Enhancement of TCR-engineered T-cells Targeting MAGE-A4 Antigen by Co-expression of CD8α and Inhibition of AKT Signaling during *ex vivo* T-cell Expansion

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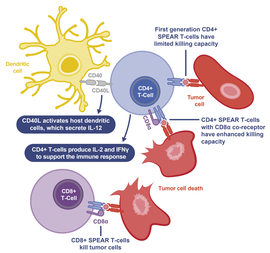
Introduction

- Autologous specific peptide enhanced affinity receptor (SPEAR) T-cells targeting MAGE-A4 can be effective treatment for solid tumors[1–3]
- To enhance efficacy, we developed a next-generation SPEAR T-cell targeting MAGE-A4 co-expressing CD8α (ADP-A2M4CD8), which is under investigation in the Phase 1 SURPASS trial (NCT04044859)
- Enhancements have also been made to the SPEAR T-cell manufacturing process with addition of an AKT inhibitor (AKTi) during *ex vivo* expansion to provide a greater proliferative potential and enhanced memory phenotype[4]

ADP-A2M4CD8 SPEAR T-cells



- SPEAR T-cells are a mix of CD4+ and CD8+ T cells engineered with a TCR recognizing an intracellular tumor antigen in an HLA-restricted fashion
- ADP-A2M4CD8 are next-generation SPEAR T-cells targeting MAGE-A4 with a CD8α co-receptor introduced into T-cells alongside the TCR
- The co-expression of CD8α adds CD8+ killer cell capability to CD4+ helper cells, while also maintaining/enhancing their helper cell capabilities
- The enhanced TCR interaction results in a more potent response because the ADP-A2M4CD8 next-generation CD4+ SPEAR T-cells can now both kill tumor cells as well as engage the broader immune system including dendritic cell activation

SURPASS trial design

- Eligible patients in the SURPASS trial are HLA-A*02 positive with MAGE-A4-expressing tumors
- Patients undergo leukapheresis to collect autologous T-cells for processing and manufacture
- SPEAR T-cells were manufactured using a lentiviral vector with CD8α and MAGE-A4 targeted TCR genes, and AKTi was added during the *ex vivo* expansion process
- Patients receive ADP-A2M4CD8 SPEAR T-cell doses between 1.0–10 × 10^9 transduced T-cells after lymphodepleting chemotherapy
- *In vitro* ADP-A2M4CD8 SPEAR T-cell attributes were evaluated by Incucyte (Sartorius, Goettingen, Germany) and flow cytometry
- *In vitro* attributes of afami-cel manufactured with and without AKTi were examined using single-cell RNA sequencing (scRNAseq; Fluidigm, San Francisco, CA)
- Post-infusion activity of ADP-A2M4CD8α including serum cytokine levels and presence of transduced T-cells in peripheral blood was assessed by Meso Scale Discovery assays (Meso Scale Diagnostics, Rockville, MD), and quantitative polymerase chain reaction, respectively

Responses per RECIST v1.1 (presented at ESMO 2021)

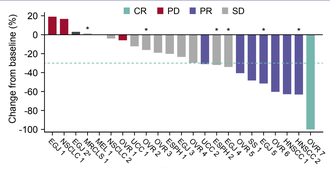
- As of August 2, 2021 (ESMO 2021 data cut-off), 22 of 25 patients with 9 different primary tumor types treated with ADP-A2M4CD8 were evaluable for RECIST v1.1 responses
- Disease control rate was 86% (1 complete response [CR], 7 partial response [PR], and 11 stable disease [SD]; out of 22 evaluable patients; **Figure 1**)
- Overall response rate was 36% (1 CR and 7 PR; out of 22 evaluable patients)

Table 1. Patients treated with ADP-A2M4CD8 SPEAR T-cells as of the data cut-off (Aug 2, 2021)

