Generation of Functional CD8ab T Cells from iPSCs in a TCR-Signal Independent Manner Via Supra-Physiological Notch Signaling Provided by DLL4/VCAM-Coupled Microbeads

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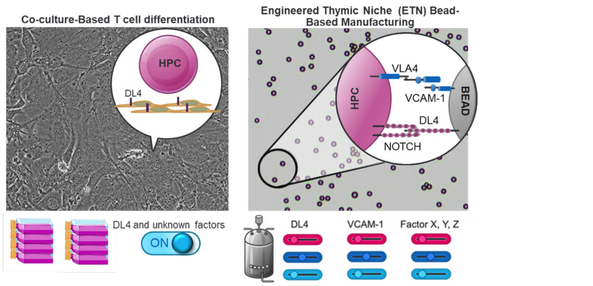
INTRODUCTION & BACKGROUND

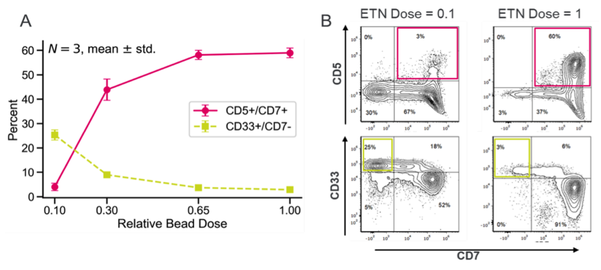
Engineered Thymic Niche (ETN) Technology



- The use of clonally-derived, induced pluripotent stem cells (iPSCs) as starting material for therapeutic T cell manufacturing would overcome many limitations of autologous Chimeric Antigen Receptor T cell (CAR-T) therapies.
- Activation of Notch signaling via the ligand DL4 is required for T cell differentiation from Hematopoietic Progenitor Cells (HPCs). Current in vitro differentiation technologies rely on co-culture with DL4-expressing stromal cells in tissue culture plates.
- We have created DL4/VCAM-conjugated, magnetic microbeads, enabling precise and temporal control of Notch signaling in suspension culture; "Engineered Thymic Niche (ETN)" technology.
- This technology enables us to modulate the intensity and duration of Notch signal activation, and explore the quantitative relationship between Notch signaling and T cell differentiation.

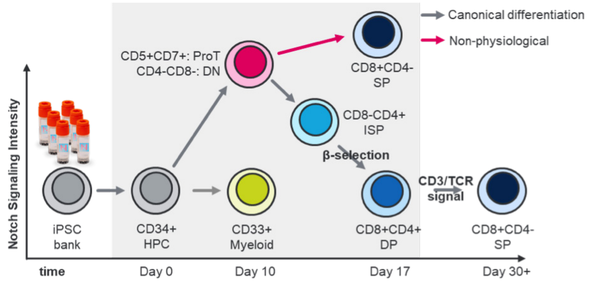
RESULTS

T cell lineage induction from iPSC-derived HSPCs is dependent upon ETN-mediated Notch signaling intensity



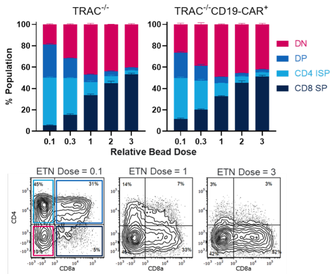
A. Progenitor T (ProT; CD5+CD7+) vs. Myeloid (CD33+CD7-) cell generation from iPSC-derived HSPCs at Day-10 of culture is dose-responsive to ETN bead 'dose'. ETN bead doses are normalized to tissue culture plate surface area.
B. Representative flow cytometry plots of CD5, CD7, and CD33 expression at 0.1 and 1 ETN bead doses.

Experimental Workflow & Summary of Findings



- iPSC-derived, CD34+ HPCs were cultured with a range of ETN bead-doses for 10-days in serum-free suspension media.
- Progenitor T cell (ProT; CD5+CD7+) to Myeloid (CD33+CD7-) cell yield is ETN dose-dependent.
- Reduction of Notch signaling via reduced ETN-dose specifies differentiation of ProTs through the canonical, thymic development pathway to CD4+CD8+ (Double Positive: DP) cells.
- These require TCR rearrangement (B-selection) and CD3/TCR signaling for differentiation to cytotoxic, CD4-CD8+ (Single Positive: SP) T cells.
- Sustained, supra-physiological Notch signaling mediated by high ETN-dose culture yields a population of functional CD8-SP T cells from ProTs after just 7 days, bypassing intermediate differentiation stages and the necessity of TCR/CD3 signaling.

Sustained, supra-physiological Notch signaling for 7-days yields a population of CD8a+CD4- (SP) cells from ProTs, independent of TCR-signaling.

