ABSTRACT NO.
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Controlled and Scalable T Cell Differentiation from Induced Pluripotent Stem Cell (iPSC)-Derived Blood Progenitor Cells Using the Engineered Thymic Niche Technology

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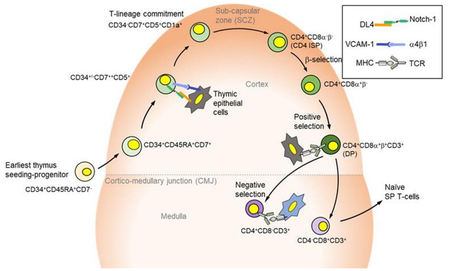
OVERVIEW

- 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 challenges that limit the utility and accessibility of this cellular immunotherapeutic.
- 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. T-lineage commitment occurs in the thymus through Notch signaling activation in hematopoietic stem/progenitor cells (HSPC) by ligands such as Delta-like 4 (DL4) expressed on adjacent epithelial cells.
- *In vitro*, Notch signaling in HSPCs can be activated by co-culture with DL4-expressing cell lines, or by culture on DL4-coated tissue culture plates. 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 +/- vascular cell adhesion molecule 1 (VCAM-1). ETN enables differentiation of iPSC-derived CD34+ HSPC to progenitor T-cells (ProT).

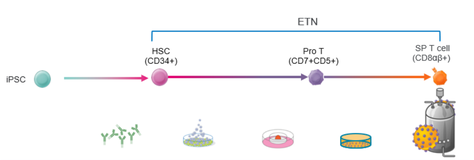
Notch signaling is required for T-cell development in the thymus

Figure 1. Notch signaling drives T-cell lineage commitment and differentiation from hematopoietic progenitor cells in the thymus.
T cell generation during development and adult homeostasis occur in the thymus. CD34+ progenitors in circulation seed the thymic cortex. Notch signaling, initiated by cell-cell interactions between Notch1+ hematopoietic progenitors and DLL4+ epithelial cells, is required for T-lineage commitment and differentiation. VCAM:ITG signaling functionally synergizes with Notch signaling to support efficient T-cell lineage commitment, though the exact mechanisms remain unclear. 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.

Generation of functionally mature T-cells in vitro from stem or progenitors requires synthetically recapitulating these interactions, controlling Notch and VCAM signaling via surface presentation of ligands.



Clinical T-cell manufacturing requires a xenogeneic-free, scalable culture platform: Notch's Engineered Thymic Niche (ETN)



	Soluble DL4-FC	OP9-DL4	Artificial Thymic Organoids	Notch 2D ETN	Notch 3D ETN
T Cell Production	✗	✓	✓	✓	✓
Xenogenic-free	✓	✗	✗	✓	✓
Tunable	✗	✗	✗	✓	✓
Readily Scalable	✗	✗	✗	✓	✓✓

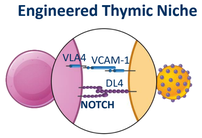
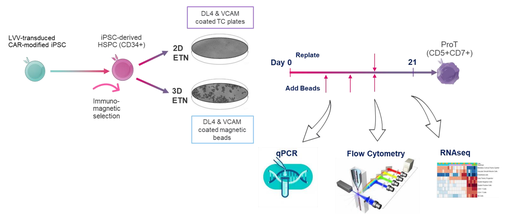


Figure 2. Notch ETN technology enables tunable control of ligand timing, binding and density.
Experimental systems for inducing T-cell differentiation from hematopoietic progenitors rely on co-culture with DL4-expressing cell lines. While suitable for research purposes, these are insufficient for clinical-scale manufacturing of T-cell based therapeutics.

Building on our discovery that immobilized DL4/VCAM supports T-cell differentiation in vitro, we have designed the 3D Engineered Thymic Niche (ETN) platform for scalable T-cell manufacturing.

METHODS

Figure 3. iPSC-derived CD34+ cells are cultured with the Engineered Thymic Niche (ETN), either with DL4 and VCAM immobilized on tissue culture plates (2D ETN) or on magnetic beads (3D ETN). For the 2D ETN, cells are passaged onto fresh plates after 14 days. With the 3D ETN, beads were easily added at additional timepoints (e.g. day 7, day 10) to modulate Notch-1 signaling. Cells were harvested for gene expression analysis of the Notch regulatory network (day 14) and flow cytometric phenotype analysis (day 14, 21). Cell culture protocol was performed using unmodified (iPSC-11) cell lines, or iPSC starting material transduced with CD19-CAR Lenti-viral Vectors (LVV).