Characteristic (N=25)	Overall
Sex, n (%), male	13 (52.0)
Median age, years (range)	58 (31, 75)
Median H score,[a] (range)	267.5 (130–300)
ECOG performance status at baseline of 0, 1, n (%)	8 (32), 17 (68)
Transduced cells, range	1.0–9.9 billion

[a]N = 24

Figure 1. Tumor shrinkage seen in 18 patients with 8 responses

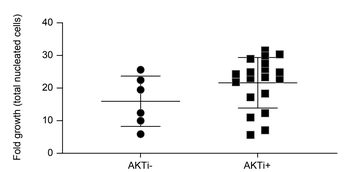


AKTi, AKT inhibitor; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease
*Patient had a post-baseline scan that did not meet the ≥4 week duration for SD.
†Indicates ADP-A2M4CD8 SPEAR T-cells were manufactured without AKTi. No significant difference in change from baseline in patients treated with cells manufactured in the absence of AKTi (n=6) versus the presence of AKTi (n=12) was observed

In vitro SPEAR T-cell attributes

- Addition of AKTi during manufacturing resulted in a trend towards greater fold expansion, although statistical significance was not reached (P=0.13; **Figure 2**)

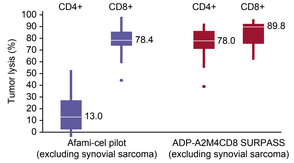
Figure 2. The addition of AKTi during *ex vivo* expansion of ADP-A2M4CD8 resulted in a trend towards increased growth



AKTi, AKT inhibitor

- CD4+ SPEAR T-cells from manufactured ADP-A2M4CD8 demonstrated direct *in vitro* tumor cell killing similar to CD8+ T-cells, confirming the positive benefit of CD8α in these CD4+ cells (**Figure 3**)

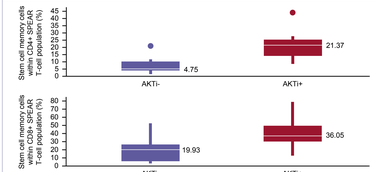
Figure 3. ADP-A2M4CD8 SPEAR CD4+ T-cells lyse target tumor cells *in vitro* (presented at ESMO 2021)



SPEAR, specific peptide enhanced affinity receptor
Lysis: 100–0% tumor cells remaining in presence of SPEAR T-cells) at 72 h, compared with no T-cells. Includes all samples with paired CD4+ and CD8+ data at the time of data cut-off. Medians are shown next to boxes (n=21, blue; n=18, red)

- Flow cytometry of infused ADP-A2M4CD8 SPEAR T-cells demonstrated increased stem cell memory content (**Figure 4**) of the transduced population in samples manufactured in the presence of AKTi (n=14) relative to those manufactured without (n=7)

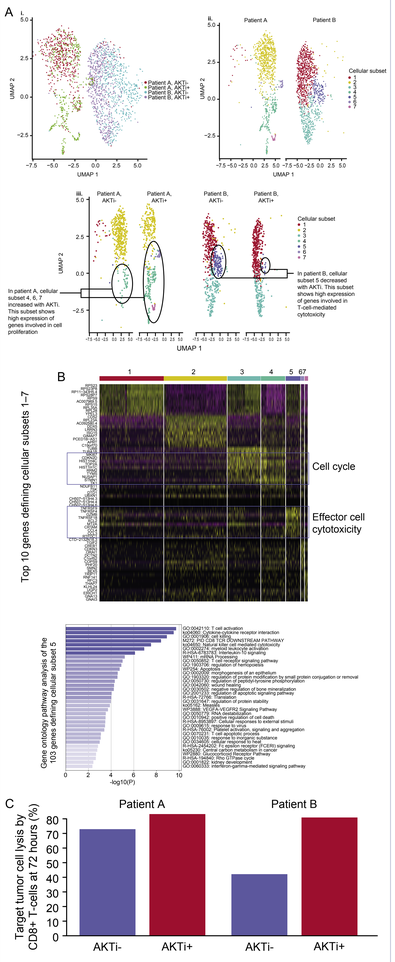
Figure 4. AKTi-expanded ADP-A2M4CD8 SPEAR T-cells showed enhanced stem cell memory phenotype[a]



