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 (TCRs) 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 Vα and Vβ 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 TCRα/β were amplified and barcoded. Amplified DNA containing CDR3 regions of paired TCRα/β were sequenced using the next generation technology (Figure 1).

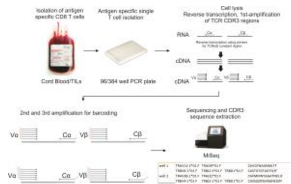

Figure 1. Strategy for single T Cell TCR cloning

Generation of the reporter system

A reporter system was generated using TCRβ-deficient Jurkat (JRT3-T35) cells genetically modified to (i) enforce expression of CD8αβ, (ii) conditionally express GFP under *NR4A1* promoter, and (iii) disrupt expression of endogenous TCRα chain.

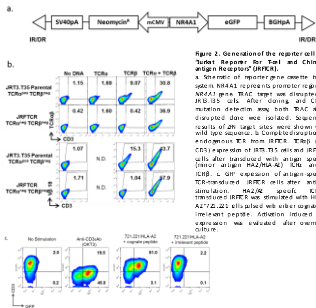

Figure 2. Generation of the reporter cell line "Jurkat Reporter For T-cell and Chimeric antigen Receptors" (JRFTCR).
a. Schematic of reporter gene cassette in SB system. NR4A1 represents promoter region of *NR4A1* gene. TRAC target was disrupted in JRT3.T35 cells. After cloning and CEL-1 mutation detection assay, both TRAC allele disrupted clone were isolated. Sequencing results of ZFN target sites were shown with wild type sequence. b. Complete disruption of endogenous TCR from JRFTCR. TCRαβ (and CD3) expression of JRT3.T35 cells and JRFTCR cells after transduced with antigen specific (minor antigen HA2/HLA-A2) TCRα and/or TCRβ. c. GFP expression of antigen-specific TCR-transduced JRFTCR cells after antigen stimulation. HA2/A2 specific TCRαβ transduced JRFTCR was stimulated with HLA-A2⁺721.221 cells pulsed with either cognate or irrelevant peptide. Activation induced GFP expression was evaluated after overnight culture.

GFP expression correlate with affinity and antigen density

JRFTCR were genetically modified with either 1G4 wild type (Wt) TCR (low-affinity) or mutant (Mut) TCR (high-affinity α95:LY/β51:AI) against NY-ESO-1/A2. These cells were co-cultured with HLA-A2⁺ 721.221 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).

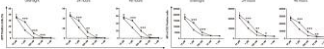

Figure 3 Assay identifies high-avidity and low-avidity TCRs targeting peptide SLLMWITQC derived from NY-ESO-1.
After incubation with antigen presenting cells, live and CD3 expression cells were gated and analyzed for GFP expression. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Construction of TCR expression plasmid using TCRα/β plasmid libraries

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

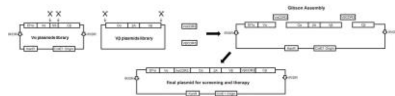

Figure 4. Synthesis of SB-derived DNA transposons expressing TCRαβ constructs using TCRα and TCRβ plasmids libraries

High-throughput TCR transduction and screening of identified TCR

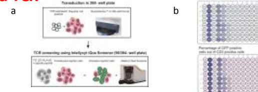

Figure 5. High-throughput screening of antigen specific TCR.
a. High-throughput screening of antigen-specific TCR using reporter cells. TCR expressing plasmids were introduced into JRFTCR using high-throughput Nucleofection system in a 384-well format. Transduced cells were incubated with peptide pulsed HLA A2+721.221 cells and GFP expression was analyze using Intellycyt iQue Screener, which is able to analyze a 96-well plate in 3 minutes and a 384-well plate in 12 minutes. b. An example of heatmaps for analyzing GFP expression using iQue screener. Reporter cells were introduced with either 1G4 wild type (Wt) TCR (low-affinity) or mutant (Mut) TCR (high-affinity α95:LY/β51:AI) against NY-ESO-1/A2. These cells were co-cultured with HLA-A2+721.221 cells loaded with graded doses of cognate peptide overnight. GFP expression were analyzed using iQue Screener.

Conclusion and future direction

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

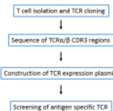

Figure 6 High-throughput system for TCR screening
All steps involved in our high-throughput system to identify and characterize antigen specific TCRs.

Relationship Disclosures

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