Enhanced Activity of Second-Generation MAGE-A4 SPEAR T-Cells Through Co-Expression of a CD8α Homodimer

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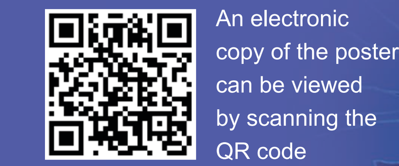

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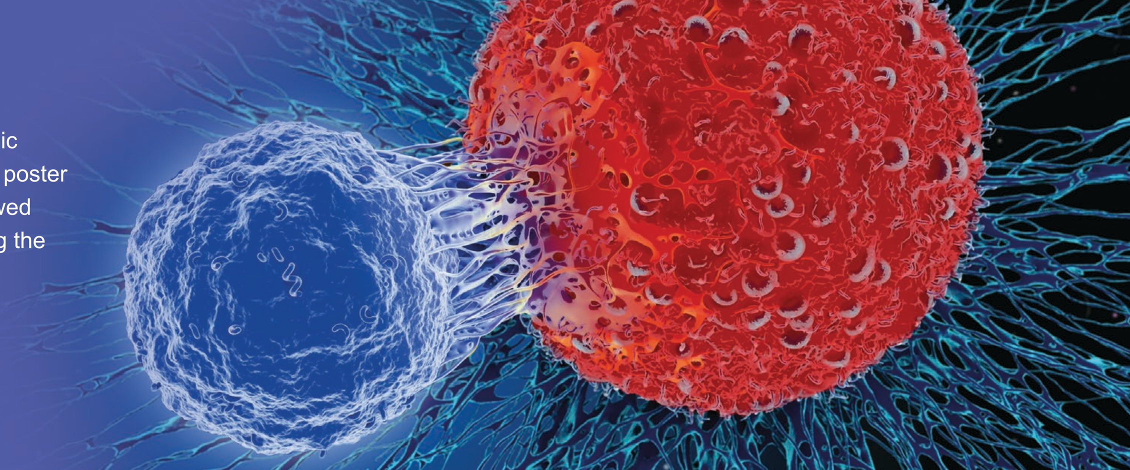
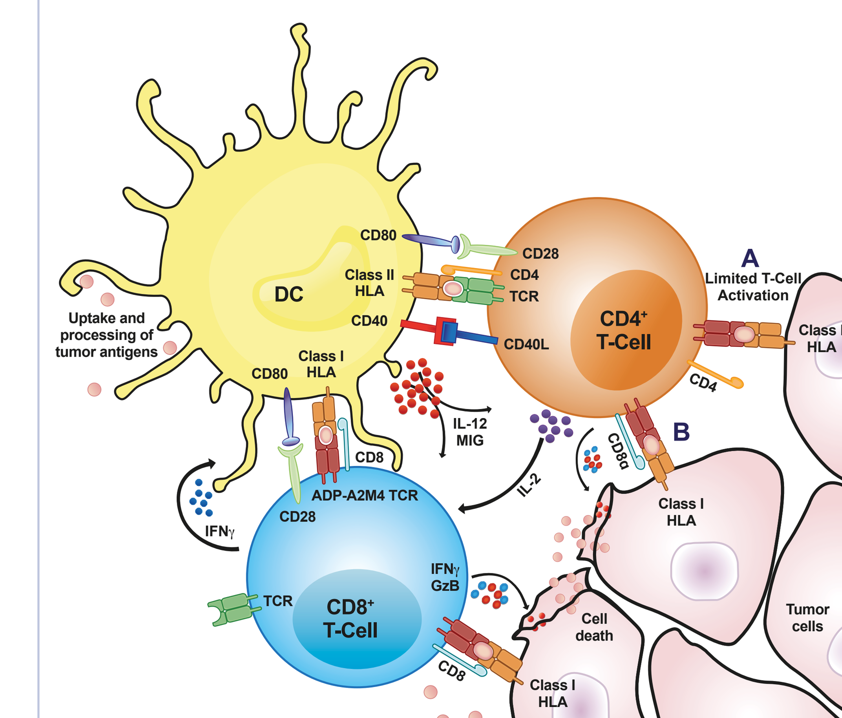


Introduction

- Affinity-enhanced TCRs have shown promise in the clinic against solid tumors such as sarcoma,[1] and a first-generation TCR targeting MAGE-A4 (ADP-A2M4; MAGE-A4^{c1032}) is being tested in a clinical trial against multiple solid tumor indications (NCT03132922)
- Next-generation strategies to enhance T-cell function may improve the depth and durability of anti-tumor responses
- The addition of a CD8α co-receptor into CD4⁺ T-cells alongside the engineered TCR (ADP-A2M4CD8) is anticipated to increase TCR binding avidity and enhance the polyfunctional response of CD4⁺ T-cells against tumor antigens[2]
- This approach is intended to widen the immune response to the tumor through DC activation and enhanced cytotoxicity (**Figure 1**)

