Scalable Production of Induced Pluripotent Stem Cell-Derived CD8+ Cells in Stirred Tank Reactors Using DLL4/VCAM-1 Coated Beads

Notch THERAPEUTICS

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OVERVIEW

Adoptive T-cell therapies are effective for some hematological malignancies and could transform the treatment of many other cancers. Despite early clinical success, use of genetically modified patient-derived T cells poses production and logistical challenges that limit the utility and accessibility of this cellular immunotherapy.

T cells derived from genetically modified, clonal, induced pluripotent stem cells (iPSC) can enable a defined, reproducibly manufacturable cell product. However, inducing T-lineage commitment in a scalable and controlled format suitable for clinical manufacturing remains a major obstacle.

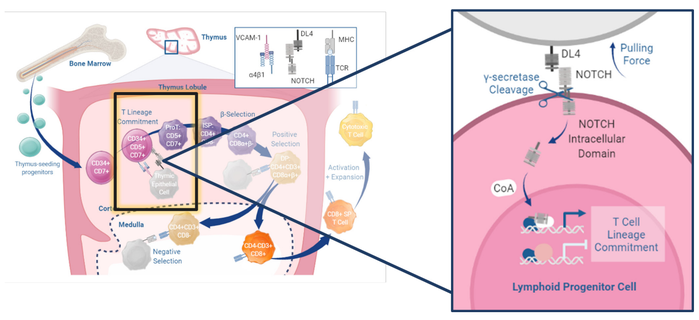
Notch signaling is required to drive T-lineage differentiation and in conventional static culture protocols is delivered by DL4-expressing feeder cells or DL4 protein-coated tissue culture vessels. Such systems lack control of the intensity and dynamics of Notch signaling needed for robust T-cell development (especially for iPSC derived HSPC) and are not easily scalable to meet the needs of clinical manufacturing. To address these limitations, we developed the Engineered Thymic Niche (ETN) platform, consisting of magnetic beads coated with DL4 and vascular cell adhesion molecule 1 (VCAM-1).

Using the ETN we now demonstrate a scalable, feeder-free suspension bioreactor-based process using fully defined custom reagents, allowing for control of strength and timing of Notch signaling and efficient generation of CD8+ CAR-T cells. The iPSC derived CD8+ cells demonstrate equivalent function as primary CD8+ cells in serial killing assays, respond to antigen like primary T cells, have a majority central / stem cell memory phenotype and are less prone to exhaustion.

Notch signaling is required for T cell development in the Thymus

CD34+ progenitors in circulation seed the thymic cortex. Notch signaling, initiated by interactions between Notch1+ hematopoietic progenitors and DLL4+ epithelial cells, is required for T-lineage commitment and differentiation. VCAM-Integrin signaling functionally synergizes with Notch signaling to support efficient T-cell lineage commitment. T-cell induction is marked by successive expression of CD5 and CD7 and loss of CD34. CD5+CD7+ Progenitor T-cells (ProTs) then differentiate into CD8+ and CD4+ ('single positive') T cells, before exiting the thymus and entering circulation as naïve T cells.

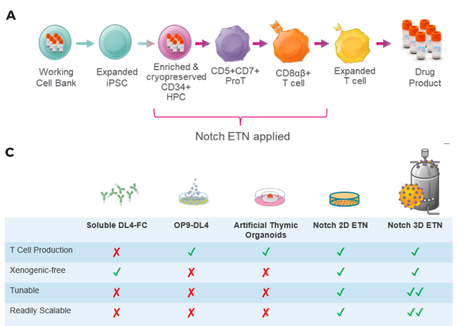
Figure 1. Notch signaling drives T-cell lineage commitment and differentiation from hematopoietic progenitor cells in the thymus.



Clinical T-cell manufacturing requires a Xenogeneic-free, scalable culture platform

Building on our discovery that immobilized DLL4/VCAM supports T-cell differentiation in vitro, we have designed the Engineered Thymic Niche (ETN) platform for scalable T-cell manufacturing (B). The ETN is utilized during differentiation from CD34+ HPCs to CD8 single positive T cells (A). The Notch ETN is uniquely amenable to scalable processes (C).

Figure 2. Notch ETN technology enables tunable control of ligand timing, binding and density.



