Specific Peptide Enhanced Affinity Receptor (SPEAR) T-cells Targeting MAGE-A4

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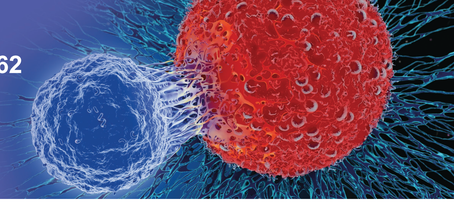


Introduction

- Melanoma-associated antigens-A (MAGE-A) are members of an extensive family of cancer/testis (CT) antigens
- MAGE-A4 is an attractive target for SPEAR T-cell therapy because it has a high frequency of expression in a broad spectrum of solid tumors according to The Cancer Genome Atlas[1]
- MAGE-A4^{c1032} SPEAR T-cells are autologous CD4$^+$ and CD8$^+$ T-cells genetically engineered to express an affinity-enhanced T-cell receptor (TCR) that recognizes the HLA-A2-restricted peptide MAGE-A4$_{230\text{-}239}$ (GVYDGREHTV)
- Here we describe the frequency of MAGE-A4 expression in non-small cell lung cancer (NSCLC) and the assessment of MAGE-A4^{c1032} T-cell potency and safety using our state-of-the-art *in vitro* preclinical safety package designed to establish TCR specificity, alloreactivity, and potency

Objectives

- Determine the frequency of MAGE-A4 expression in NSCLC to identify patients most likely to benefit from SPEAR T-cell therapy
- Evaluate the specificity, potency, and safety of MAGE-A4 SPEAR T-cells

Methods

NSCLC cohort selection

- A total of 534 resected NSCLC cases (stage I to IV) with clinicopathological information including overall survival and recurrence were analyzed for MAGE-A4 expression

MAGE-A4 immunohistochemistry (IHC) analysis

- IHC was performed using the MAGE-A4 mouse monoclonal (Clone: OTI1F9) antibody on formalin fixed paraffin embedded (FFPE) tissue microarrays (TMA)
  - TMAs included 3 separate cores of each resected NSCLC case
- All stained cores were evaluated and scored by a pathologist
  - Percentage of tumor cells expressing MAGE-A4 were captured at each intensity (0: null, 1+: weak, 2+: moderate, and 3+: strong)
  - H-score was calculated (1 × [% cells 1+] + 2 × [% cells 2+] + 3 × [% cells 3+]) for each core and averaged
  - Positive samples: intensity 1+ in ≥10% of tumor cells (H-score ≥10 is considered positive)

Preclinical evaluation of optimal-affinity MAGE-A4 SPEAR T-cells

- Potency/efficacy testing of the TCR-transduced T-cells by antigen-driven proliferation, cytokine release, and cytotoxicity assays
- *In vitro* testing against panels of primary normal cells from multiple organ systems in 2-D, 3-D, and induced pluripotent stem cell culture formats to identify cross-reactivities in more physiologically relevant cultures
- Molecular mapping of the TCR peptide-major histocompatibility complex (MHC) binding preferences to identify potential cross-reactive peptides, verification of identified peptides by loading candidates on antigen-presenting cells, and expression of source proteins in antigen-presenting cells to confirm lack of candidate peptide processing and presentation

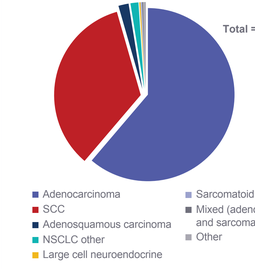
Results

NSCLC cohort clinicopathological details

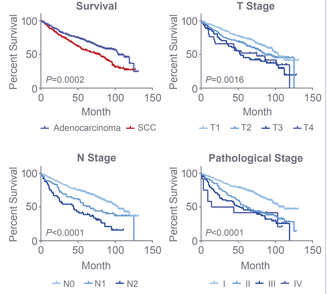
- Of the 534 resected NSCLC cases, the majority were either adenocarcinoma (61%) or squamous cell carcinoma (SCC) (34%) (**Figure 1A**)
- Histological diagnosis, T stage, N stage, and pathological stage were independent prognostic factors in the cohort (**Figure 1B**)

Figure 1. NSCLC cohort clinicopathological information

1A. Histological characterization of the NSCLC cohort



1B. Log-rank (Mantel-Cox) analysis by histological diagnosis, T stage, N stage, and pathological staging