Figure 1. Impact of next-generation T-cells on relevant immune cell interactions in the tumor microenvironment

- Tumor cells present intracellular antigens to T-cells via their class I HLA:peptide complex, which is recognized by the TCR (ADP-A2M4 TCR in red, endogenous TCR in green)
- CD4⁺ T-cells normally have limited functional activation through the interaction with HLA class I (**A**)
- In the next-generation ADP-A2M4CD8, the recruitment of an armed effector CD4⁺ T-cell becomes available through the HLA class I interaction (**B**), promoted by expression of the introduced CD8α co-receptors
- The engineered CD4⁺ T-cells can effectively kill tumor cells, as well as stimulate DCs to mature (eg, through CD40L/CD40 interaction), upregulate co-stimulatory molecules on the cell surface (eg, CD80), and induce IL-12 secretion
- These mechanisms in turn boost CD8⁺ T-cell activation, clonal expansion, and differentiation into effector and memory T-cells. ADP-A2M4CD8 may enhance tumor cytotoxicity via improved CD4⁺ T-cell effector and helper functions



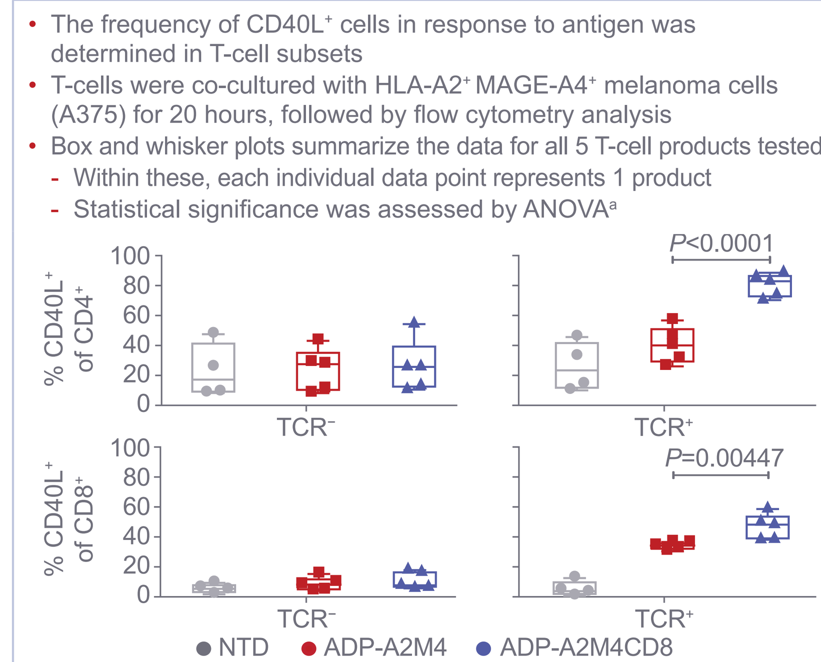
Objectives

- Investigate preclinical proof-of-concept of adding a CD8α homodimer to ADP-A2M4 SPEAR T-cells, focusing on CD4⁺ T-cell function, using *in vitro* assays
- Confirm that addition of the CD8α homodimer does not result in additional cross-reactivity of the ADP-A2M4 product through preclinical evaluations

Results

Figure 2. ADP-A2M4CD8 T-cells show enhanced upregulation of CD40L in response to antigen *in vitro*

