240 PRESENTATION OF DLL4 AND VCAM ON PARAMAGNETIC BEADS REPLICATES THYMIC NOTCH SIGNALING AND ENABLES DIFFERENTIATION OF FUNCTIONAL OF IPSC DERIVED TCR T-CELLS IN VITRO

[1]Siddarth Chandrasekaran*, [1]Melanie Kardel, [1]Elisa Martinez, [2]Nabil Zeidan, [1]Avisek Deyati, [3]Valerie Wall, [2]Dylan Lee, [1]Negin Farahani, [2]Amanda AuYeung, [2]Omar Subedar, [3]Michael Cadell, [3]Sommer Apelu, [3]Laura Prochazka, [2]Zahra Sahaf, [1]Angela Duong, [1]Cole Zmurchok, [1]Boyoung Yoon, [3]Dan Kirouac, [2]Muluken Belew, [3]Jasdeep Mann, [3]Deepika Rajesh, [3]Chris Bond. *[1]Notch Therapeutics, Vancouver, BC, Canada; [2]Notch Therapeutics, Toronto, ON, Canada; [3]Notch Therapeutics, Seattle, WA, USA*

Background Adoptive TCR T-cell therapies from autologous donor are expensive, time-consuming and depends on quality of T-cells in patients. One of the key challenges for autologous TCR therapy is the requirement of cell numbers that are orders of magnitude higher than CAR-T cells. This has several implications including the inability to manufacture enough cells for a repeat dosing regimen. To this end, we have developed a scalable method by which TCR+ CD8+ lymphocytes can be generated from induced pluripotent stem cells (iPSCs) using 3D engineered thymic niche (ETN) comprised of DLL4- and VCAM-conjugated paramagnetic microbeads.

Methods We have successfully developed a gene-editing workflow for generation and characterization of iPSC clones engineered to express an exogenous TCR. We utilized a defined scalable differentiation process to generate lymphoid competent CD34+ HPCs from multiple iPSC clones. Cryopreserved CD34 cells were differentiated to Pro T cells and CD8+TCR + cells by leveraging stage specific control of Notch signalling using the 3D ETN. Flow cytometry and single-cell RNA sequencing was used to characterize the phenotypic and transcriptional state of iPSC derived effector cells. Incucyte-based cytotoxic killing assay was used to demonstrate antigen specific effector cell proliferation and cytotoxic function. We have developed a computational tool to quantify the average number of target cells killed by each input effector cell per unit time.

Results Multiple iPSC engineered clones expressing a TCR were successfully differentiated to hematopoietic precursor cells with an average efficiency of ~2.5 lympho-competent HPCs per input iPSC without magnetic sorting. HPCs were further differentiated in the presence of proprietary ETN beads to generate mature single positive T cells expressing TCR. The end stage cells expressed T-cell markers mimicking mature peripheral blood T-cells with a 90% TCR expression detected by an antigen-loaded tetramer. Transcriptional profiling of iPSC-derived and peripheral blood-derived CD8+ T-cells revealed similar gene expression signatures. Cryopreserved end-of-process CD8+ cells displayed target-specific cytotoxic activity against antigen expressing tumor cell lines *in vitro* across 4 rounds of stimulation in a long-term serial killing assay. iPSC derived CD8 TCR T-cells secreted perforin, granzyme B, IFNg, TNFa and GM-CSF and expanded ~1000-fold in response to target antigen.

Conclusions We have successfully generated CD8+TCR+ T-cells from iPSCs using a defined process that is amenable to scale up. iPSCs derived TCR T-cells, when combined with genetic manipulations to enhance persistence in a suppressive tumor microenvironment and overcome allogeneic rejection, could lead to a new generation of TCR T-cell therapies.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0240