Driving ADP-A2M4 SPEAR expression from an endogenous hematopoietic lineage promotor for off-the-shelf T-cell therapy for MAGE-A4+ solid tumors

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Herein, we describe progress with our allogeneic T-cell lines (iT-cell) derived from directed differentiation of human induced pluripotent stem cells (hiPSCs). We demonstrate that by using targeted genetic engineering we can produce clonal iT-cell populations that express the ADP-A2M4 SPEAR (Specific Peptide Enhanced Affinity Receptor) driven from an endogenous promotor. ADP-A2M4 SPEAR expressing iT-cells are shown to exhibit a range of effector functions upon exposure to tumor lines expressing cognate MAGE-A4 peptide (*GVYDGREHTV*) presented by HLA-A*02.

The treatment of cancer with adoptive T-cell therapy is an expanding area of therapeutic intervention. Most current approaches use autologous or patient-derived T-cells that must be harvested from the patient, transferred to a manufacturing facility, and usually genetically modified prior to expansion and transfusion back into the patient. These autologous therapies have performed well in the clinic; however, there are advantages to moving away from patient-derived cells, and developing "off-the-shelf" or allogeneic approaches. An "off-the-shelf" approach can significantly reduce the vein-to-vein times and logistical challenges associated with autologous cell manufacturing. It also provides the opportunity to develop a more consistent T-cell product that does not vary from patient to patient.

One source of allogenic T-cell products are those differentiated from hiPSCs. HiPSCs are an attractive starting material as they can proliferate indefinitely, in an undifferentiated state, allowing the production of large cell banks that in turn can be differentiated to produce multiple therapeutic doses. HiPSCs are amenable to genetic engineering and can also be directed to differentiate into various lineages that are clinically useful, such as T-cells or NK cells.

The challenge with any allogeneic approach is to ensure that the final product expresses a defined T-cell receptor (TCR) to reduce the risk of graft-versus host disease (GvHD). Here we describe a novel approach via targeted knock-in of the ADP-A2M4 SPEAR in hiPSC, exemplified in two separate clones. This results in the formation of a multi-cistronic expression cassette where the native gene and ADP-A2M4 SPEAR expression are both regulated by the endogenous promoter. The temporal pattern of expression of both genes is linked throughout the differentiation process. Each edited clone was characterized to have one edited and one wild type allele. We demonstrate that edited hiPSC lines can produce iT-cells via a series of defined progenitor populations: $CD34^{+/}CD45^{+}$ hematopoietic progenitor cells (HPCs), common lymphoid precursors (CLP), pro T-cells, double negative (DN) cells, $CD4^{+}/CD8^{+}$ double positive (DP) cells, and $CD3^{+}/CD8^{+}/TCR^{+}$ single positive (SP) T-cells without the use of a feeder or stromal cell line. Flow cytometry shows that iT-cells expressing CD3 and ADP-A2M4 SPEAR upregulate CD69/CD25 and produce cytokine (IFNγ/TNFα) in response to antigen. These differentiated cells express high levels of granzyme and are capable of killing antigen positive tumor lines.

Overall, this work represents the development of an allogeneic hiPSC derived platform, with limited genome editing, that permits the production of SPEAR iT-cells with potential therapeutic value. The identification of a suitable locus for targeted integration of a defined TCR/SPEAR enables the future production of multiple iT-cell banks directed against specific tumor associated antigens in a defined and reproducible manner.

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