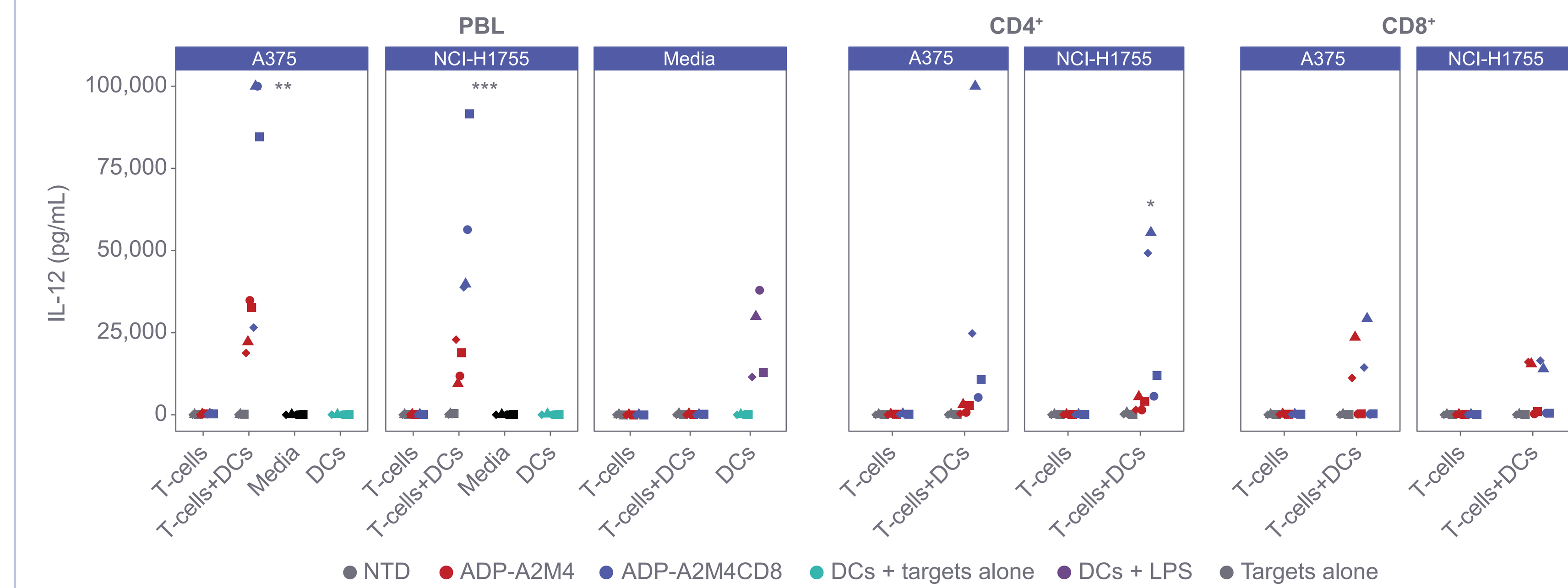
- The frequency of CD40L⁺ cells in response to antigen was determined in T-cell subsets
- T-cells were co-cultured with HLA-A2⁺ MAGE-A4⁺ melanoma cells (A375) for 20 hours, followed by flow cytometry analysis
- Box and whisker plots summarize the data for all 5 T-cell products tested
  - Within these, each individual data point represents 1 product
  - Statistical significance was assessed by ANOVA[a]



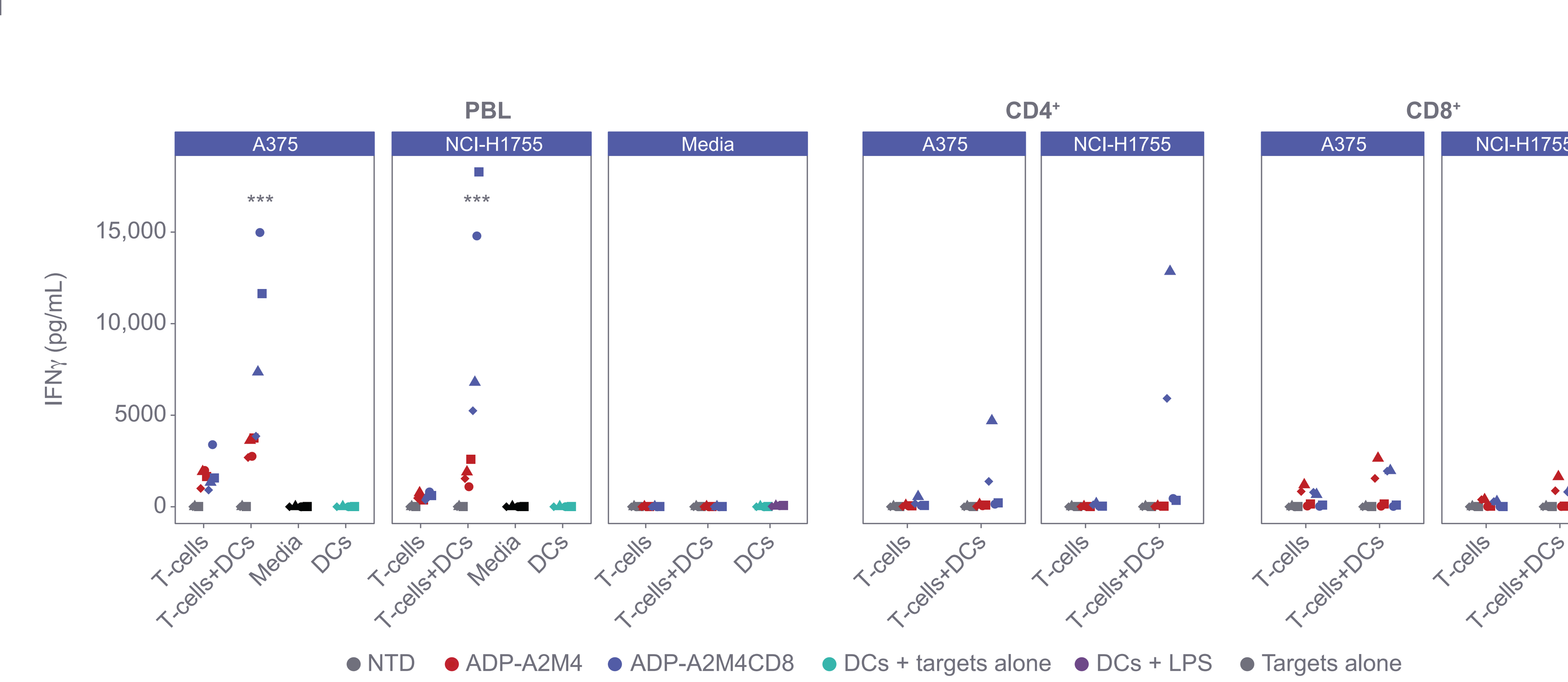
[a]Three-way repeated-measures ANOVA with subset (ie, CD4/8), TCR+/-, TCR transduction as within-subject factors followed by pairwise *post hoc* tests for each combination of transduction within a subset/TCR combination, and *P* values adjusted using the Holm method