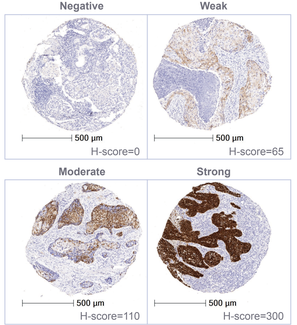


MAGE-A4 expression in NSCLC

- From initial IHC TMA screening of 534 NSCLC cases, 472 cases (88%) were successfully scored for MAGE-A4 (**Figure 2A**)
- MAGE-A4 expression was observed in 24% of all NSCLC cases, with higher frequency observed in SCC (51%) versus adenocarcinoma (8%) (**Figure 2B**)

Figure 2. MAGE-A4 expression screening in the NSCLC cohort

2A. TMA cores of MAGE-A4 cytonuclear IHC staining and corresponding H-score

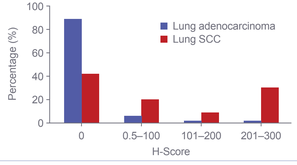


2B. MAGE-A4 expression frequency observed across the NSCLC cohort (positivity threshold for frequency H-score ≥10)

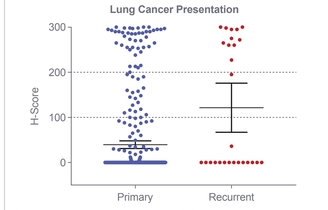
Tumor Type	MAGE-A4 Expression Frequency
NSCLC (all)	**24%** (114/472)
SCC	**51%** (83/162)
Adenocarcinoma	**8%** (22/289)

Figure 3. MAGE-A4 distribution of expression across the NSCLC cohort

3A. Percentage of negative (H-score=0), weak (H-score=0.5–100), moderate (H-score=101–200), and strong (H-score=201–300)



3B. Distribution of expression across primary and recurrent NSCLC cases

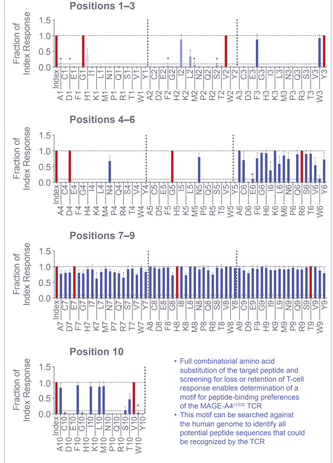


H-scores for primary and recurrent tumors assessed by IHC; individual samples represented by blue dots (primary; 23% positive) and red dots (recurrent; 48% positive)
Horizontal bars and whiskers represent mean (± 95% confidence interval); dashed horizontal lines represent cut-offs for weak (<100), moderate (>100 and <200), and strong staining (>200)

Specificity testing of the HLA-A*02-restricted TCR specific for MAGE-A4$_{230\text{-}239}$ peptide GVYDGREHTV (Figure 4A–C)

Figure 4. Specificity testing for MAGE-A4$_{230\text{-}239}$ peptide GVYDGREHTV

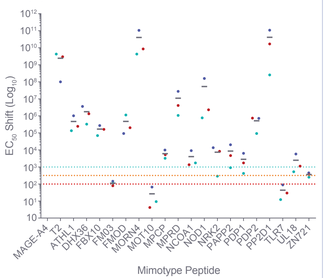
4A. Full X-scan results for MAGE-A4 SPEAR T-cells



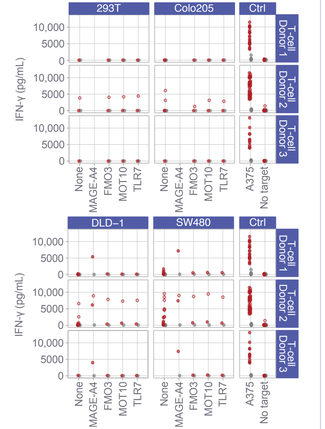
- Full combinatorial amino acid substitution of the target peptide and screening for loss or retention of T-cell response enables determination of a motif for peptide-binding preferences of the MAGE-A4^{c1032} TCR
- This motif can be searched against the human genome to identify all potential peptide sequences that could be recognized by the TCR

Data are presented as the mean ± SEM response relative to the IFN-γ ELISpot response to the index peptide at 10^{-5}M (red bars)
*Marginal positives (residues that were tolerated in ≥1 repeat, but whose average response did not reach cut-off over all repeats)

4B. Only 3 identified peptides were found to stimulate MAGE-A4^{c1032} SPEAR T-cells with a potency less than 100× weaker than the index peptide



