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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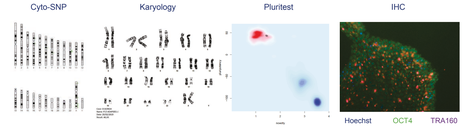
Introduction

- Adoptive T-cell therapy is now widely recognized as an important therapeutic intervention for the treatment of cancer. Most current approaches use autologous or patient-derived T-cells. These include TILs (tumor infiltrating lymphocytes) or T-cells that have been virally transduced to express a CAR (chimeric antigen receptor) or enhanced affinity TCR (T-cell receptor)
- One drawback is the complexity of the logistics associated with their manufacture. An alternative "off-the-shelf" approach is the production of engineered T-cells from an allogeneic source, such as healthy donor-derived T-cells or T-cells that have been differentiated from a human-induced pluripotent stem cell (hiPSC). The "off-the-shelf" approach is particularly advantageous as it significantly reduces vein-to-vein time, ie, patients get treatment faster, and facilitates the generation of a more defined T-cell phenotype that does not vary from patient to patient
- Adaptimmune is currently developing an hiPSC-derived allogeneic platform that allows the expression of TCRs in hiPSC-derived T-cells (iT-cells), including expression of SPEAR (specific peptide enhanced affinity receptor) TCRs
- Several groups have described the *in vitro* production of iT-cells using OP9/DL1 expressing stromal cells[1-5] or hiPSC-derived artificial thymic organoids.[6] A prerequisite for any allogeneic T-cell product that uses an αβTCR is the production of a clonal T-cell population. This is required to mitigate the risk of graft versus host disease (GvHD). iT-cells with restricted expression of a defined αβTCR have been successfully produced from clones of hiPSC that were transduced with lentiviral vectors encoding a specific TCR.[5,6] An alternative method is to derive hiPSC clones from reprogrammed T-cells (TiPSC) that encode an αβTCR of the desired specificity.[1,2,5] These reports have suggested that restriction of αβTCR expression in differentiated iT-cells is driven by TCR allelic exclusion, which prevents erroneous rearrangement of endogenous TCR genes

Objective

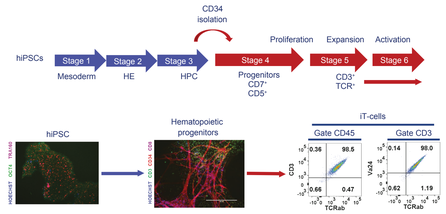
- We present data based on a minimal editing strategy that targeted insertion of the ADP-A2M4 SPEAR sequence permits ADP-A2M4 SPEAR expression in differentiated iT-cells
- We describe a serum- and feeder-free approach for the generation of iT-cells expressing a defined αβTCR. Starting with a GMP-compliant hiPSC source, we knocked-in the ADP-A2M4 SPEAR that recognizes the MAGE-A4 (melanoma-associated antigen A4) peptide (GVYDGREHTV) presented by HLA-A*02. We show that ADP-A2M4 iT-cells specifically express the ADP-A2M4 SPEAR as measured by anti-TCR Vα24 and dextramer staining. Edited ADP-A2M4 iT-cells up-regulate activation markers, including CD25 and CD69, when incubated with HLA-A*02-expressing tumor lines that express the cognate antigen, and exhibit potent antigen-dependent killing of these 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 anticipated therapeutic value

Figure 1. Generation of the ADAPi001 GMP-compliant hiPSC lines



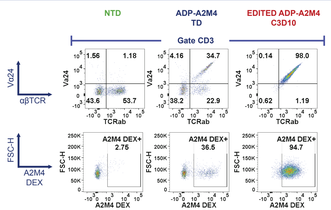
The ADAPi001 hiPSC lines were created via re-programming of CD34^{+} progenitors isolated from umbilical cord blood using the pEB-C5 and pEB-TG episomal plasmids.[7] All ADAPi001 clones are traceable and produced under GMP conditions. Nine ADAPi001 hiPSC clones were characterized with a small working cell bank of early passage number (p10) produced for each clone. Genomic stability was assessed via cyto-SNP analysis, karyotyping and WGS (whole genome sequencing). Pluripotency was determined with IHC (immunohistochemistry), flow cytometry and Pluritest.[8] Differentiation of iT-cells from ADAPi001 hiPSC clones was also confirmed.