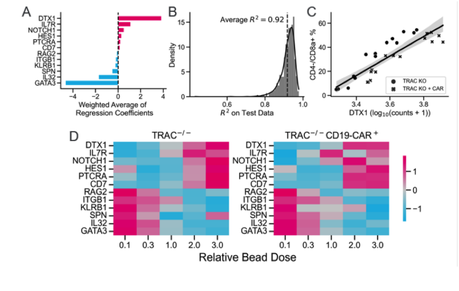


- CD8-SP cell yield at Day-17 is responsive to ETN dose in TRAC-deficient cell lines, in both CD19-CAR engineered and lines lacking CD19-CAR.
- High ETN doses yields CD8-SP cells, while low (physiological) levels yield CD8+CD4+ (DP) cells.
- Representative flow cytometry plots of CD4 vs. CD8a expression shown for 0.1, 1, and 3 ETN bead doses, n=3 technical triplicates/cell line.

RESULTS CONT'D

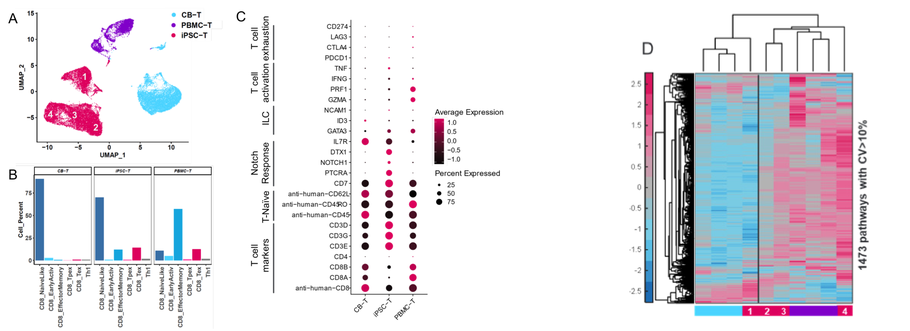
Immediate transcriptional responses to ETN-dose are predictive of CD8-SP cell yield from T cell progenitors (ProTs)

A. Transcripts were measured using a custom NanoString panel. A machine learning workflow predicts CD8-SP phenotype at Day-17 based on Notch-responsive gene expression at Day-12 and identifies the Notch-responsive genes associated with CD8-SP phenotype.
B. The machine learning workflow randomly splits the data into 1000 50-50 train-test splits and fits a regularized linear model to predict CD8-SP from gene expression data with 5-fold cross validation. All models are highly accurate (average R2 = 0.92 on the test data).
C. DTX1 is the most positively predictive feature and is tightly correlated with CD8-SP
D. ETN-responsive gene-expression trends are similar for both cell lines (z-score normalized mean expression shown, n=3).



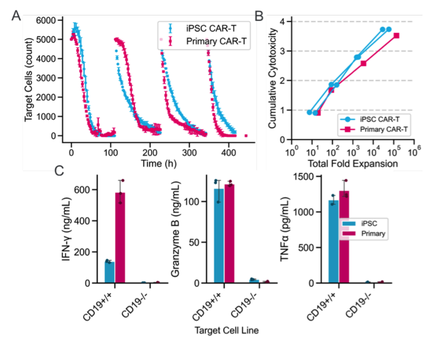
Single cell transcriptome characterization of iPSC-derived CD8-SP cells in comparison to CD8+ T cells derived from peripheral blood and cord blood

A. UMAP of single cell transcriptomes; iPSC-derived T cells, CD8+ T cells from peripheral blood (PBMC) & cord blood (CB) are transcriptionally distinct.
B. ProjecTILs algorithm classifies iPSC-derived cells as predominantly T-naïve, similar to cord blood-derived T cells.
C. Select expression of canonical T cell and naïve T cells markers, Notch response-genes, Innate lymphoid cells (ILCs), T cell activation and exhaustion transcripts.
D. 1473 gene signatures differentially enriched between cell clusters. Single sample GSEA was applied at single cell resolution, means computed for each cluster, and represented via hierarchical clustered z-scores. Three of the iPSC-T cell clusters share signaling similarity with PBMC cells, and one with CB cells.



iPSC-derived, CD19-CAR expressing CD8SP cells are capable of in vitro serial target-mediated cell killing, proliferation and cytokine secretion comparable to Primary CARTs

A. Target cell (CD19-expressing A549) dynamics over 4 rounds of in vitro killing assay with Primary CARTs vs. iPSC-CD8 cells. Cell density readout using Incucyte live cell imaging, wherein Effector T cells were added at 2:1 E:T ratios (mean ± std, n=3).
B. In vitro cytotoxicity vs. Effector cell expansion, Primary CARTs vs. iPSC-CARTs (n=2 biological replicates). Cumulative cytotoxicity is the sum of relative change in AUC from a target cell only control on each stimulation (not shown).
C. iPSC-CARTs secrete inflammatory cytokines upon co-culture with antigen expressing target cells at levels comparable to Primary CARTs.



CONCLUSIONS

Supra-physiological, sustained Notch signaling provided via Engineered Thymic Niche technology generates a population of CD8+ T cells, bypassing the necessity of TCR/CD3 signaling and intermediate differentiation states associated with canonical T cell differentiation.

Using an iPSC cell line with a CAR knocked in at the TRAC locus (TCR-deficient), we apply this approach to generate iPSC-CARTs, capable of multiple rounds of in vitro tumor cell lysis, proliferation and cytokine secretion comparable to primary T cells.