SYNTHETIC RECEPTOR ENABLED DIFFERENTIATION (SHRED), A NOVEL PLATFORM FOR MANUFACTURING OF IPSC-DERIVED CYTOTOXIC INNATE LYMPHOCYTES FOR “OFF-THE-SHELF” CANCER IMMUNOTHERAPIES

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Background Induced pluripotent stem cells (iPSCs) are a renewable, modifiable, and scalable starting material for manufacturing cell-based therapies. However, current approaches for differentiating iPSCs into therapeutic immune effector cells, such as natural killer (NK) cells, require complex growth factors and feeder cells to achieve sufficient yields. Here, we present Synthetic Receptor Enabled Differentiation (ShRED), a directed differentiation and expansion process controlled by the Rapamycin-Activated Cytokine Receptor (RACR). RACR is activated via the addition of its synthetic ligand rapamycin, which induces a JAK/STAT signal that drives differentiation and expansion of cells into hematopoietic progenitors (HPs) and then into immune effector cells, termed RACR-induced Cytotoxic Innate Lymphocytes (RACR-iCILs). Furthermore, because rapamycin is a safe, effective, and approved therapeutic for immune suppression, we believe RACR can also be engaged in vivo through rapamycin dosing to increase the persistence of RACR-iCILs, while simultaneously protecting these cells from allogeneic rejection.

Methods RACR was introduced into iPSCs through CRISPR/Cas9 editing, enabling consistent expression of RACR throughout differentiation and expansion. Next, RACR was activated at the start of the manufacturing process, and the resulting differentiated cells were phenotyped by flow cytometry and assessed functionally in cytotoxicity assays.

Results Activating RACR in our engineered iPSCs resulted in enhanced HP differentiation, consistently producing a 300x yield of HPs (CD43+/CD45+/CD34+ cells) from iPSCs, two orders of magnitude greater than conventional protocols (figure 1). Continued engagement of RACR during differentiation resulted in >300,000x yield of RACR-iCILs (CD45+/CD56+/LFA1+ cells) from iPSCs in a completely feeder-free process (figure 1). These cells were highly pure and expressed receptors associated with NK cytotoxicity (figure 2A). Functionally, RACR-iCILs demonstrated potent cytotoxicity against solid tumors, and activation of RACR enhanced the cells’ ability to clear tumor cells in a manner similar to potency-enhancing cytokines (figure 2B). Lastly, in a preclinical tumor xenograft model, RACR-NK cells demonstrated robust rapamycin-mediated expansion and clearance of breast adenocarcinoma tumors, highlighting RACR’s ability to replace cytokine support and potentially increase cell persistence in vivo.

Conclusions These data demonstrate the potential of RACR to solve both the manufacturing and in vivo persistence challenges of iPSC-derived cell therapies. ShRED improves the manufacturing of iPSC-derived cells, producing unprecedented yields of immune effector cells (RACR-iCILs) and eliminating the need for complex cytokines and feeder expansions. In vivo, RACR activation increases the proliferation of RACR-expressing NK cells, potentially enhancing the persistence of cell immunotherapies and eliminating the need for cytokine dosing and lymphodepletion.