Figure 3. Cytokine production is increased in 48-hour co-cultures of DCs, ADP-A2M4CD8 T-cells, and MAGE-A4⁺ cancer cell lines

A. DCs in co-culture with ADP-A2M4CD8 (blue) and MAGE-A4⁺ cancer cell lines (A375 or NCI-H1755) produce significantly more IL-12 than those in culture with first-generation ADP-A2M4 SPEAR T-cells (red); LPS was included as a positive control for DC activation. Left, center, and right panels show data from standard T-cell product (PBLs), or CD4⁺/CD8⁺ cells purified from the T-cell product. Each point is the mean of 2 replicates for 1 T-cell product (4 total), with each product indicated by a different shape. Statistical significance was assessed by ANOVA.[a] Only significant comparisons between ADP-A2M4 and ADP-A2M4CD8 conditions are shown



B. ADP-A2M4CD8 T-cells (blue) produce significantly more IFNγ than ADP-A2M4 (red) (also see **Figure 3A**)



[a]Three-way repeated-measures ANOVAs were run separately for each cytokine and positive-control target, with TCR transduction, T-cell fraction, and presence or absence of DCs as within-subject factors, followed by pairwise *post hoc* tests for each combination of transduction within a transduction/T-cell fraction/DC combination, and *P* values adjusted using the Holm method
*P<0.05, **P<0.01, ***P<0.005

Figure 4. DCs mature when co-cultured with ADP-A2M4CD8 in the presence of antigen-positive cells

- MAGE-A4⁺ A375 cells were co-cultured with immature DCs and PBLs, CD4⁺ or CD8⁺ T-cells for 48 hours, after which DCs were harvested for flow cytometry analyses
- Each point represents 1 of 3 or 4 T-cell products tested