4C. The peptides identified in **Figure 4B** were not able to stimulate MAGE-A4^{c1032} SPEAR T-cells after proteins containing the identified peptide sequences were overexpressed in tumor cell lines. The MAGE-A4^{c1032} SPEAR T-cells did not elicit IFN-γ responses, as assessed by ELISA, indicating the transfected proteins are not natively processed and presented

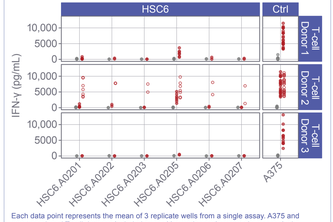


Grey circles: non-transduced T-cells; red circles: MAGE-A4^{c1032} SPEAR T-cells; open circles: MAGE-A4 peptide added as positive control

Human cell testing

- MAGE-A4^{c1032} SPEAR T-cells were screened against a wide panel of normal primary cells from multiple organ systems, induced pluripotent stem cell-derived cells (iCell®), and autologous whole blood to test for off-target reactivity, and against a panel of EBV-derived B-lymphoblastic cell lines expressing a wide range of HLA molecules to assess the risk of alloreactivity (**Figure 5**)
- Alloreactivity was observed in antigen-negative cells expressing HLA-A*02:05, and therefore this allele is exclusionary

Figure 5. Response of MAGE-A4^{c1032} SPEAR T-cells (red circles) and non-transduced T-cells (grey circles) to Schwann cells transduced with common HLA-A2 alleles or non-transduced Schwann cells



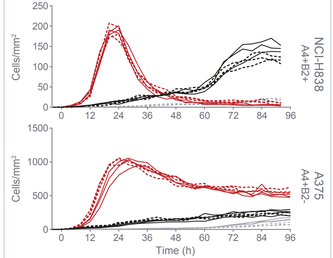
Each data point represents the mean of 3 replicate wells from a single assay. A375 and non-transduced T-cell data are included as positive and negative controls, respectively; MAGE-A4 peptide as positive control (open circles)

MAGE-A4^{c1032} SPEAR T-cell potency testing

- MAGE-A4^{c1032} SPEAR T-cell potency was assessed by a variety of *in vitro* assays, including proliferation, IFN-γ release and cytotoxicity in response to antigen-positive tumor lines in 2-D and 3-D culture, and cytokine release in response to freshly prepared antigen-positive primary tumor material (**Figure 6**)

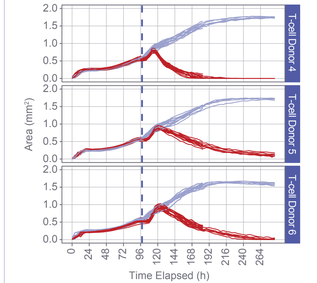
Figure 6. MAGE-A4^{c1032} SPEAR T-cell cytotoxicity *in vitro*

6A. Cytotoxicity of antigen-positive tumor lines by MAGE-A4^{c1032} SPEAR T-cells by time-lapse microscopy



Plots represent the time course of cell death after addition of MAGE-A4^{c1032} T-cells (red lines) or non-transduced T-cells (black lines); certain wells also included 10^{-6}M MAGE-A4 peptide (dashed lines). Background wells without T-cells (grey lines). Dashed lines represent addition of exogenous peptide. Cell death is measured by caspase-activation by Incucyte™ (Essen Biosciences)

6B. Cytotoxicity of 3-D microtissues derived from MAGE-A4$^+$ melanoma cell line A375, as determined by time-lapse imaging of GFP-labeled cells



Plots represent microtissue areas before and after addition (vertical dashed line) of MAGE-A4^{c1032} T-cells (red lines) or non-transduced controls (blue lines)

Conclusions

- MAGE-A4 is a promising target for SPEAR T-cell therapy
- We have performed an extensive *in vitro* preclinical safety assessment and identified no major safety concerns for MAGE-A4 SPEAR T-cell reactivity
- Patients with HLA-A*02:05 are excluded for potential alloreactivity, due to findings of IFN-γ activity by some MAGE-A4 negative HLA-A*02:05 positive cells *in vitro*
- A clinical trial opened this year to treat patients with inoperable or metastatic (advanced) NSCLC (SCC, adenosquamous, or large cell carcinoma); ovarian cancer; head and neck SCC; gastric or esophageal cancer (SCC or adenocarcinoma); urothelial tumors; and melanoma (NCT03132922; https://clinicaltrials.gov/ct2/show/NCT03132922)

Reference

1. The Cancer Genome Atlas, Version 2. https://cancergenome.nih.gov/. Accessed Nov 23, 2017

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Disclosures

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