RESULTS

iPSC-derived CD34+ progenitors cultured in the ETN acquire lymphocyte-associated gene signatures

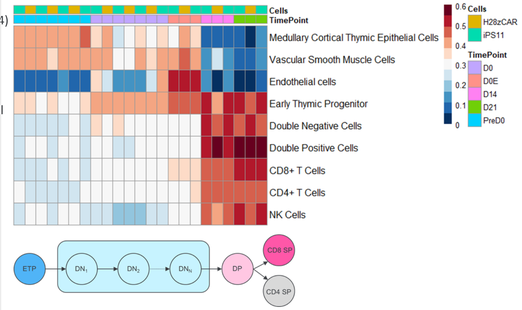


Figure 4. iPSC-derived CD34+ progenitors cultured on 2D ETN acquire lymphocyte potential.
Following 10-days hematopoietic differentiation of iPSCs, CD34+ cells were selected via magnetic enrichment, plated on 2D ETN and cultured for 21 days. RNAseq expression profiling was performed on iPSCs, hematopoietic differentiated iPSCs (D0), CD34+ enriched cells (D0E), and following 14 and 21-days of cell culture on immobilized DLL4/VCAM culture plates (D14, D21).

Gene signatures of thymic cell populations were taken from Park et al. (2020) and single sample GSEA implemented to compute enrichment scores for the samples.

Signatures for lymphocyte populations are enriched following 14 and 21 days of culture on the ETN, indicating lymphocyte lineage commitment and differentiation. Conversely, stromal gene signatures are reduced during ETN culture, indicating loss of epithelial potential from iPSCs. Endothelial cell potential is enriched in CD34+ selected cells, consistent with known endothelial/hematopoietic potential of CD34+ iPSCs.

Park et al (2020) A cell atlas of human thymic development defines T cell repertoire formation. Science 367:eaay3224.

T-lineage commitment is controlled by DLL4 & VCAM exposure on the 3D ETN beads

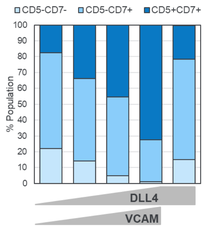


Figure 5. T-lineage commitment, as measured by progenitor T-cell generation is controlled by DLL4 and VCAM exposure.
(A) iPSC-derived CD34+ cells were cultured 21 days in 3D ETN, with DLL4/VCAM input tuned by both varying the amount of ligand loaded/bead, and the number of beads added. DLL4 exposure was increased successively from 0.25x to 4x relative concentration, with and without corresponding increases in VCAM. ProT induction (%CD5+CD7+) is dose-proportional to DLL4 and is significantly enhanced by VCAM addition.

ETN bead concentration modulates Notch target gene expression and resultant ProT differentiation

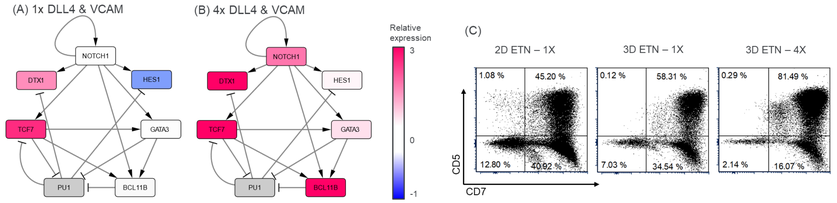


Figure 6. Notch signaling, inferred by Gene Regulatory Network activation, can be modulated by changing the bead ETN concentration.
(A), (B) qPCR measurement of Notch regulated genes: Notch1, Hes1, GATA3, TCF7, DTX1, and BCL11B at 14-days of culture on the 3D ETN with expression normalized to that observed in 2D ETN culture. Relative expression values are overlaid on Notch Gene Regulatory Network. ETN beads were added to provide 1x and 4X total DLL4 & VCAM, where total ligand immobilization area at the 1x bead ETN concentration is equal to the microwell area for 2D ETN (n=3).
(C) Resultant CD5 vs CD7 phenotype at day 21. Enhanced Notch gene regulatory network activity is associated with greater ProT induction.