AKTi, AKT inhibitor; SPEAR, specific peptide enhanced affinity receptor
[a]CD3+/Dextramer+/CD4+CD8+/CD45RA+/CCR7+

- To evaluate the impact of AKTi on SPEAR T-cell manufacturing, surplus pre-infusion apheresis material from patients in the Phase I pilot trial of afamitresgene autoleucel ("afami-cel" [formerly ADP-A2M4]) was remanufactured into research-grade afami-cel with (+) or without (-) AKTi during *ex vivo* expansion. This research-grade AKTi+ and AKTi- afami-cel was used for *in vitro* functional analyses
- Afami-cel from Patient A's original infusion showed good *in vitro* cytotoxicity but relatively low growth during manufacturing and poor persistence post-infusion (data not shown). Patient A's research-grade AKTi+ afami-cel compared with research-grade AKTi- afami-cel showed increased production of cellular subset 4, 6, and 7 (**Figure 5A**), which highly expresses cell proliferation genes (**Figure 5B**), and it proliferated better *in vitro* in response to antigen[4] and killed tumor cells (**Figure 5C**) to a similar degree compared with research-grade AKTi- afami-cel
- Afami-cel from Patient B's original infusion showed relatively low *in vitro* cytotoxicity but good growth in manufacturing and good persistence post-infusion (data not shown). Patient B's research-grade AKTi+ afami-cel compared with research-grade AKTi- afami-cel showed enhanced stem cell memory T-cell production (**Figure 4**) and reduced production of cellular subset 5 (**Figure 5A**), which highly expresses T-cell mediated cytotoxicity genes (**Figure 5B**)
- Together these data imply a less differentiated phenotype of AKTi+ afami-cel. Despite this, Patient B's research-grade AKTi+ afami-cel killed target tumor cells *in vitro* to a greater degree, compared with research-grade AKTi- afami-cel (**Figure 5C**)

Figure 5. Manufacturing SPEAR T-cells with AKTi can remodel gene expression in favour of improved proliferation or cytotoxicity

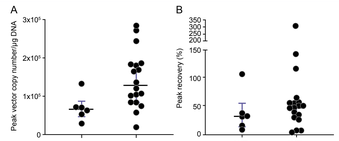


AKTi, AKT inhibitor; SPEAR, specific peptide enhanced affinity receptor

Post-infusion activity of ADP-A2M4CD8 SPEAR T-cells

- Post-infusion, ADP-A2M4CD8 SPEAR T-cells manufactured with AKTi had nearly 2-fold increase in peak vector copy number (median 128808 for lots manufactured with AKTi vs. 65639 for lots without AKTi, P=0.012, Mann Whitney U test) in peripheral blood as measured by qPCR detection of lentiviral DNA in peripheral blood mononuclear cells (**Figure 6A**)
- The addition of AKTi to the manufacturing process increased peak recovery (dose-normalized peak persistence) by ~ 55% post-infusion, although the difference was not statistically significant (median 49.7% with AKTi vs. 31.95 without AKTi, P=0.28). Peak recovery is notably higher in some patients infused with SPEAR T-cells manufactured with AKTi (**Figure 6B**)

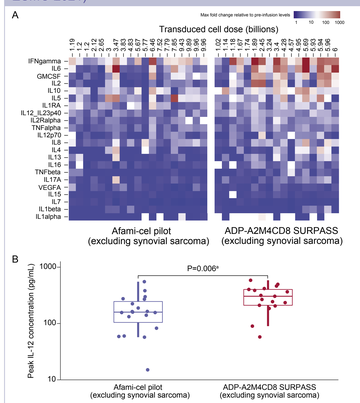
Figure 6. ADP-A2M4CD8 SPEAR T-cells manufactured with AKTi shows higher median persistence of T-cells in peripheral blood



