# Modulation of Single-Cell Gene Expression and Cell Function in Evolving Manufacturing Processes for Clinical Trials With Enhanced-Affinity T-Cell Receptor T-Cell Therapy Targeting the MAGE-A4 Antigen in Solid Tumors

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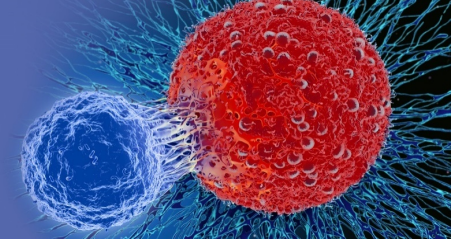


## Introduction

- Affinity-enhanced T-cell receptor (TCR) T-cell therapies targeting the intracellular cancer testis antigen melanoma-associated antigen A4 (MAGE-A4) have shown encouraging results in adults with advanced solid cancers[1,2]
- ADP-A2M4CD8, the next generation counterpart of afamitresgene autoleucel (afami-cel, formerly ADP-A2M4), co-expressing CD8α with the same MAGE-A4 targeting TCR, was developed to enhance anti-tumor activity
- Enhancements to manufacture ex vivo expansion were also made; evolving from rocking platform Xuri bioreactor culture to G-Rex (static culture, gas permeable membrane) and inclusion of an AKT inhibitor (AKTi), hypothesized to generate less differentiated cells with increased functional potential[3]
- Here we explore how manufacturing process evolution may modulate the function of ADP-A2M4CD8 using single-cell RNA sequencing (scRNA-seq) and in vitro functional assessment of manufactured products generated for the Phase 1 afami-cel (NCT03132922) and SURPASS (NCT04044859) trials

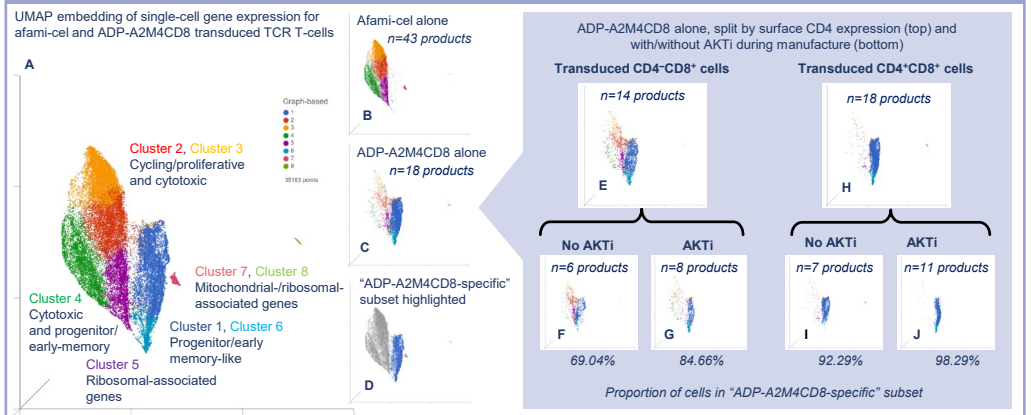
## Methods

- Autologous T-cell products were manufactured from patient leukapheresis material by transduction of a self-inactivating Lentiviral vector expressing either a MAGE-A4-specific TCR alone (afami-cel), or the same MAGE-A4-specific TCR plus an additional CD8α co-receptor (ADP-A2M4CD8), followed by ex vivo expansion
- Afami-cel T-cells were expanded for 11–15 days in a Xuri bioreactor. ADP-A2M4CD8 T-cells were expanded for 10 days in a G-Rex bioreactor, with or without addition of an AKTi
- Transduced CD4$^-$CD8$^+$ T-cells were isolated from retains of afami-cel (N=43) and ADP-A2M4CD8 (N=18) products and evaluated for differences in gene expression profile (GEP) using scRNA-seq (Fluidigm C1 platform, Standard BioTools, South San Francisco, CA)
- For ADP-A2M4CD8 products, transduced CD4$^+$CD8$^+$ T-cells were also profiled as they demonstrate increased tumor cell lysis via the CD8α co-receptor
- Transduced ADP-A2M4CD8 subsets were assessed for their capacity to directly lyse tumor cells in vitro
- Translational correlation between the dose of transduced ADP-A2M4CD8 subsets identified in scRNA-seq and the percentage of infused transduced T-cells recovered at peak expansion in peripheral blood after infusion was explored

## Results

- Unsupervised analysis of the scRNA-seq data revealed a novel subset of cells with a distinct GEP in transduced T-cells from ADP-A2M4CD8 infusion products compared with afami-cel (**Figure 1 A–D**)
- Both transduced CD4$^+$CD8$^+$ and CD4$^-$CD8$^+$ ADP-A2M4CD8 T-cells demonstrate this profile, and products manufactured with AKTi contain a greater proportion of cells within the novel "ADP-A2M4CD8-specific" subset (**Figure 1 E–J**)

