INTRAEPITHELIAL GROUP 1 INNATE LYMPHOID CELLS GENERATED *IN VITRO* EXHIBIT ENHANCED CYTOTOXICITY AND INFILTRATION INTO SOLID TUMOROIDS

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Background Immunotherapy approaches have shown striking success in the liquid tumor setting but have been unable to demonstrate similar efficacy against solid tumors. Cell-based therapies, in particular, struggle to overcome the harshly immunosuppressive tumor microenvironment. Additionally, cells for adoptive therapy are often generated from immune cells circulating in the peripheral blood of patients or healthy donors, rather than isolating them from solid tissues. Designing immunotherapies using insights from tissue-resident cells represents a novel method for enhancing trafficking into and retention within solid tumors.¹ To this end, we developed methodology for differentiating peripheral blood natural killer cells (pbNKs) into cells resembling intraepithelial group 1 innate lymphoid cells (ieILC1s) *in vitro* and assessed their potential for immunotherapy.

Methods We co-cultured irradiated squamous cell carcinoma (SCC) cells and pbNKs, isolated from blood of healthy human donors, to generate cells exhibiting an ieILC1 phenotype. We assessed the differentiation using traditional flow cytometry and further profiled the cells using cytometry by time of flight (CyTOF)² to obtain higher-dimensional data about their surface and intracellular phenotypes. We then tested the cells for their cytotoxicity against a variety of target cell lines using the xCELLigence platform. Next, we grew three-dimensional tumoroids from single-cell suspensions of SCC cell lines in basement membrane extracts and added fluorescently labeled pbNKs and ieILC1s to them. We assessed their infiltration capacity into the tumoroids using confocal microscopy.

Results The ieILC1-like cells generated *in vitro* had a comparable surface and intracellular phenotype to ieILC1s in healthy tissue. These cells exhibited significantly increased cytotoxicity against multiple SCC cell lines and were also capable of antibody-dependent cellular cytotoxicity, which we tested using anti-epidermal growth factor receptor (EGFR) antibody (figure 1). Importantly, the ieILC1-like cells efficiently infiltrated the tumoroids in a manner consistent with their tissue-resident phenotype (figure 2A) and at higher rates than the conventional pbNKs (figure 2B).

Abstract 181 Figure 1 Cytolysis of pbNKs and ieILC1-like cells was compared using the xCELLigence platform to monitor killing of adherent SCC target cells over 36 hours. Cells were cultured at a 1:4 E:T ratio with or without 10 ug/mL cetuximab.

Abstract 181 Figure 2 Tumoroid infiltration rates of pbNKs and ieILC1-like cells were compared using confocal microscopy. A: Representative cross-sections of tumoroids, with nuclei labeled with DAPI (blue) and infiltrating cells labeled with CellTrace Far Red (pink). B: Infiltrating cells within each tumoroid were counted and then normalized to the area of the tumoroid.

Conclusions Our data show that ieILC1-like cells can be generated from pbNKs using a co-culture system with irradiated epithelial tumor cells. These ieILC1-like cells provide a novel platform for adoptive cell therapy, as they exhibit strong natural cytotoxicity and ADCC against multiple cell lines. Finally, the ieILC1-like cells have an enhanced capacity for infiltration into solid tumoroids. Future work will include *in vivo* modeling of tumor infiltration and efficacy as well as optimization of the co-culture platform to maximize expansion and cytotoxicity of the cells as they differentiate.

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Ethics Approval The studies reported here were approved by the Stanford Institutional Review Board (IRB 11402) and the Stanford Administrative Panel on Laboratory Animal Care (APLAC 20547).

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