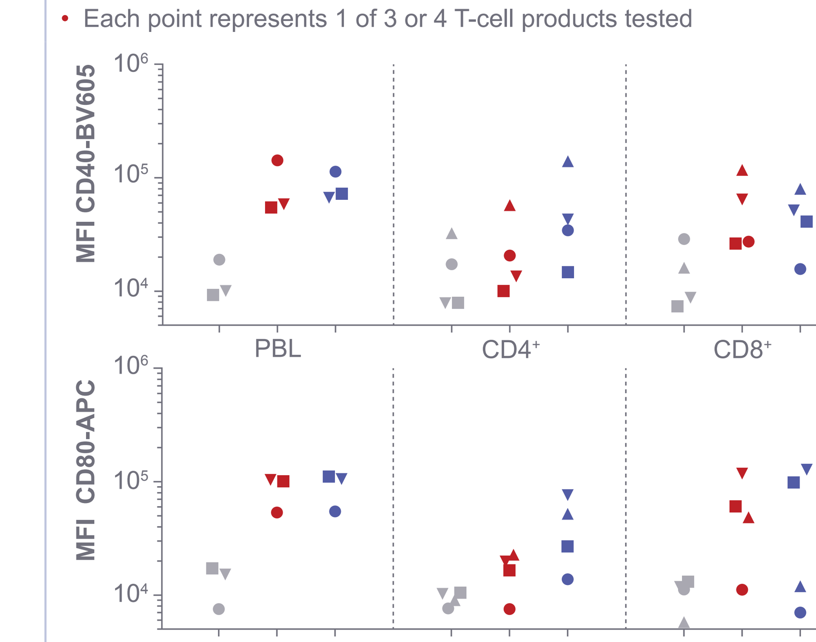
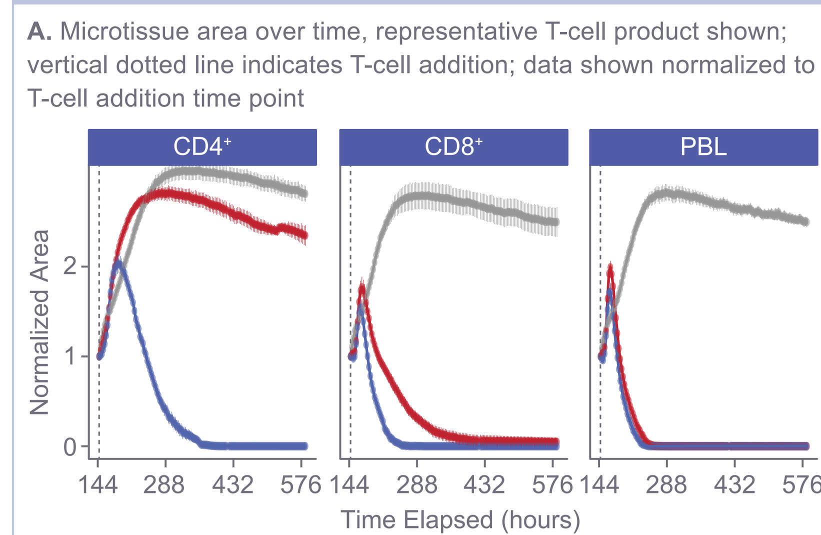
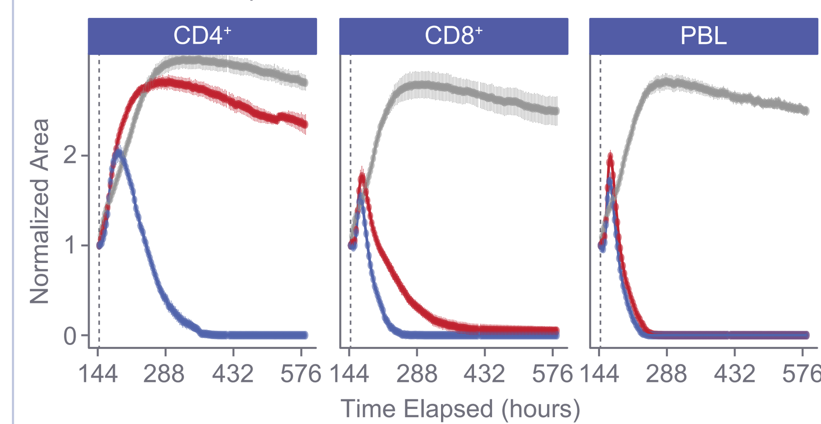
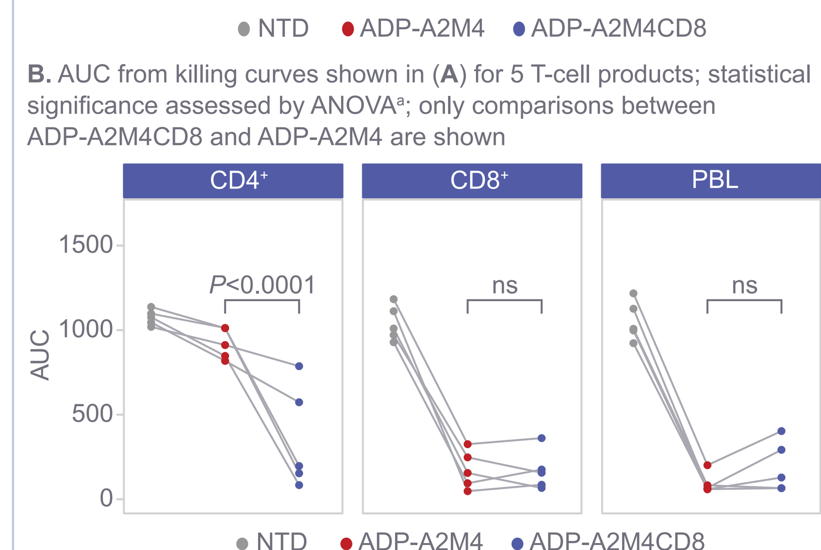
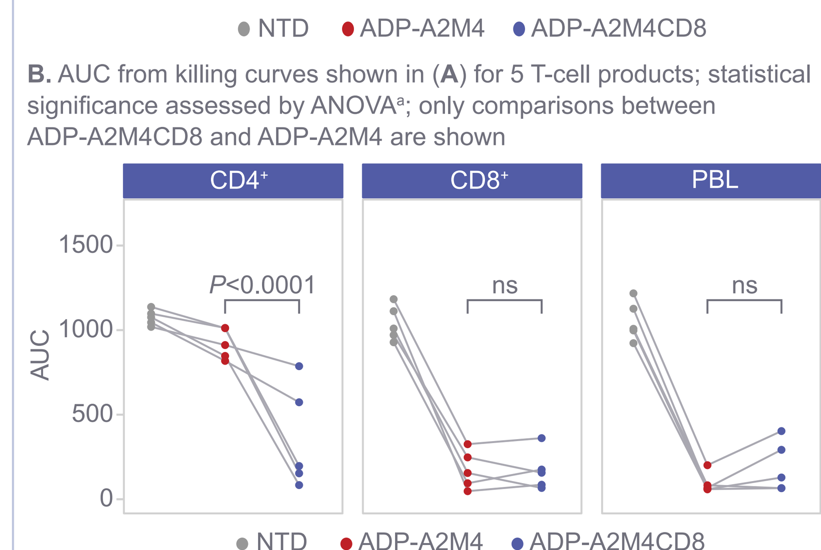