METHODS

Figure 3. T cell differentiation scheme in microplates and STR

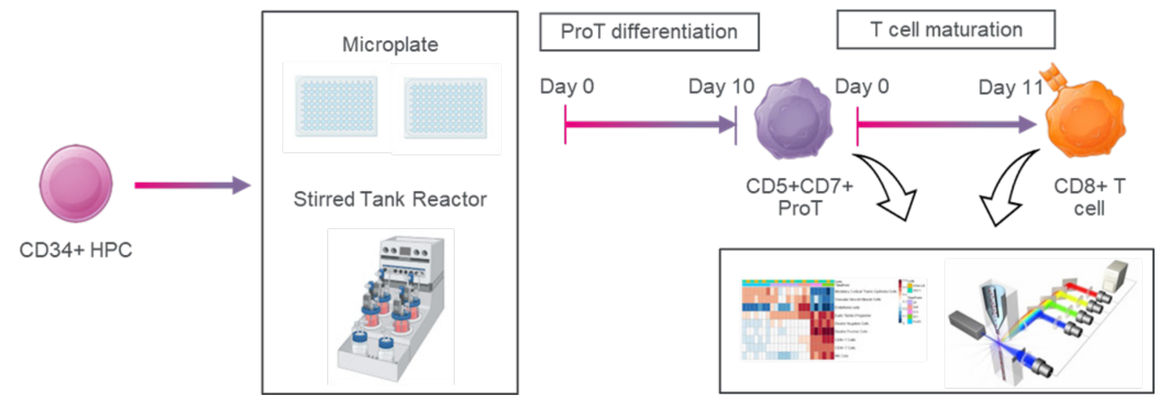
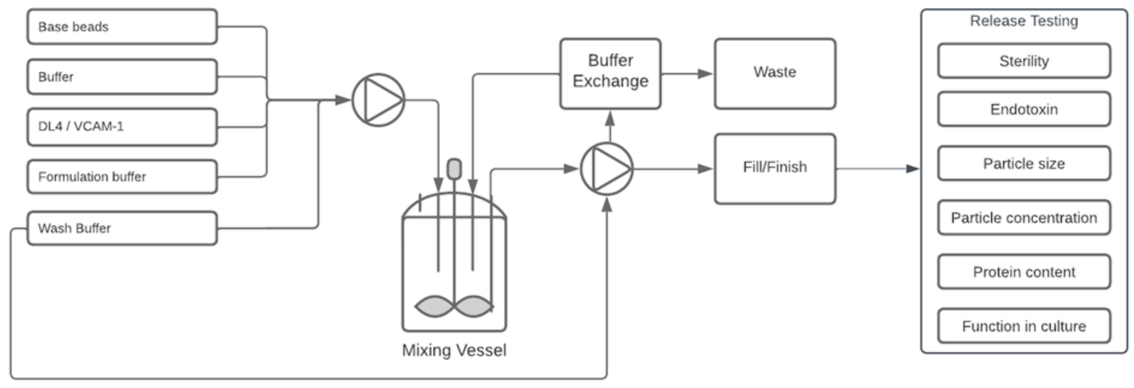


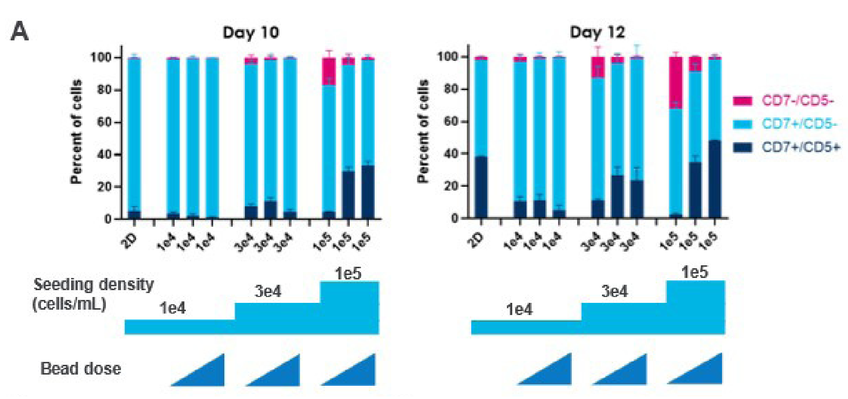
Figure 4. ETN bead manufacturing and testing workflow.



RESULTS

Cells seeded at three different cell densities (1e4, 3e4, and 1e5 cells/mL) in combination with 3 bead doses (10-fold range; 9 conditions total) were assessed for CD5 and CD7 (ProT phenotype) expression after 10 and 12 days for differentiation. High seeding density with medium to high bead dose resulted in the highest CD7+/CD5+ population.