Mechanism-based model of Notch signaling at the bead: cell interface

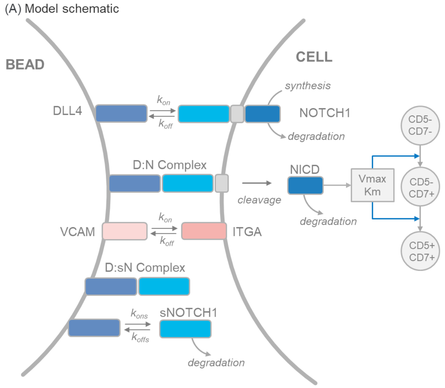
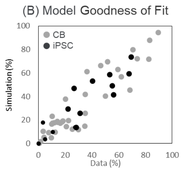


Figure 7. Dynamic molecular interactions at the bead:cell interface.
As a tool to optimize ETN design and employment, we have developed a dynamic ordinary differential equation-based model of Notch signaling as represented in the schematic (A). Fixed parameters were taken from literature, based on generic cell biology, or specified by experimental measurements. Notch signaling is empirically linked to T-cell differentiation using a Michaelis-Menton equation driving CD5 and CD7 expression. Free parameters were estimated from experimental measurements of phenotype (CD5/CD7 expression) under a variety of DLL4/VCAM input conditions using both CB and iPSC-derived cells (B), and VCAM is assumed to drive differentiation via both avidity and Notch-independent signaling.

NICD; notch intracellular domain. sNOTCH1; soluble Notch1 ECD



In silico optimization of ETN design and deployment

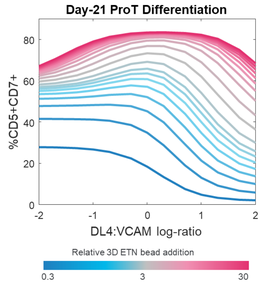


Figure 8. Model simulations predict optimal DLL4:VCAM ratio and bead ETN addition.
After parameterizing the model to experimental data, cell culture simulations were performed with ETN beads over a range of DLL4:VCAM ratios and bead ETN addition concentrations (normalized relative to 2D ETN surface area). Simulations predict increasing differentiation as a function of increasing ETN surface area with the optimal DL4:VCAM ratio around 1:1.

Robust and tunable expansion of CD5+CD7+ Progenitor T-cells (ProTs) using the 3D ETN

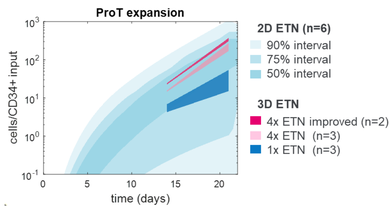
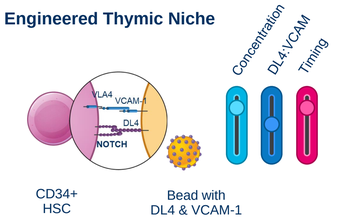


Figure 9. Expansion of CD5+CD7+ (ProT) cells over 21-days in culture from CAR-transduced-iPSC-derived CD34+ cells.
CD5+CD7+ cell yield (75% interval) from 3D ETN cultures dosed with 1x and 4x 3D ETN, and 4x 3D ETN with an improved cell seeding scheme, overlaid on distribution of yields from 2D (1x area) ETN culture. The 3D ETN enables tunable ProT induction and reduced variability likely due to tighter control of Notch signaling.

CONCLUSIONS

- 3D Engineered Thymic Niche (ETN) serves as surrogate thymic stromal tissue, inducing Notch signaling in iPSC-derived CD34+ cells and resultant T-cell lineage commitment to CD5+CD7+ progenitor T-cells
- Notch signaling and T-cell lineage commitment controlled by varying DLL4 and VCAM protein/bead, and bead concentration in culture.
- A mechanism-based computational model of Notch signaling has been developed to guide the design and implementation of the 3D ETN
- 3D ETN enables robust and controllable induction of T-cell lineage commitment from CAR-transduced iPSC cells
- The ETN technology provides a foundation for time and dose-dependent Notch signaling in a manner that enables repeatable manufacturing of iPSC-derived T cells



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