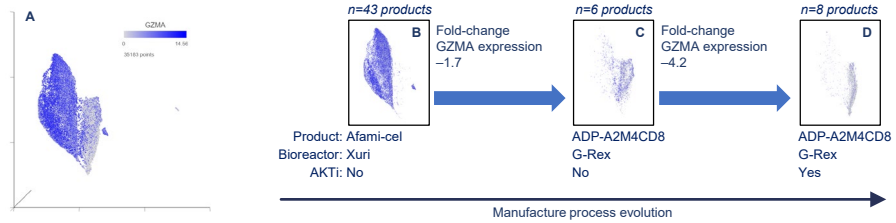
### Figure 1. Unsupervised analysis of afami-cel and ADP-A2M4CD8 transcriptomes reveals a novel "ADP-A2M4CD8-specific" cell subset




**A**: UMAP embedding following unsupervised analysis of afami-cel and ADP-A2M4CD8 transduced TCR T-cell transcriptional profiles. Single-cell RNA sequencing was used to profile transduced CD4$^-$CD8$^+$ T-cells (afami-cel) and transduced CD4$^-$CD8$^+$ and CD4$^+$CD8$^+$ T-cells (ADP-A2M4CD8). Cluster assignment from graph-based clustering are indicated and annotated. **B**: UMAP embedding showing afami-cel alone. **C**: UMAP embedding showing ADP-A2M4CD8 alone. **D**: UMAP embedding including both afami-cel and ADP-A2M4CD8 with the novel "ADP-A2M4CD8-specific" cell subset highlighted (blue). **E**: UMAP embedding showing transduced CD4$^-$CD8$^+$ T-cells from ADP-A2M4CD8 alone. This is further divided to show only cells for patients whose product was generation without (**F**) and with (**G**) inclusion of an AKTi. **H**: UMAP embedding showing transduced CD4$^+$CD8$^+$ T-cells from ADP-A2M4CD8 alone. This is further divided to show only cells for patients whose product was generated without (**I**) and with (**J**) inclusion of an AKTi. AKTi, AKT inhibitor; TCR, T-cell receptor; UMAP, uniform manifold approximation and projection

- The "ADP-A2M4CD8-specific" subset has a less cytotoxic GEP, and transduced CD4$^-$CD8$^+$ T-cells exhibited a progressive decrease in cytotoxic GEP with manufacture process evolution, demonstrated through step-wise decrease in granzyme A (GZMA) expression (**Figure 2**)
- This is also observed between a patient's afami-cel product and research-grade re-manufactures, from the same apheresis material, expanded in a G-Rex bioreactor with/without an AKTi (fold-change GZMA expression Xuri>G-Rex –2.76, G-Rex>G-Rex + AKTi –19.3), suggesting this effect is not solely associated with the co-expressed CD8α co-receptor
- Graph-based Cluster 6, located in the "ADP-A2M4CD8-specific" subset, has the lowest expression of cytotoxicity-related genes and highest expression of progenitor/early memory-associated genes, and is strongly enriched in products manufactured with an AKTi

### Figure 2. The novel "ADP-A2M4CD8 specific" subset has an apparently less cytotoxic gene expression profile




**A**: UMAP embedding following unsupervised analysis of afami-cel and ADP-A2M4CD8 TCR T-cell transcriptional profiles showing transduced CD4$^-$CD8$^+$ T-cells only. Color intensity scale represents level of GZMA gene expression. **B–D**: UMAP embedding of afami-cel and ADP-A2M4CD8 (transduced CD4$^-$CD8$^+$ T-cells shown) divided by manufacture process, which evolves from ex vivo expansion in a Xuri bioreactor (**B**) to ex vivo expansion in a G-Rex bioreactor (**C**) to ex vivo expansion in a G-Rex bioreactor with the inclusion of an AKTi (**D**). AKTi, AKT inhibitor; GZMA, granzyme A; TCR, T-cell receptor; UMAP, uniform manifold approximation and projection

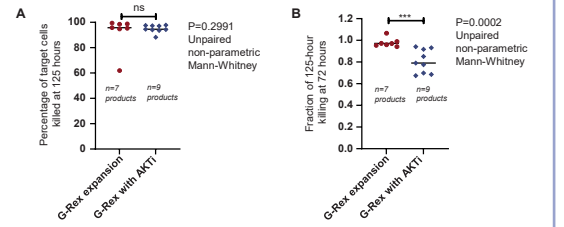
- Functional differences in transduced CD4$^-$CD8$^+$ T-cells from ADP-A2M4CD8 products manufactured with or without an AKTi were explored in an in vitro cytotoxicity assay
- Transduced CD4$^-$CD8$^+$ T-cells manufactured with or without an AKTi killed a similar percentage of target cells after 125 hours (**Figure 3 A**)
- However, transduced CD4$^-$CD8$^+$ T-cells manufactured with an AKTi demonstrated a delay in their cytotoxicity with a significantly lower fraction of the 125hr killing achieved by 72hrs (**Figure 3 B**)
  - A similar effect is observed with transduced CD4$^+$CD8$^+$ T-cells (data not shown)
- This delay in cytotoxic activity is consistent with the production of less differentiated cells with the inclusion of an AKTi during ex vivo expansion