Figure 2. Summary of the iT-cell differentiation protocol



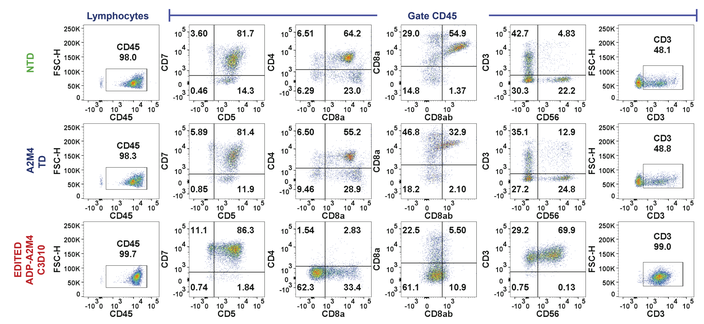
iT-cells have been differentiated from three different hiPSC lines. This process is independent of stromal lines and is serum- and feeder-free. The intracellular expression of T-cell markers (CD3 and CD8α) can be detected in differentiated colonies early in the process. As differentiation progresses, defined progenitor populations can be identified throughout the differentiation process: CD34^{+} hematopoietic progenitors, CD7^{+}CD5^{+} progenitor T-cells and differentiated CD3^{+}CD8^{+}TCR^{+} iT-cell populations. We have been investigating the production of iT-cell phenotypes after hiPSC gene editing or ADP-A2M4 SPEAR lentiviral transduction. Edited hiPSC clones have been generated in ADAPi001-J and other research-grade hiPSC lines. The edited ADAPi001-J ADP-A2M4 clone, 3D10, has been characterized after undergoing this differentiation process. Lentiviral transduction was performed on proliferating CD7^{+}CD5^{+} progenitor T-cells that arise partway through the differentiation process.

Figure 4. Expression of ADP-A2M4 SPEAR in differentiated iT-cells



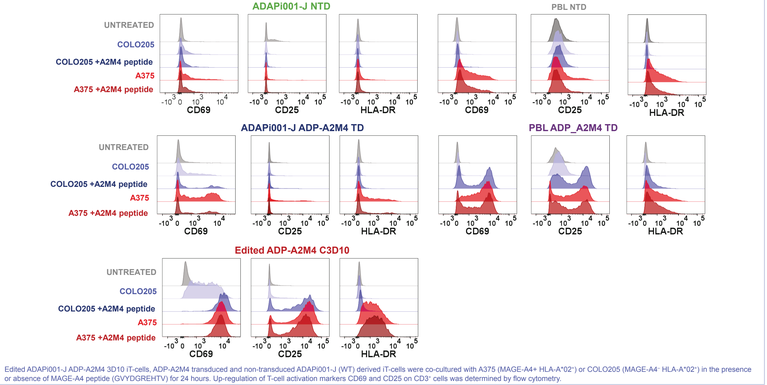
iT-cells were differentiated from WT ADAPi001-J or edited ADAPi001-J ADP-A2M4 clone 3D10. CD7^{+}CD5^{+} progenitors differentiated from WT ADAPi001-J were lentivirally transduced with ADP-A2M4 SPEAR. Expression of ADP-A2M4 SPEAR on CD3^{+} cells after differentiation (**Figure 3**) was measured by flow cytometry with anti-TCR Vα24 and MAGE-A4 GVYDGREHTV/HLA-A*0201 dextramer.

Figure 3. ADAPi001-J iT-cell phenotypes after differentiation and expansion



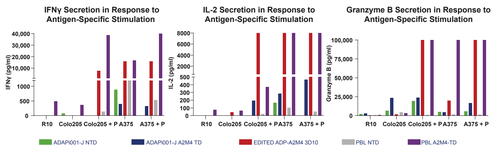
iT-cells were differentiated from WT ADAPi001-J or edited ADAPi001-J ADP-A2M4 clone 3D10. CD7^{+}CD5^{+} progenitor T-cells were differentiated from WT ADAPi001-J and lentivirally transduced with ADP-A2M4 SPEAR. iT-cells were phenotyped by flow cytometry, and expression of CD45, CD7, CD5, CD4, CD8, CD56 and CD3 is shown.

Figure 5. Edited ADP-A2M4 iT-cells are activated in an antigen-dependent manner



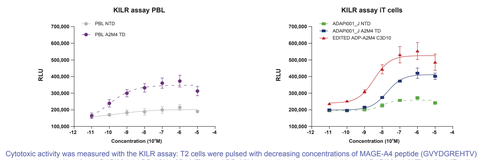
Edited ADAPi001-J ADP-A2M4 3D10 iT-cells, ADP-A2M4 transduced and non-transduced ADAPi001-J (WT) derived iT-cells were co-cultured with A375 (MAGE-A4+ HLA-A*02^{+}) or COLO205 (MAGE-A4^{-} HLA-A*02^{+}) in the presence or absence of MAGE-A4 peptide (GVYDGREHTV) for 24 hours. Up-regulation of T-cell activation markers CD69 and CD25 on CD3^{+} cells was determined by flow cytometry.