Figure 5. Progenitor T cell induction can be modulated by ETN bead dose and cell seeding density.



RESULTS - CONT'D

Figure 6. T cell phenotype is modulated by ETN bead dose. iPSC-derived ProT cells were cultured in microplates for 7 days with 30- fold range of ETN dose (from 0.1 to 3 relative bead concentration). (A) The resulting cell phenotype was assessed by flow cytometry. High bead doses produced more CD8 SP cells than lower bead doses, while low bead doses produced more CD4 ISPs and double positive cells (DP). (B) Expression of CD8A, IL7R, CD4, and BCL11A genes measured at ETN Dose 2 and 3 by Nanostring over 7 days, show CD8A (T cell) and ILR7 (lymphoid) expression increasing over time and CD4 (T cell) and BCL11A (hematopoietic progenitor) decreasing over time.

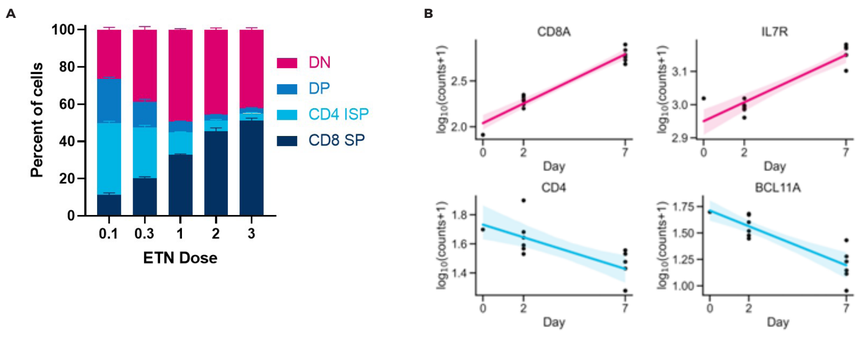


Figure 7. STR culture with ETN generates equivalent proportion of Progenitor T cells as 24-well plate culture and is highly reproducible. iPSC-derived CD34+ cells were differentiated into ProT at the high bead dose as identified in Figure 5. (A) Two (2) CD34+ cell banks were seeded in duplicate at low or high cell density in Dasbox STR and 24-well plates. The % ProT at D10 was equivalent in STR and microplates for both cell banks, with higher % ProT for conditions seeded at high cell density. The number of ProT produced per input CD34+ cell was higher for STR culture. (B) Eight replicate bioreactors were seeded at the high cell density. Differentiation and expansion were reproducible with 28 ± 4% CD5+CD7+ cells at D10 and 23 ± 6 ProT generated per input CD34+ cell (mean ± 1 SD).

