#1772

A novel approach for autologous pan-cancer cellular immunotherapy reveals dramatic expansion of αβ and γδ TCR T cell clonotypes indicative of an antigen-driven response

Frank Borriello, MD, PhD*[1], Joshua Keegan, BS[1], Brandon Hancock, BS[2], Fei Guo, PhD[2], and James Lederer, PhD[1,2]

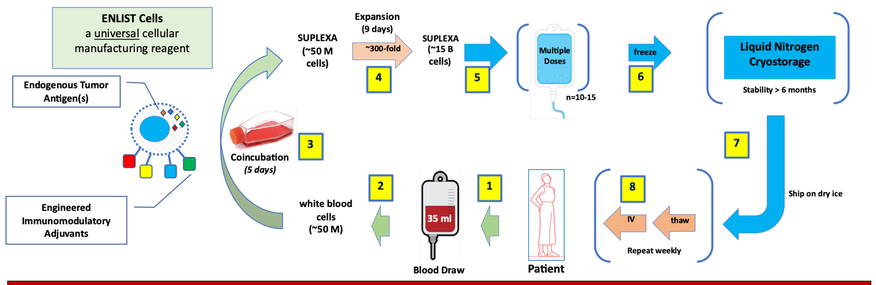
[1]Alloplex Biotherapeutics, Inc., Woburn, MA, [2]Department of Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

*Corresponding Author, fborriello@alloplexbio.com

Background

Alloplex Biotherapeutics has developed a novel cellular therapeutic that uses ENgineered Leukocyte ImmunoSTimulatory cell lines called **ENLIST** cells to activate and expand a heterogeneous population of tumor killing effector cells from human peripheral blood mononuclear cells (PBMCs). This process results in a 300-fold cellular expansion containing NK cells, CD8+ T cells, γδ T cells, TCR variant NKT-like cells and CD4+ T cells, collectively called **SUPLEXA** therapeutic cells. **In this study, SUPLEXA cells and matched donor PBMCs underwent comprehensive TCR sequencing analysis in bulk and at single-cell resolution to determine clonality, diversity, and specific paired TCR sequences with matched transcriptomes.**

The SUPLEXA cells manufacturing process uses peripheral blood mononuclear cells (PBMCs) from cancer patients. PBMCs are then stimulated with ENLIST cells for a 5-day induction period, which is then followed by a 9-day cytokine-induced expansion period. SUPLEXA cells are then cryopreserved for later use as an autologous adoptive immunotherapy. A first-in-human clinical trial for this novel adoptive cellular therapeutic for cancer is projected to begin later this year.



Methods

ENLIST cells: Engineered SK-MEL2 melanoma cell line that expresses curated sets of > 20 different immunomodulatory proteins that are engineered for membrane expression.

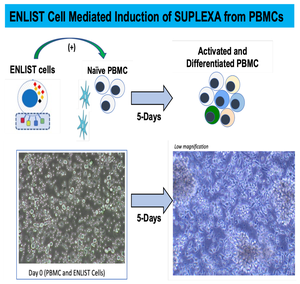
SUPLEXA: PBMCs from normal healthy volunteers were co-incubated with freeze/thaw dead ENLIST cells for 5 days followed by expansion in culture medium containing cytokine support. After 9 days, SUPLEXA cells were harvested and cryopreserved.

iRepertoire TCRαβ and TCRγδ clonality and diversity analysis: RNA prepared from SUPLEXA cells and donor matched PBMCs underwent the dimer-avoided multiplexed (DAM)-PCR analytical workflow to sequence human TCRαβ and TCRγδ sequences in PBMCs and SUPLEXA cells. The data was analyzed for clonality and diversity analysis to generate plots to visualize clonotype frequency among CD4+, CD8+, and γδ T cells.

Single-cell RNA sequencing with paired αβ and γδ TCR identification: SUPLEXA cells were thawed and sorted by flow cytometry into live CD3+ T cells. 30,000 T cells were input into the 10X genomics platform to generate single cell RNA preparations. cDNA libraries were made using the 10X 5' V(D)J kit, which uses primers to amplify TCRαβ and TCRγδ sequences. Data was analyzed using CellRanger, Seurat, and the cLoupe/vLoupe browser platforms for dimensional reduction, single-cell TCR overlays, and gene expression analysis.

