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Engineering CAR-expressing innate lymphoid cells (ILCs) from human induced pluripotent stem cells (iPSCs) for cancer immunotherapy

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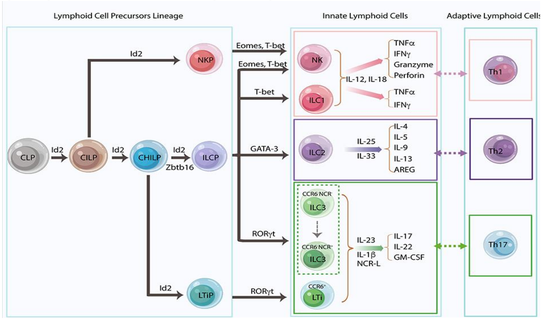
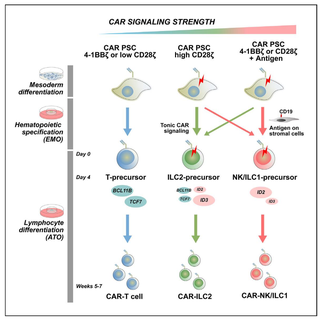
Introduction

Innate lymphoid cells (ILCs) are newly discovered tissue-resident innate immune cells that share similar developmental and transcriptional programs with T cells but, notably, lack antigen-specific receptors. They develop into functionally diverse subsets as pivotal frontline effectors similar to T cells and play crucial roles in maintaining tissue integrity as well as fighting infectious diseases and cancer. Notably, in solid tumors, ILCs have demonstrated tumor-infiltrating characteristics and anti-tumor properties through multiple mechanisms including direct cytotoxicity, secretion of inhibitory cytokines, antigen presentation, and immune cell recruitment. Harnessing and directing these ILC functions may offer novel approaches to cancer treatment that overcome the current hurdles CAR T-cell therapy has encountered in treating solid tumors. However, developing a suitable methodology for expanding human ILCs bearing CARs (CAR-ILCs) for immunotherapy requires further investigation. We are developing a rationalized strategy involving the serum- and feeder-free in vitro generation of CAR-expressing type 2 and 3 ILCs (ILC2 and ILC3s) from engineered pluripotent stem cells (PSCs).



An, Z., Flores-Borja et al. *(2020) Frontiers in Immunology, 10, 501889*.



Li et al. (2023). Cell Reports. 42:112241

Method

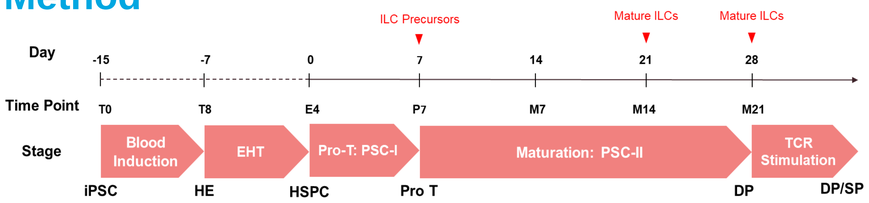


Figure 01. Culture Protocol for Serum- and Feeder Cell-Free Differentiation of iPSCs to T cells utilized for screening ILC precursors and mature ILCs.

Hemogenic endothelial cells (HECs) were enriched from iPSCs or CAR-iPSCs through the blood induction protocol and used fresh or cryopreserved for later use. On day -7 (T8), $CD34^+$ HECs were seeded into a DLL4-VCAM1 coated 96-well plate at 1.0 x 10^4 cells/mL in 100 uL StemPro 34 Base Media + EHT supplement per well.

After undergoing endothelial-hematopoietic transition (EHT), the hematopoietic stem and progenitor cells (HSPCs) generated on day 0 (E4) were seeded into a new DLL4-VCAM1 coated 96-well plate at 1.0 x 10^4 cells/mL in 100 uL JAK Ultra Base Media + PSC1 supplement per well. Cells were cultured for 7 days, with a top-up of 100 uL per well on day 3 - 4.

On day 7 (P7), the cells were harvested, counted, sampled for ILCPs using flow cytometry, and seeded into a new DLL4-VCAM1 coated 96-well plate at 2.0 x 10^5 cells/well in 100 uL JAK Ultra Base Media + PSC2 Supplement per well. $CD7^+CD5^{+/-}$ cells were cultured for 14 days, with an initial 100 uL top-up and subsequent half-medium changes every 3 - 4 days.

On day 21 (M14), cells were harvested, sampled for flow cytometry, CITE-Seq and re-seeded into a 96-well plate in 200 uL JAK Ultra Base Media + PSC2 Supplement per well and cultured for 7 days, with a half-medium change after 3 or 4 days. On day 28 (M21), cells were harvested and analyzed for expression of mature ILC cell surface markers.

Flow Cytometry

Cultured cells were analyzed by flow cytometry for expression of stage-specific markers on lineage negative and CD45+ cells: ILC progenitors ($CD7^+$ $CD45RA^+$ $CD117^+$ $CD127^+$ $CD121a^+$), mature $CD117^{+/-}$ ILC2s ($CD7^+$ $CD127^+$ $CD161^+$ $CRTH2^+$) and mature ILC3s ($CD7^+$ $CD127^+$ $CD161^+$ $Nkp46^+$). Dead cells were excluded by light-scatter profile and fixable NearIR viability staining. The number of ILC progenitor cells, mature type 2 and type 3 ILCs were calculated based on the fraction of $CD45^+$ Lin^- cells expressing the requisite markers.