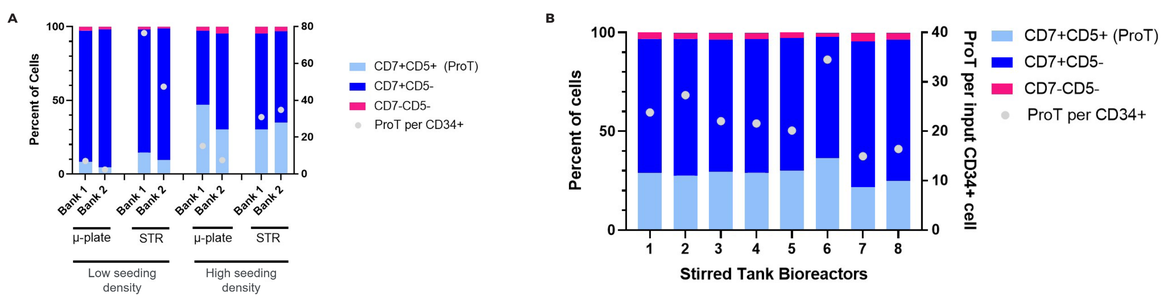


Figure 8. STR culture reproducibly generates CD8+ cells. STR generated ProT cells (Figure 7) were re-seeded at 1e6 cells/mL and cultured for 11 days with ETN at a 2.5x relative dose, based on the optimal conditions (Figure 6) in microplate culture. (A) Maximum CD8+ cell yield occurred after 8 days of maturation with an average of 41 ± 1.5% across all 8 bioreactors. The number of CD8+ SP cells produced per input CD34+ cell was 35 ± 5. The phenotype is equivalent to that seen in Figure 4 for microplate culture (B) The proportion of CD8ab+ cells at Day 8 of maturation was consistent across all 8 STR at 28 ± 1 %. (C) Gene expression dynamics for CD8A, IL7R, CD4 and BCL11A are similar for STR and microplate cultures (Figure 5). (D) Microwell and STR gene expression dynamics are significantly correlated (Pearson's r = 0.71, p<0.001) for a panel of 48 lymphocyte development and Notch signaling genes. Gene expression dynamics were quantified by the sign of the regression coefficient of a linear model fit to each gene's expression over time and the logarithm of the Benjamini/ Hochberg adjusted p-values.

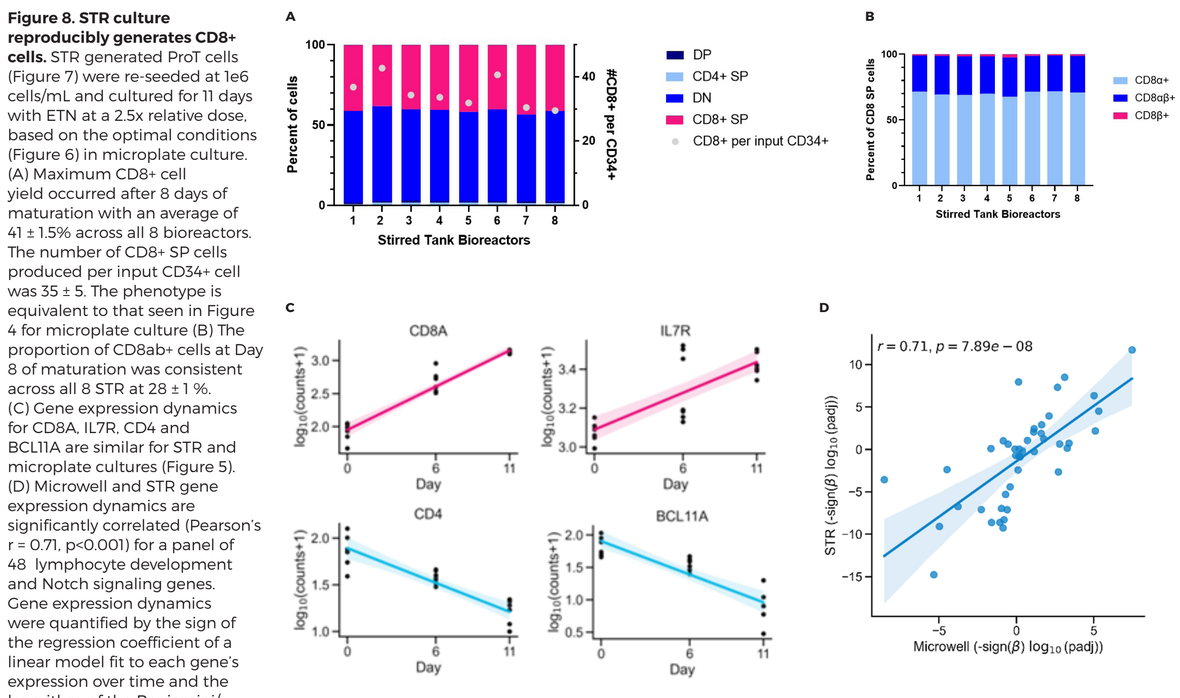
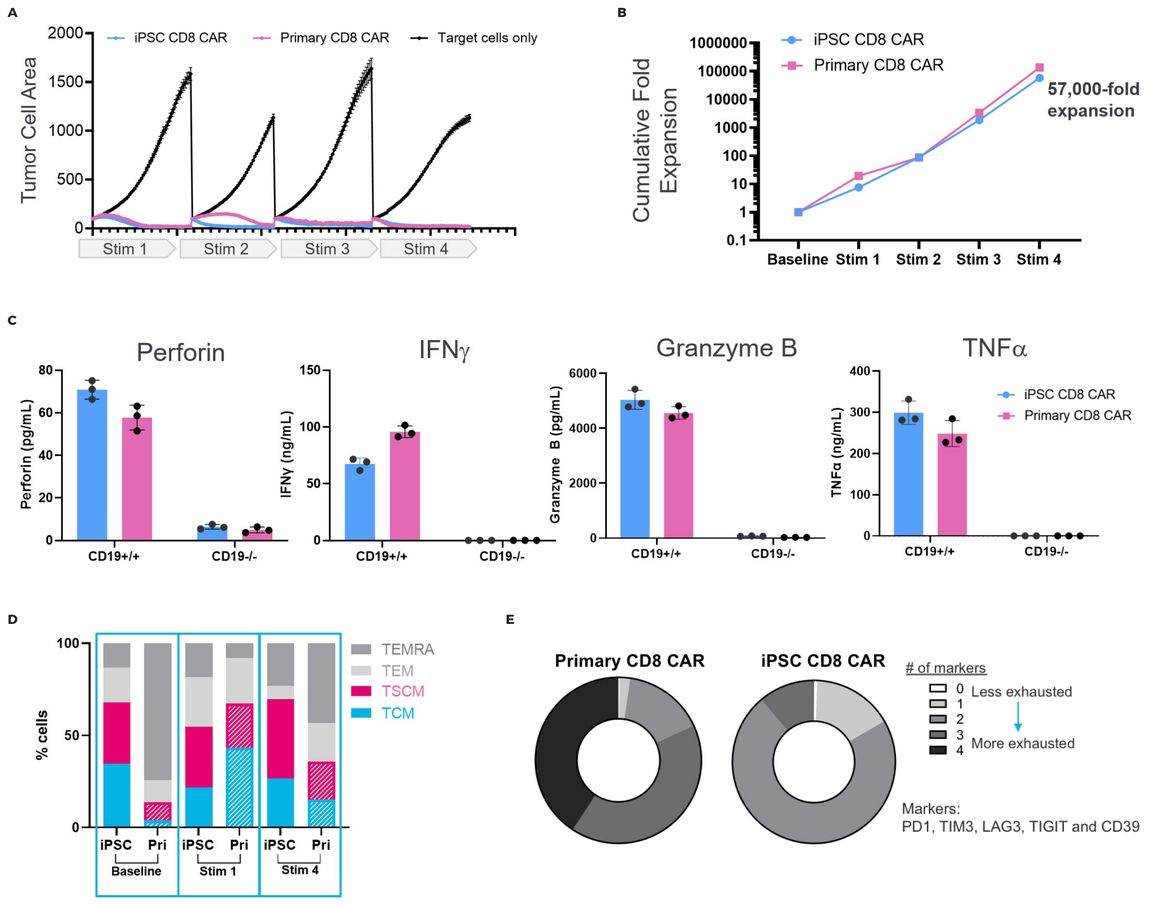