Results

Figure 1: SUPLEXA Cell Manufacturing. ENLIST immunomodulatory cells are mixed with PBMCs and cultured for 5 days to activate PBMCs. Activated cells are then expanded for 9 days in IL-7 and IL-15. Photomicrographs of 5-day activated PBMCs are shown along with a table listing broad tumor cell killing activity.



Qualitative Tumor Cell Killing Survey

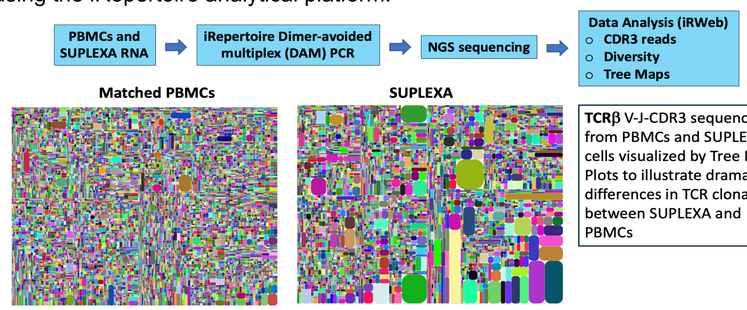
Unmodified Cell Lines Used in Secondary Effector Assay	PBMC coincubated with *Unmodified SK-MEL-2* (% Killing)	PBMC coincubated with *ENLIST Cells* * (% Killing)
Melanoma Cell Lines		
• SK-MEL-2	0%	>95%
• M14	0%	>95%
• SK-MEL-28	0%	>95%
Non-Melanoma Cell Lines		
• COLO205 - colorectal	0%	>95%
• HOP62 - lung	0%	>95%
• IGR-OV1 - ovarian	0%	>95%
• SKOV - ovarian	0%	>95%
• PC3 - prostate	0%	>95%
• SN12C - renal	0%	>95%
• K562 - leukemia	0%	>95%
Autologous Leukemia Cells	0%	>95%

Quantitative Tumor Killing Assay

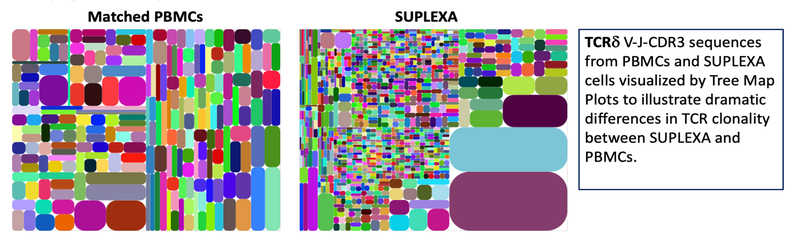
E:T ratio	melanoma M14	prostate PC-3	leukemia K562	colorectal COLO
>16:1				
16:1				
8:1				
4:1				
2:1				
1:1				
1:2				
1:4				

Interpretation:
- The induced cytolytic activity is broad and extends beyond melanoma, suggesting **HLA independence**
- **All tumor cell lines tested to date are susceptible**
- SUPLEXA cells do not kill each other or healthy PBMC

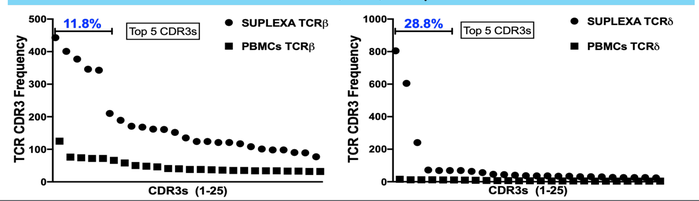
Figure 2: iRepertoire Analysis of TCRα/β TCRγ/δ Clonality and Diversity Comparing PBMCs and SUPLEXA Cells. SUPLEXA cells and donor-matched PBMCs were analyzed to detect all TCRα/β and TCRγ/δ gene expression profiles using the iRepertoire analytical platform.