Figure 5. ADP-A2M4CD8 CD4⁺ T-cells show significantly improved killing of 3D microtissues compared to T-cells transduced with ADP-A2M4 TCR alone

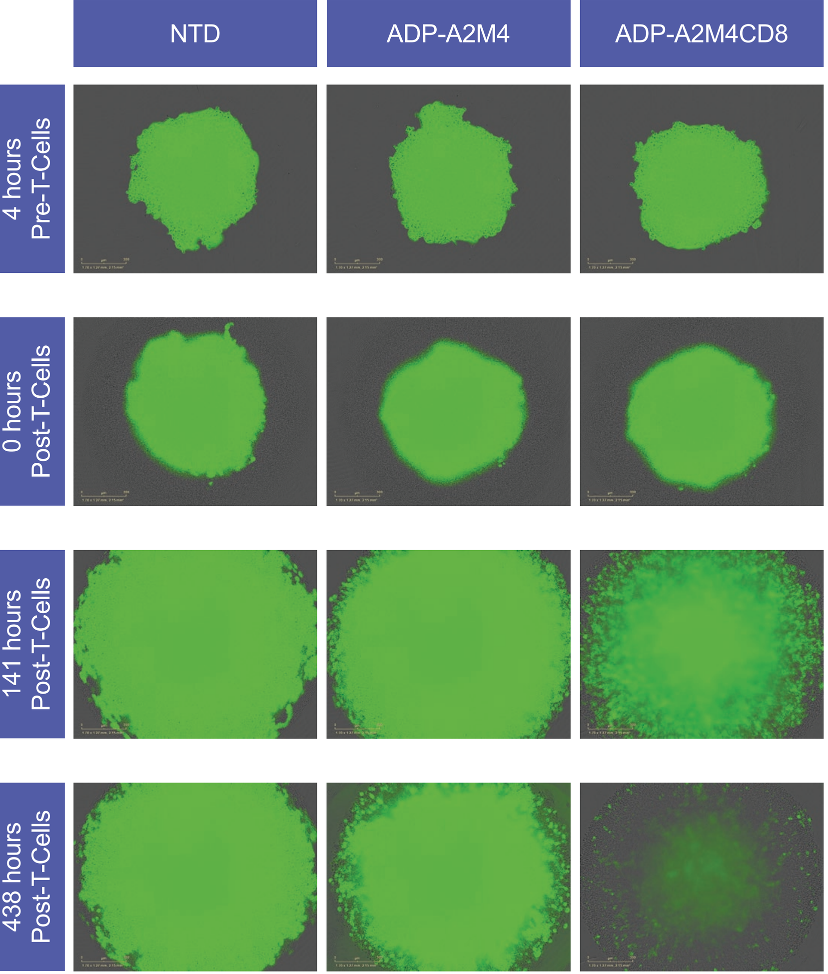
A. Microtissue area over time, representative T-cell product shown; vertical dotted line indicates T-cell addition; data shown normalized to T-cell addition time point

B. AUC from killing curves shown in (**A**) for 5 T-cell products; statistical significance by ANOVA[a]; only comparisons between ADP-A2M4CD8 and ADP-A2M4 are shown

