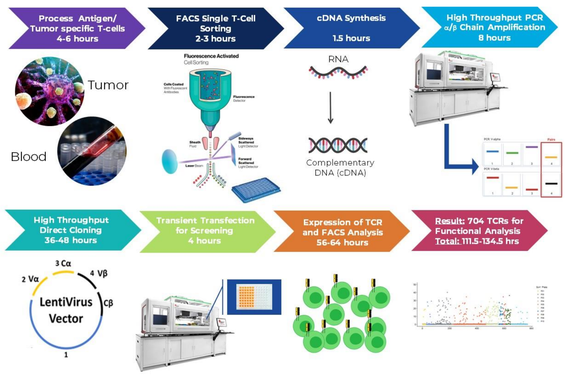
- TCR-T adoptive cell therapy (ACT) shows promise as an alternative to CAR-T ACT due to the ability to identify both surface and intracellular tumor antigens leading to a broader range of targets for treatment
- It is also believed that with no or minimal engineering, TCR-T therapeutics may prove to be a safer autologous treatment for patients than other ACTs
- However, challenges with TCR-T ACT still exist, including determination of lead antigen candidates for tumor specificity, persistence within the tumor microenvironment, and killing functionality
- Currently, generating TCR-T libraries to address these issues uses methods such as ex vivo cell expansion prior to isolation and sequencing TCRs from isolated tumor infiltrating lymphocytes (TILs) prior to creating synthetic DNA fragments for cloning
- These strategies often prove to be costly and time consuming while generating fewer options for downstream analysis and candidate identification
- We have developed a TCR discovery platform, TCXpress™, that can quickly and efficiently produce functional TCRs in a high throughput format.
- Furthermore, by applying the in-house developed bioinformatics platform iTCXpress™, we can quickly analyze and identify a broad range of unique antigen-specific TCRs for downstream processing and functional testing

Methods

TCX-101 Liquid Tumor Program

- Donors for Target 1 or Target 2 TCR isolation were identified using whole exome sequencing (WES)
- CD8⁺Target 1 or CD8⁺Target 2 multimer⁺ cells were directly isolated from peripheral blood mononuclear cells (PBMCs) by single-cell flow cytometry activated cell sorting (FACS)
- In separate experiments, antigen specific cells were expanded by co-culturing antigen presenting cells (APCs) pulsed with Target 1 or Target 2 peptide and PBMCs for one week and underwent single-cell FACS for CD8⁺Target 1 or CD8⁺Target 2 multimer⁺ cells
- TCXpress™ was utilized to clone TCRs isolated from the single cells into a lentiviral vector and expressed in a HEK293 reporter cell line to perform Target 1 or Target 2 multimer binding (**Figure 1**)
- In the case of Target 1, TCRs that bound multimer above background in HEK293 screening were re-expressed in a Jurkat (JRT) screening cell line
- The JRT screening cell lines containing TCRs of interest were co-cultured with APCs loaded with various concentrations of Target 1 peptide and CD69 upregulation was used to determine EC50s

Figure 1. Proprietary TCXpress™ Platform Produces TCRs in Direct, Streamlined Process

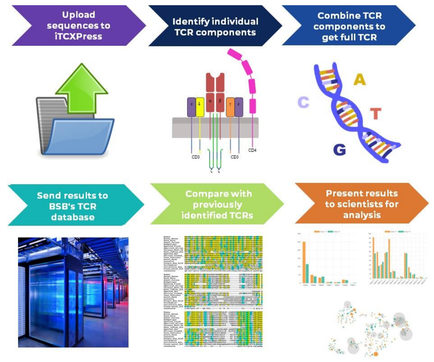


Methods (continued)

TCX-201 Solid Tumor Program

- Fresh tumors were received within 24 hours of excision and underwent mechanical and enzymatic dissociation to isolate TILs for both sorting and expansion
- Following dissociation, CD8⁺ T-cells were single-cell sorted via FACS and processed through TCXpress™
- Resulting lentiviral/TCR constructs were transfected into an HEK293 reporter line to analyze the efficiency of the library by assessing the number of samples that had double positive CD3 and TCR antibody binding
- Stable JRT screening cell lines for functional testing were generated from transduction with lentiviral/TCR constructs
- For databank purposes, TCR library sequences were generated by Sanger sequencing of the amplified TCR product and repertoire analysis was done using the iTCXpress™ platform (**Figure 2**)

Figure 2. Graphical Display of iTCXpress™: TCR Analysis Engine/Database and User Interface for Bench Scientists



Results

- 704 TCRs can be produced and screened for TCR surface expression and/ or specificity as early as 5–6 days (**Figure 1**)
- Single cell sorting through purification of the assembled plasmid costs approximately $5/ TCR (**Table 1**)

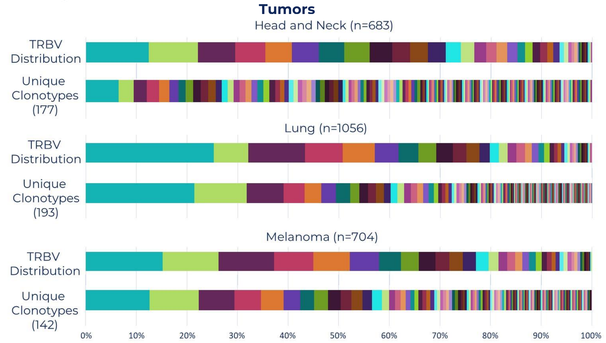
Table 1. TCXpress™ High-Throughput, Cost-Effective Production of TCRs