Tree Map: Each spot in the plot represents a unique entry: V-J-CDR3, where the size of a spot denotes the relative frequency. The entire plot area is divided into sub-area according to V usage, which is subdivided according to J usage and then CDR3 frequency, subsequently. The unevenness of spots reflects the bias of the underlying immune repertoire.

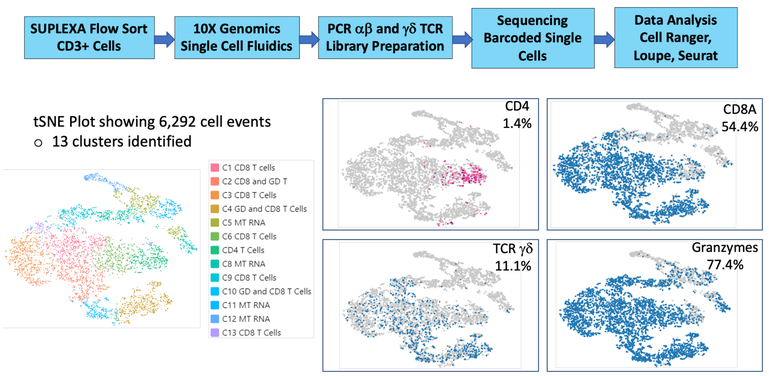


TCRs on SUPLEXA T cells show clonal expansion indicative of antigen-specific TCR activation of CD4, CD8, and γδ T cells.

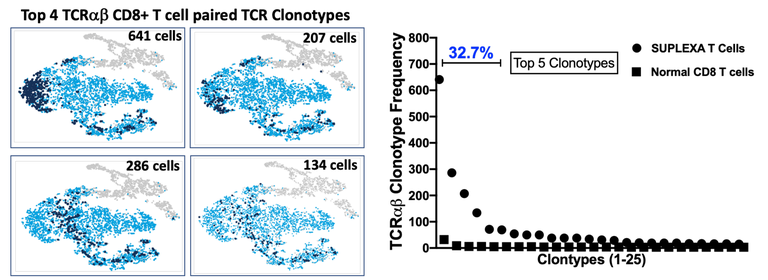


Results

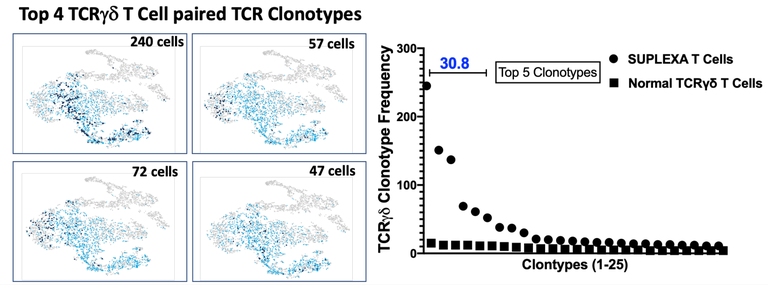
Figure 3: Single-Cell RNA Sequencing Analysis of SUPLEXA with Paired TCR α/β and γ/δ Sequences. The 10X genomics V(D)J single cell sequencing platform was used to analyze TCRs and the transcriptome of SUPLEXA T cells.



Clonal Expansion of CD8+ T Cell Paired TCR Clonotypes



Clonal Expansion of γδ T Cell Paired TCR Clonotypes



Conclusions

1. SUPLEXA cells demonstrate dramatic clonal expansions of TCRα/β and TCRγ/δ T cells with an unanticipated increase in TCRδ diversity.
2. Single cell RNA sequencing of SUPLEXA cells validates clonal expansions of TCRα/β and TCRγ/δ T cells and identifies paired TCR sequences for future antigen specificity and identification studies.
3. Collectively, these findings indicate that ENLIST cells provide antigen-driven activation and expansion of αβ and γδ TCR+ T cells that may acquire pan-tumor antigen specificities.