Figure 6. Edited ADP-A2M4 iT-cells release cytokine in an antigen-dependent manner



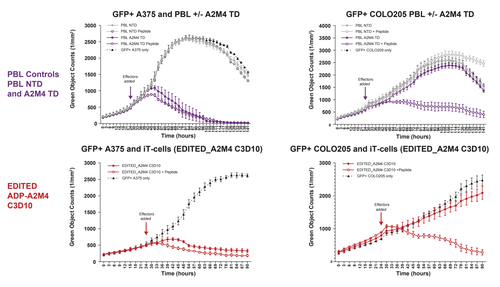
Edited ADAPi001-J ADP-A2M4 3D10 iT-cells, ADP-A2M4 transduced and non-transduced ADAPi001-J (WT) derived iT-cells were co-cultured with A375 (MAGE-A4^{+} HLA-A*02^{+}) or COLO205 (MAGE-A4^{-} HLA-A*02^{+}) in the presence or absence of MAGE-A4 peptide (GVYDGREHTV) for 24 hours. Cytokines (IFNγ and IL-2) and Granzyme B secretion were measured by ELISA (enzyme-linked immunosorbent assay). The activation of iT-cells was compared to ADP-A2M4 SPEAR transduced and non-transduced PBL (peripheral blood lymphocyte) controls.

Figure 7. Edited ADP-A2M4 iT-cells kill peptide-pulsed T2 cells



Cytotoxic activity was measured with the KILR assay: T2 cells were pulsed with decreasing concentrations of MAGE-A4 peptide (GVYDGREHTV) and co-cultured with edited ADAPi001-J ADP-A2M4 3D10 iT-cells, ADP-A2M4 transduced and non-transduced ADAPi001-J (WT) derived iT-cells. Increased signal indicates increased cell death.

Figure 8. Edited ADP-A2M4 iT-cells kill tumor lines in an antigen-dependent manner



Killing activity measured by IncuCyte® assay: GFP^{+} A375 (MAGE-A4^{+} HLA-A*02^{+}) or GFP+ COLO205 (MAGE-A4^{-} HLA-A*02^{+}) cells were co-cultured with edited ADAPi001-J ADP-A2M4 3D10 iT-cells for 90 hours in the presence or absence of MAGE-A4 peptide. The growth of GFP^{+} tumor cell lines was followed using the IncuCyte® Live-Cell Imaging platform. A decrease in green object count (GFP) is a result of reduced cell proliferation and increased tumor cell death. The cytolytic activity of iT-cells was compared to ADP-A2M4 SPEAR transduced and non-transduced PBL controls to represent autologous ADP-A2M4 SPEAR T-cell product.

Conclusions

- We have presented a novel editing strategy that has enabled the differentiation of ADP-A2M4 SPEAR expressing iT-cells. Edited ADP-A2M4 iT-cell activation and cytolytic effector function is antigen dependent. The phenotype of edited ADP-A2M4 iT-cells including CD56 and CD8α expression, potent cytokine and cytotoxic activity suggests that edited ADP-A2M4 iT-cells may possess an innate-like phenotype as previously described[2,3] (**Figures 3, 5–8**)
- Interestingly, expression of ADP-A2M4 SPEAR at later time points appears to alter the differentiation of these cells as previously noted (**Figure 5**).[9] Although dextramer staining suggests that edited ADP-A2M4 iT-cells only express the ADP-A2M4 TCR (**Figure 4**), full TCR repertoire analysis in edited iT-cells is in progress
- The edited ADP-A2M4 iT-cells exhibit potent cytolytic and effector function, which is at least comparable or increased compared with SPEAR transduced PBL from healthy donors. This suggests that, like autologous SPEAR T-cells, ADP-A2M4 iT-cells should be an efficacious cell therapy and could bring clinical benefit to patients
- The generation of ADP-A2M4 iT-cells is an important milestone in producing an iPSC-derived allogeneic platform. The ability to promote TCR expression in iT-cells via genetic knock-in at a single, defined locus offers an opportunity to produce multiple clonal iPSC banks encoding specific SPEARs against a range of tumor antigens. Understanding how to manipulate the differentiation process should allow control and skewing of the phenotype of differentiated iT-cells to produce desired functional characteristics. In the future, this "off-the-shelf" platform could deliver a range of defined and consistent T-cell therapies to patients based on their tumor antigen expression profile in a timely manner

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