Differentiating T-cells from hiPSCs to create off-the-shelf SPEAR T-cell therapies

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Harnessing the power of T-cells to fight cancer has generated many different experimental treatment options for patients. The majority of these therapies use the patients' own T-cells that must be expanded *ex vivo* to generate large numbers of cells, which can recognize tumor either via enrichment of endogenous tumor-infiltrating lymphocytes (TILs) or by the introduction of an engineered receptor. Using patient cells as starting material introduces inherent variability into any manufacturing process, as the functionality of these cells can be affected by health, age, and prior treatment regimens. Individualized manufacturing also leads to a complex chain of identity and a lag time in patient treatment while the personal cell product is being manufactured.

Human induced pluripotent stem cells (hiPSCs) offer an alternative source of T-cells that can be used to treat patients by enabling pre-manufacturing and freezing of T-cell product stock, thus shortening the lead time between patient screening and treatment. hiPSCs have the ability to divide indefinitely in an undifferentiated state and are amenable to genetic editing to produce stem cell banks that can be characterized for clinical use. hiPSCs can be directed to differentiate through multiple precursor stages towards a mature T-cell phenotype over a prolonged culture period *in vitro.*

We directed differentiation of cells from a pluripotent state ($SSEA4^+OCT4^+TRA\text{-}160^+$) through various intermediate stages: $CD34^+CD45^+$ hematopoietic progenitor cells (HPC), pro/preT $CD7^+CD5^+$cells, $CD4^+CD8^+$ double positive cells towards $CD3^+CD8^+TCR^+$ single positive T-cells. TCR^+ cells generated by this process show many similarities to mature T-cells from peripheral blood. Single cells were isolated and assessed by PCR using a panel of 96 genes to discern lineage and differentiation stage. Unedited hiPSCs can express either αβ or γδ TCR after differentiation; both subsets show multiple effector functions and release cytokines (IFNγ and TNFα) and lytic granules (Granzyme A and B, CD107a) when stimulated. Lentiviral transduction of progenitors with SPEAR (specific peptide enhanced affinity receptors) promotes surface expression of CD3 and SPEAR on the differentiated cells, which show antigen-specific activation.

These TCR^+ cells show functional characteristics that make them ideal to produce allogeneic, off-the-shelf T-cell therapies for oncology, where consistent batches can be manufactured to treat multiple patients. This approach, when combined with genetic engineering to express SPEAR TCRs, thus enhancing tumor recognition and preventing GVHD through endogenous TCR, could accelerate the next generation of SPEAR T-cell therapies.