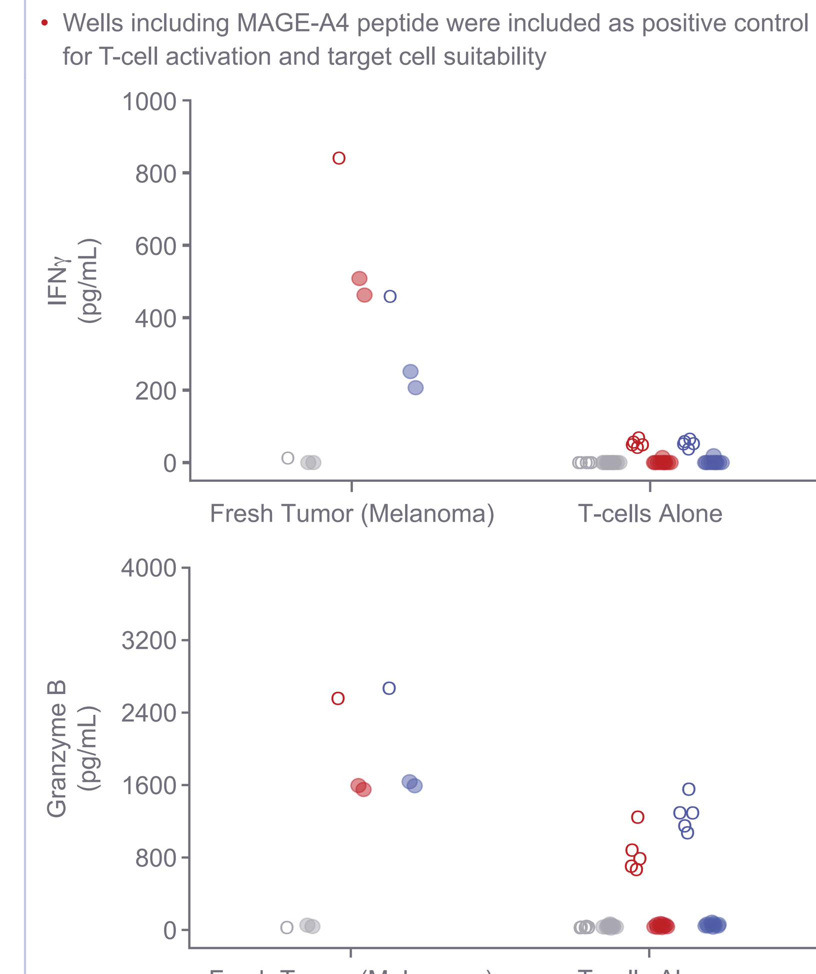
C. Images of A375-GFP microtissues after challenge with isolated CD4⁺ T-cells, representative of 5 T-cell products tested



[a]Repeated-measures ANOVA with T-cell fraction, TCR transduction and tissue size as within-subject factors followed by pairwise *post hoc* tests for each combination of transduction within a transduction/T-cell fraction/tissue size combination, and *P* values adjusted using the Holm method
ns=not significant

Figure 6. ADP-A2M4CD8 T-cells produce IFNγ and Granzyme B in response to MAGE-A4⁺ primary tumor material

- T-cells were co-cultured with fresh tumor for 48 hours, after which supernatants were harvested for ELISA analysis
- Wells including MAGE-A4 peptide were included as positive control for T-cell activation and target cell suitability



Open circles represent the corresponding product with peptide

Safety

- Fine peptide specificity of the ADP-A2M4 TCR has previously been mapped; all potential peptide hits were retested with ADP-A2M4CD8 (**Figure 7**)
- The ADP-A2M4 TCR was tested against 129 human primary cell lines, 18 primary blood products, and 19 human MAGE-A4⁻ tumor cell lines[3]
  - Previously identified low-level cross-reactivities with ADP-A2M4CD8, including melanocytes and small airway epithelia, were re-tested
  - A subset of primary normal cell lines were re-tested in 2D culture (**Figure 8**)
- ADP-A2M4CD8 T-cells showed no response to organotypic models of lung airway or skin (data not shown)

Figure 7. Addition of CD8α homodimer does not change the peptide specificity of the ADP-A2M4 TCR

- Scatter plot comparison of the EC_{50} response to select putative mimotypes and MAGE family homologous peptides for ADP-A2M4 and ADP-A2M4CD8
- Data are combined geometric mean from 3 T-cell products
  - Grey identity line indicates exact correlation, y=x
  - Dotted lines indicate 2 log shift from MAGE-$A4_{230\text{-}239}$ index peptide response

