

Personalization of T-cell Therapy Using a High-throughput Platform to Identify Tumor-specific T-cell Receptors

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Background

- Introduction of T-cell receptors (TCR) can redirect T-cells specificity to tumors.
- Tumor-specific mutation-derived "neoantigens" have emerged as promising targets for alpha/beta TCR-mediated gene therapy.
- Majority of neoantigens identified so far are derived from patient-tumor specific mutations.
- High-throughput identification and preparation of neoantigen-specific TCRs will be needed for human application
- Next-generation sequencing identifies TCR Va and Vb from single T cells.
- Sleeping Beauty* (SB) transposons/transposase system enables low cost and rapid expression of TCRs.

Aims

- Establish a high-throughput platform to identify tumor-specific T-cell receptors
- Establish sequencing of individual TCRs with next generation platform
- Generate reporter cell that can be used to evaluate antigen specificity and affinity of introduced TCR
- Generate plasmid library for rapid generation of TCR gene construct

Single T cell TCR cloning

Individual antigen-specific T cells were isolated by flow sorting into 96- or 384- well plates. Cells were lysed and mRNA was reverse-transcribed. CDR3 regions of TCR α were amplified and barcoded. Amplified DNA containing CDR3 regions of paired TCR α and β were sequenced using the next generation technology (Figure 1).

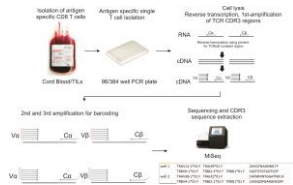
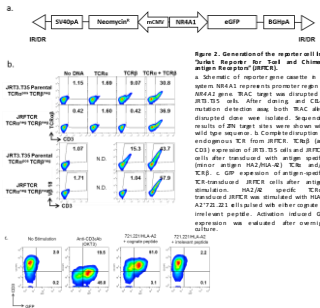


Figure 1. Strategy for single T cell TCR cloning

Generation of the reporter system

A reporter system was generated using TCR β -deficient Jurkat (JRT3-T35) cells genetically modified to (i) enforce expression of CD8 α β , (ii) conditionally express GFP under NR4A1 promoter, and (iii) disrupt expression of endogenous TCR α chain.



GFP expression correlate with affinity and antigen density

JRTCR were genetically modified with either 1G1 wild type (Wt) TCR (low-affinity) or mutant (Mut) TCR (high-affinity: LY/BS1:A1) against NY-ESO-1/A2. These cells were co-cultured with HLA-A2*72:221 cells loaded with graded doses of cognate peptide. The expression of GFP was analyzed at overnight, 24 hours and 48 hours. The percentage and intensity of GFP expression was positively correlated with avidity of TCRs (Figure 3).

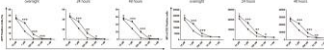


Figure 3. Affinity-dependent low-avidity TCRs binding peptide. SIMWIT101, derived from NYESO-1. After association with antigen presenting cells, he and CB expression cells were gated and analyzed for GFP expression. * p < 0.05, ** p < 0.01, *** p < 0.001

Construction of TCR expression plasmid using TCR α / β plasmid libraries

Upon synthesis of CDR3 sequences, Individual SB-derived DNA transposons expressing TCR α / β constructs were constructed by Gibson Assembly using TCR α / β plasmid libraries. The TCR α / β plasmid libraries contains 45 of Va plasmids and 48 of Vb plasmids (Figure 4).

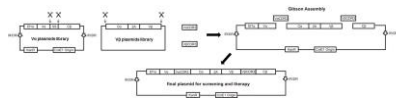


Figure 4. Synthesis of SB-derived DNA transposons expressing TCR α / β constructs using TCR α and TCR β plasmid libraries

High-throughput TCR transduction and screening of identified TCR

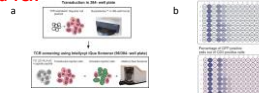


Figure 5. High-throughput screening of antigen-specific TCR. A high-throughput screening of antigen-specific TCR using reporter cells. TCR expressing plasmids were introduced into JRTCR using high-throughput nucleofection system in a 384-well format. Transduced cells were incubated with peptide pulsed HLA-A2*72:221 cells and GFP expression was analyzed using Image Cytometer, which is able to analyze a 96-well plate in 3 minutes and a 384-well plate in 12 minutes. An example of heatmap for analyzing GFP expression using the cytometer. Reporter cells were cocultured with either 1G4 wild type (Wt) TCR (low-affinity) or mutant (Mut) TCR (high-affinity: NY-ESO-1/A2). These cells were co-cultured with HLA-A2*72:221 cells loaded with graded doses of cognate peptide overnight. GFP expression were analyzed using Image Cytometer.

Conclusion and future direction

Our high-throughput system can identify and characterize, based on specificity and avidity, Ag-specific TCR α / β rapidly (Figure 6). This system will be used to generate neoantigen-specific TCR α / β for human application.

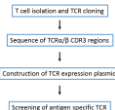


Figure 6. High-throughput system for TCR screening. All steps involved in our high-throughput system to identify and characterize antigen specific TCR.

Relationship Disclosure

L.J.N.C. has an equity ownership from Ziopharm Oncology, Intexion, Targyline, Inc. and Immunix. L.J.N.C. has Patents & Royalties from Zapharm Oncology, City of Hope and Sangamo Biosciences. SZ, HT have equity ownership, Patents & Royalties from Zapharm Oncology, Intexion and Immunix. GM has equity ownership Patents & Royalties from Zapharm Oncology, Intexion. On May 7, 2015, L.J.N.C. was appointed as the Chief Executive Officer at ZDnARM. L.J.N.C. is now a Visiting Scientist at the MD Anderson Cancer Center. GM is a consultant in immuno-oncology field.