- Persistence of ADP-A2M4CD8 transduced TCR T-cells for patients in the Phase 1 SURPASS trial was assessed from blood draws taken at multiple time points after infusion
- Total transduced T-cells in peripheral blood at each time point was inferred from the vector copy number (VCN) per microgram of peripheral blood mononuclear cell (PBMC) genomic DNA, measure by quantitative PCR, using the following equation:

$$\frac{\text{Average VCN/µg PBMC DNA} * \text{no. of PBMC/µL blood}}{\text{Average VCN/transduced cell in infused product}} * 5\text{L blood volume}$$

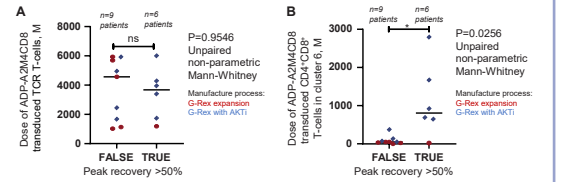
- Peak recovery is the highest total transduced T-cells recorded for each patient over the time period to the data cut (February 24, 2023), represented as a percentage of the infused dose of transduced T-cells
- Patients who received a higher dose, compared with a lower dose, of ADP-A2M4CD8 transduced CD4$^+$CD8$^+$ T-cells within Cluster 6 of **Figure 1** were more likely to have a peak recovery of >50% of their infused transduced T-cell dose (**Figure 4**). A non-significant trend is seen for dose of transduced CD4$^-$CD8$^+$ T-cells in Cluster 6 (data not shown), and this is specific to Cluster 6 as the same effect is not seen with transduced dose (**Figure 4**)
- This may reflect the enhanced proliferative potential of less differentiated cells in products manufactured with an AKTi

### Figure 3. ADP-A2M4CD8 cells manufactured with an AKTi demonstrate a delayed cytotoxicity with ultimately similar capacity to lyse target cells in vitro




In vitro target-cell killing by ADP-A2M4CD8 transduced CD4$^-$CD8$^+$ T-cells manufactured with or without inclusion of an AKTi during ex vivo expansion in a G-Rex bioreactor. **A**: The percentage of target cells killed at 125 hours. **B**: The fraction of target-cell killing at 125 hours that is achieved at 72 hours. AKTi, AKT inhibitor; TCR, T-cell receptor

### Figure 4. Patients who received a higher dose, compared with a lower dose, of ADP-A2M4CD8 transduced CD4$^+$CD8$^+$ T-cells within Cluster 6 are more likely to have a peak recovery of >50% of their infused transduced T-cell dose




Peak recovery of ADP-A2M4CD8 transduced TCR T-cells after infusion correlates with the dose of transduced CD4$^+$CD8$^+$ T-cells within Cluster 6 a patient receives. **A**: There is no correlation between the dose of ADP-A2M4CD8 transduced TCR T-cells a patient receives and their likelihood of achieving a peak recovery >50%. **B**: Patients receiving a higher dose of ADP-A2M4CD8 transduced CD4$^+$CD8$^+$ T-cells within Cluster 6 are significantly more likely to achieve a peak recovery >50%. Patients who received ADP-A2M4CD8 manufactured using a process including ex vivo expansion in a G-Rex bioreactor with no AKTi are indicated with red circles and those for which AKTi was included during expansion with blue diamonds. AKTi, AKT inhibitor; M, million; GEP, gene expression profile; TCR, T-cell receptor

## Conclusions

- Whole-transcriptome, single-cell investigation of cellular subsets produced during manufacturing of afami-cel and ADP-A2M4CD8 indicate that process changes can have significant and additive effects on the gene expression profile and function of the cells produced
- G-Rex bioreactor expansion in the presence of an AKTi produces cells with a less cytotoxic gene expression profile and a delay before they reach peak cytolytic potency, which may reflect a less differentiated state
- Infusion of TCR T-cells with a less cytotoxic gene expression profile does not prevent the broad anti-tumor activity observed with ADP-A2M4CD8 in the SURPASS clinical trial, and may correspond with robust post-infusion proliferation and beneficial persistence characteristics

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**Footnotes and abbreviations used in text**

AKTi, AKT inhibitor; GEP, gene expression profile; GZMA, granzyme A; MAGE-A4, melanoma-associated antigen A4; PBMC, peripheral blood mononuclear cell; scRNA-seq, single-cell RNA sequencing; TCR, T-cell receptor; VCN, vector copy number

**References**

1. Van Tine BA, et al. Paper (61) presented at: CTOS 2022; Vancouver, BC, Canada.
2. Hong DS, et al. *Ann Oncol*. 2022;33(suppl 7):S331. 3. Klebanoff CA, et al. *JCI Insight*. 2017;2:e95103.

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