Quarter	High-Throughput TCR Production	High-Throughput Plasmid Production
Q1 2022	10503	506
Q2 2022	10134	2300
Q3 2022 (July- Current)	6887	2753

Stage of Process	Cost per well
Sort through TCR Amplification	$1.75
Cloning through Plasmid Purification	$3.42
Total	**$5.17**

- The iTCXpress™ platform has allowed the cloning and characterization of a variety of TCR repertoires against solid tumors (**Figure 3**)

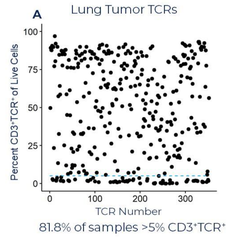
Figure 3. iTCXpress™ Identification of Unique TCR Clones in Solid Tumors



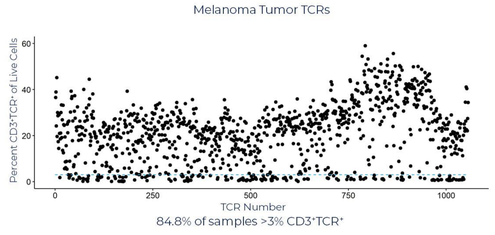
- >80% of samples from TCXpress™-generated libraries expressed TCR above background level (**Figure 4**)

Figure 4. High Efficiency of TCR Expression Achieved by TCXpress™

A) Stable JRT cell lines generated from a lung library (n=352)



B) Transient expression of TCRs from a melanoma library (n=1056) in HEK 293 screening line

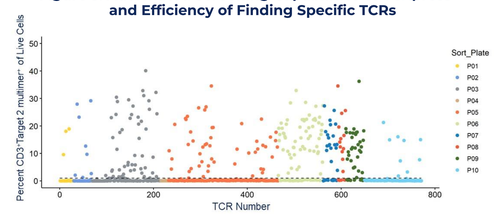


Blue dotted line indicates background threshold of 5% in (A) and of 3% in (B)

- Multiple isolation techniques were assessed for obtaining Target 2 specific TCRs
- 242 of 771 (31.4%) samples had percent CD3⁺Target 2 multimer⁺ >1%
- Successful isolation techniques were able to produce 60–70% of samples that had >1% CD3⁺Target 2 multimer⁺ of live cells (**Figure 5**)

Results (continued)

Figure 5. Automated Screening Capabilities of TCXpress™ and Efficiency of Finding Specific TCRs



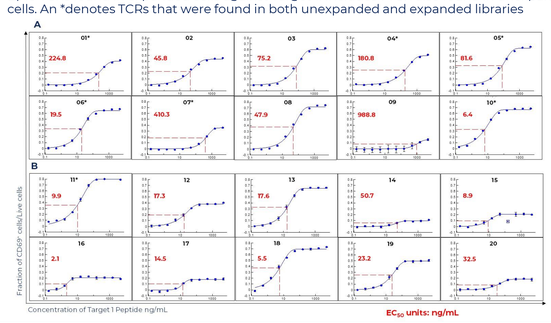
Expressed transiently in HEK293 screening cell line
Black dotted line indicates background threshold of 1%

Sort Plate	Isolation Technique	% Samples >4% CD3⁺	% Samples >1% CD3⁺Target 2 multimer⁺
P1	1	48.3%	10.3%
P2	2	47.5%	20.0%
P3	2	50.7%	34.2%
P4	2	6.3%	0.0%
P5	3	47.0%	21.8%
P6	4	96.9%	62.5%
P7	5	87.1%	61.3%
P8	5	100.0%	70.6%
P9	6	97.4%	76.9%
P10	7	25.2%	7.3%

- 20 unique Target 1 TCRs from unexpanded and expanded cultures show functionality from 2.1-988.8 ng/mL (**Figure 6**)

Figure 6. Functional Characterization of Unique Target 1 CD3⁺/Multimer⁺ Clones and Diversity of Affinity

A) 10 TCRs found in non-expanded cells against Target 1
B) 10 TCRs found in expanded cells against Target 1. Includes 1 TCR also found in unexpanded cells. An *denotes TCRs that were found in both unexpanded and expanded libraries



Conclusions

- **The functionality and versatility of TCXpress™ and iTCXpress™ provides an elegant, innovative approach to identifying TCR candidates for engineering TCR-T ACT**
- **By applying both direct and parallel cloning methods, we can rapidly produce specific, expressible TCRs from both liquid and solid tumors with efficiencies above 80%**
- **This feature combined with a cost around $5/TCR capture, creates a scenario where screening larger libraries for functional candidates is financially and logistically feasible**
- **Additionally, we can identify numerous unique clonotypes without expansion and instead can utilize expansion as a potential enhancement in process when necessary**
- **These advantages can revolutionize TCR-T discovery and make identifying lead candidates that address current TCR-T ACT challenges easier to**

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