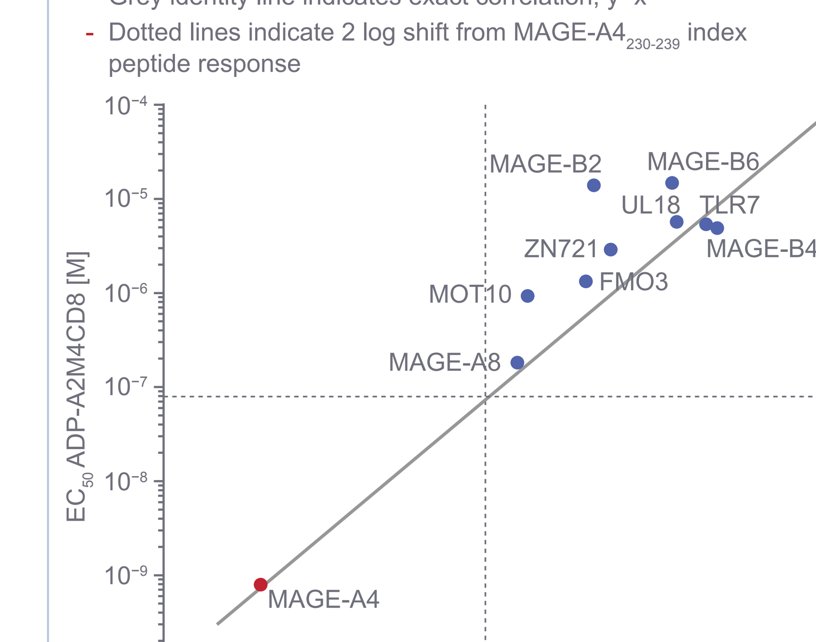
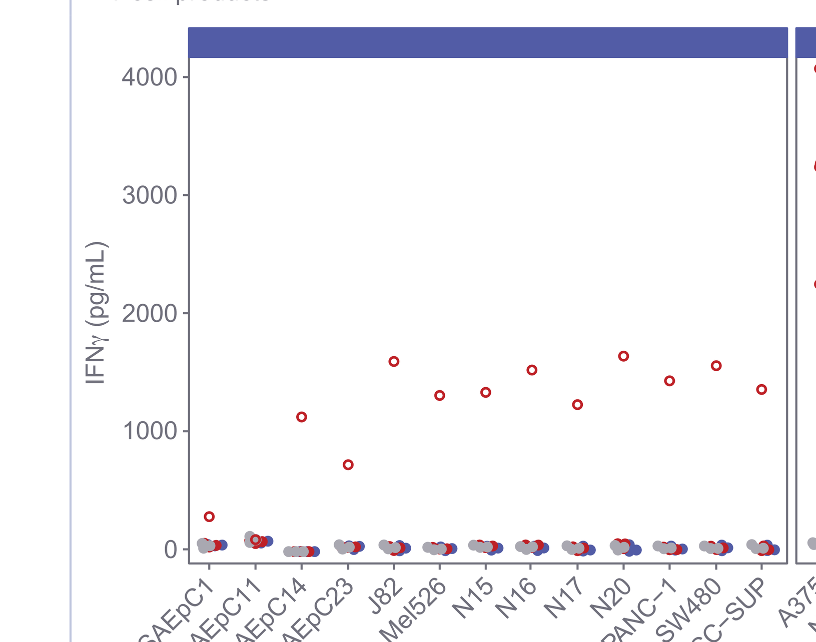


Figure 8. ADP-A2M4CD8 T-cells show no cross-reactivity *in vitro*

- Response of ADP-A2M4, ADP-A2M4CD8, and NTD T-cells to small airway epithelial cells (HSAEpC), melanocytes (N), or antigen-negative cancer cell lines (J82, Mel526, PANC-1, SW480, TCC-SUP) assessed by IFNγ cell-based ELISA
- Each data point represents the mean of duplicate assays for 1 of 3 T-cell products



Open circles represent the corresponding product with peptide

Conclusions

- These data illustrate improved engagement and function in CD4⁺ T-cells transduced with ADP-A2M4CD8, without additional off-target reactivity *in vitro*
  - Increased T-cell activation as illustrated by CD40L upregulation
  - Improved engagement with wider immune system as illustrated by improved cytokine release of both DCs and T-cells in co-culture with MAGE-A4⁺ cancer cell lines
  - Improved cytotoxic activity of CD4⁺ cells against 3D MAGE-A4⁺ tumor microtissues
  - No additional off-target reactivity, as tested by both peptide specificity and screening of selected primary "normal" cells
- Next-generation ADP-A2M4CD8 T-cells may improve long-term T-cell functions as well as immediate anti-tumor activity *in vivo*
  - Improved CD4⁺ function is expected to support clonal expansion of CD8⁺ T-cells, differentiation into effector and memory T-cells, as well as engage the wider immune system in anti-tumor response
  - Conversion of CD4⁺ cells from helper to cytotoxic function could potentially increase short-term anti-tumor activity

Abbreviations

ANOVA, analysis of covariance; **AUC,** area under the curve; **DC,** dendritic cell; **ELISA,** enzyme-linked immunosorbent assay; **HLA,** human leukocyte antigen; **IFN,** interferon; **IL,** interleukin; **LPS,** lipopolysaccharide; **MAGE-A,** melanoma-associated antigen-A; **MFI,** median fluorescent intensity; **NTD,** non-transduced; **PBL,** peripheral blood lymphocytes; **SPEAR,** specific peptide enhanced affinity receptor; **TCR,** T-cell receptor

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