Figure 9. Functional comparison of STR culture-generated iPSC-derived CD8+ CAR-T cells and healthy-donor peripheral blood CD8 CAR-T. (A) Serial restimulation assay (n=3 technical replicates) with 2:1 E:T ratio. iPSC derived CAR-T cells show comparable activity to primary CD8 CAR-Ts over 4 rounds of antigen exposure. (B) iPSC derived CAR-T cells proliferate 57,000-fold over 4 rounds of antigen exposure - comparable to primary CD8 CAR-Ts. (C) iPSC derived CD8 CAR-T cells secrete effector molecules in a target specific manner at levels comparable to primary CD8 CAR-Ts. (D) Stem cell memory (TSCM) and central memory (TCM) subsets that are associated with better performance in vivo are enriched in iPSC derived CD8 CAR-T cells at baseline and after chronic antigen exposures. T-cell subsets are classified based on expression of CD45RA, CD62L and CD95. (E) Co-expression of multiple exhaustion markers (PD1, TIM3, LAG3, TIGIT and CD39) is lower in iPSC derived CD8 CAR-T cells than in primary CD8 CAR-Ts.



CONCLUSIONS

Differentiation of iPSC derived CD34+ cell to CD8+ cells can be optimized for ETN and cell concentration in microplate culture. These optimal microplate conditions were found to translate faithfully to STR culture conditions based on both phenotype and gene expression.

iPSC-derived CD8+ CAR-T cells produced in STR are equivalent functionally to primary CAR-T cells in serial killing assays and maintain a favourable central/stem cell memory phenotype as well as lower exhaustion marker expression.

Production of functional iPSC-derived CD8+ T cells in STR using ETN is a controlled and consistent process that will allow us to address the challenge of scalability in clinical manufacturing for off-the-shelf allogeneic cell therapies.