AKTi, AKT inhibitor; SPEAR, specific peptide enhanced affinity receptor

- Maximum observed fold inductions of cytokines from pre-infusion indicate that ADP-A2M4CD8 SPEAR T-cells elicit (**Figure 7A**) pharmacodynamic effects at lower SPEAR T-cell doses relative to afami-cel. IL-12 is not known to be produced by T-cells. Increased serum IL-12 following ADP-A2M4CD8 infusion (**Figure 7B**) is consistent with engagement of the patient's antigen-presenting cells

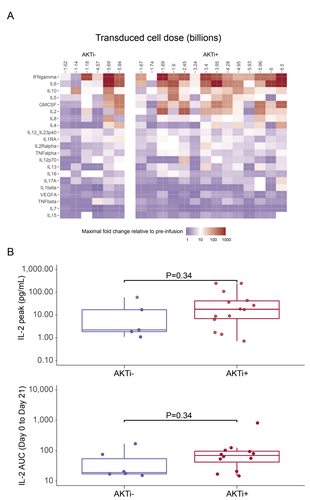
Figure 7. ADP-A2M4CD8 SPEAR T-cells show activity *in vivo*, including induction of host immune response (presented at ESMO 2021)



SPEAR, specific peptide enhanced affinity receptor
*Unpaired non-parametric Wilcoxon rank-sum test

- Serum cytokine responses were consistent among AKTi+ products (n=14) relative to AKTi- (n=6; **Figure 8A**). Induction of IL-2 reached a higher peak value (pg/ml) with AKTi+ but neither peak nor area under the curve reached statistical significance (**Figure 8B**)

Figure 8. ADP-A2M4CD8 cells manufactured with AKTi show similar or greater induction of host immune response to those with no AKTi



AKTi, AKT inhibitor; AUC, area under the curve

Conclusions

- Engineered T-cells targeting MAGE-A4 expressing tumors have been enhanced by co-expressing CD8α and adding AKTi during manufacture
  - Addition of CD8α to CD4+ SPEAR T-cells improved killing by CD4+ T-cells *in vitro*
  - Adding AKTi to the manufacturing process resulted in equivalent or better proliferation *ex vivo* with an increased proportion of the manufactured cells having a stem cell memory phenotype
  - scRNA-seq and functional analysis of research-grade afami-cel suggests AKTi can remodel gene expression programs towards better proliferation or better cytolytic potency
  - Manufacturing ADP-A2M4CD8 SPEAR T-cells with AKTi increases median persistence of T-cells in patients' peripheral blood and, in some patients, significant expansion is implied by higher peak recovery

E-poster 373: Society for Immunotherapy of Cancer (SITC), 10−14 November 2021
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Acknowledgements and Disclosures

We thank the patients and their caregivers for taking part in this trial, and we thank the investigators and their teams who participated in this work. This trial (NCT04044859) is sponsored by Adaptimmune. Editorial support and formatting assistance were provided by Gabrielle Knafler, MSc, PhD, of Excel Scientific Solutions, which was contracted and compensated by Adaptimmune for these services. All authors are employees of Adaptimmune and may hold stock/stock options in Adaptimmune.

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Abbreviations

AKTi, AKT inhibitor; AKTi+, T-cells manufactured with AKTi; AKTi-, T-cells manufactured without AKTi; PCR, polymerase chain reaction; scRNASeq, single-cell ribonucleic acid sequencing; MSD, Meso Scale Discovery; IL, interleukin; PD, progressive disease; PR, partial response; CR, complete response; SD, stable disease; IFN, interferon; RECIST, Response Evaluation Criteria in Solid Tumours; HLA, human leukocyte antigen; SPEAR, specific peptide enhanced affinity receptor; TCR, T-cell receptor; UMAP, Uniform Manifold Approximation and Projection