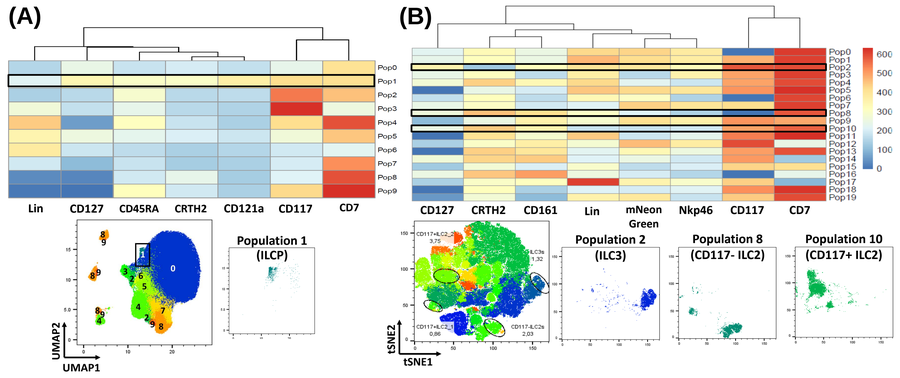


Figure 02. Heatmaps and UMAP/tSNE plots created to distinguish (A) ILC precursor population on day 7 of differentiation and (B) mature ILC2 and ILC3 populations on days 21 and 28 of differentiation.

Results

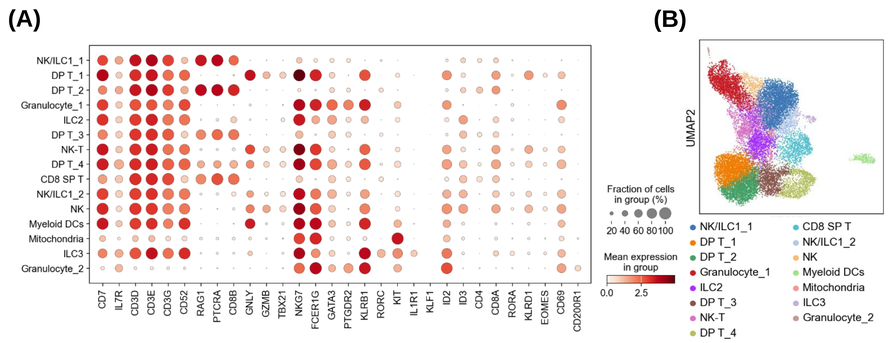


Figure 03. Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-Seq) revealed distinct populations of mature NK/ILC1, ILC2 and ILC3 on day 21 of the differentiation process. (A) The clustered dot plot represents the average gene expression profiles, revealing two distinct subpopulations within the NK/ILC1 cell group characterized by the expression of canonical genes, namely *ID2, ID3, IL7R, and NKG7*, a separate subpopulation of ILC2 which expresses signature genes including *ID3, KIT, KLRB, IL7R, TCF7, BCL11b*, and *RORA*, and one subpopulation of ILC3 expressing genes such as *ID2, RORC, GATA3, and IL7R*. (B) Clustering analysis using UMAP demonstrated a higher prevelance of ILC1 and ILC2 within the culture compared to ILC3s.

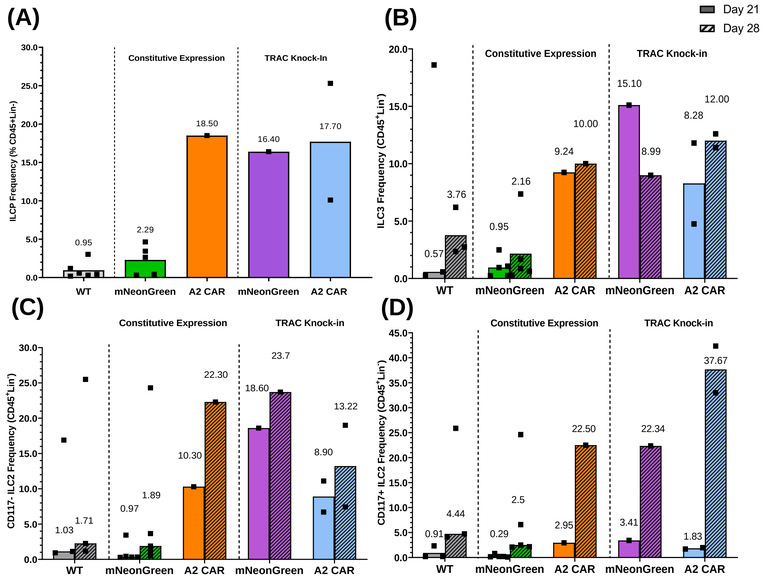


Figure 04. The frequencies of (A) ILC Precursors on day 7 of the differentiation process, mature (B) ILC3, (C) CD117- and (D) CD117+ ILC2 on days 21 and 28 of the differentiation workflow in wildtype and CAR knock-in iPSCs.

Summary

- The current CAR-iPSC differentiation workflow yields 18% of ILCP, 2% for CD117+ ILC2, and 8-10% for CD117- ILC2 and ILC3 within the CD45+ Lin- cell population.
- The introduction of a second-generation A2 CAR through constitutive expression or TRAC knock-in augments the frequencies of ILC progenitors, mature ILC2, and ILC3.
- Further protocol optimization involves cytokine polarization and modulation of Notch signaling strength.
- Functional assessment of ILCs will encompass *in vitro* cytokine quantification and *in vivo* testing in humanized mice, utilizing pancreatic and breast cancer tumor models.

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