Identifying MAGE-A4–Positive Tumors for SPEAR T-Cell Therapies in HLA-A*02–Eligible Patients

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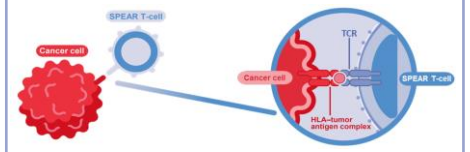
Introduction

- Autologous T-cells engineered with T-cell receptors (TCRs) targeting tumor antigens are promising therapies for metastatic solid cancers.[1-3]
- Human leukocyte antigen (HLA) molecules play a central role in TCR T-cell therapies by presenting tumor antigens to T-cells, thereby activating the immune response.
- Melanoma-associated antigen A4 (MAGE-A4) is a cancer testis antigen expressed in several solid tumors.[4]
- Specific peptide enhanced affinity receptor (SPEAR) TCR T-cell therapies (**Figure 1**) targeting MAGE-A4 in HLA-A*02–eligible patients have shown responses across multiple different cancer types.[2,3]

Figure 1. Specific Peptide Enhanced Affinity Receptor T-Cells



- T-cells scan HLA peptides presented on diseased cells, including tumor cells.
- TCRs targeting peptide antigens bind and activate the T-cell.
- Natural TCRs can target both intra- and extracellular antigens.
- Using TCRs engineered to recognize and bind to specific cancer peptides, SPEAR T-cells can target solid tumors.

HLA, human leukocyte antigen; SPEAR, specific peptide enhanced affinity receptor; TCR, T-cell receptor.

- Here we present data from a multinational, multicenter, screening study (NCT02636855) that prospectively evaluated HLA subtypes and MAGE-A4 profiles to determine patients' eligibility to enroll in clinical trials assessing the safety and efficacy of SPEAR T-cell therapy targeting MAGE-A4 in HLA-A*02–eligible patients with metastatic solid cancers.
- The screening study protocol follows a two-step testing process:
  - HLA typing via a high-resolution (allelic, 4-digit), sequence-based assay. Patients with HLA-A*02:01P, 02:02P, 02:03P, and 02:06P were eligible, and those with A*02:05P were excluded.[5] All other HLA-A*02 alleles were considered neutral. To be eligible, a patient must present at least one inclusion allele and not present the exclusion allele.
    - Blood samples were used as the source of DNA.
    - A Sanger sequencing-based typing assay was used at a central lab (American Red Cross, Philadelphia, PA).
    - Our abstract[6] reported eligibility results based on HLA criteria used at the time of testing, and those criteria were subsequently amended.
    - Here we present results based on the patients who satisfied the amended HLA criteria listed above.
  - MAGE-A4 testing of tumor samples (either an archived formalin-fixed, paraffin-embedded specimen or a fresh biopsy) in HLA-eligible patients via an immunohistochemical clinical trial assay with nuclear/cytoplasmic staining at 0, 1+, 2+, 3+ intensity.
    - MAGE-A4 expression level was defined by protein score (P score; % of cells staining at 2+, 3+).
    - Tumor samples with P score ≥30 (%) were considered MAGE-A4 positive (MAGE-A4+), which is the current cut-off[a] used to determine eligibility in our clinical trials. H-score is assessed as part of our translational research but is not used to determine eligibility, therefore it is not included with the screening protocol data presented herein.
    - Both the MAGE-A4+ rate (%) and MAGE-A4 expression level (P score, median) are reported here.

HLA

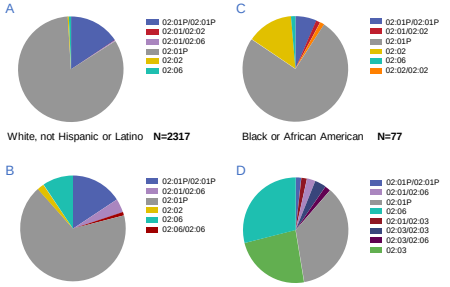
- Data from the US National Marrow Donor Program show that the percentage of individuals expressing different common HLA-A*02 alleles varies greatly among racial and ethnic backgrounds.[7,8]
  - A*02:01P is generally the most frequent allele, but its frequency is higher in White populations and lower in Asian and Black or African American populations.[7,8]
- A total of 6167 patients had their HLA-A type accurately determined; among them, 2729 (44.3%) were eligible based on the screening protocol criteria.
  - Patients who had both an inclusion allele and A*02:05P were ineligible (n=29; 0.47% of patients screened).
- Eligibility rate was different between races and ethnicities (**Table 1**).

Table 1. HLA-A Screening Results by Race and Ethnicity

	Race/ Ethnicity	Screened (N)	Eligible (N)	Screened (%)	Eligible (%)
Overall United States	White, not Hispanic or Latino	4954	2317	80.33	46.8
	White, Hispanic or Latino	228	96	3.70	41.9
	White, not specified	20	7	0.32	35.0
	Black or African American	293	77	4.75	26.3
	Asian	408	114	6.62	27.9
	American Indian or Alaska Native	19	7	0.31	36.8
	Native Hawaiian or Pacific Islander	10	1	0.16	10.0
	Not recorded	16	8	0.26	50.0
	Other	219	102	3.55	46.6
	Total	**6167**	**2729**	**100.00**	**44.3**
	White, not Hispanic or Latino	3733	1797	77.08	48.1
	White, Hispanic or Latino	213	92	4.40	43.2
	White, not specified	20	7	0.41	35.0
	Black or African American	280	72	5.78	25.7
	Asian	358	96	7.39	26.8
	American Indian or Alaska Native	17	7	0.35	41.2
	Native Hawaiian or Pacific Islander	10	1	0.21	10.0
	Not recorded	11	7	0.23	63.6
	Other	201	93	4.15	46.3
	Total	**4843**	**2172**	**100.00**	**44.8**

- While a higher percentage of White patients were eligible due to HLA-A*02:01P, the inclusion alleles A*02:02P, 02:03P, and 02:06P considerably increased the proportion of eligible patients in the Hispanic or Latino, Black or African American, and Asian populations (**Figure 2**).
  - The percentages of patients eligible due to the expression of at least 1 of these 3 alleles, without also expressing A*02:01P, were: 11.6% of Hispanic or Latino patients, 16.9% of Black or African American patients, and 57.9% of Asian patients.

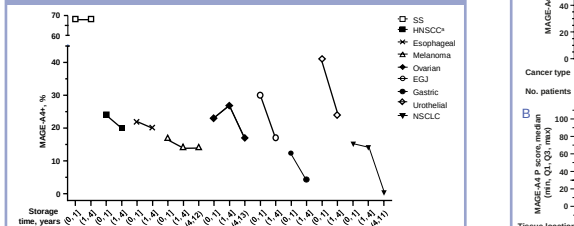
Figure 2. Distribution of HLA-A Alleles by Race and Ethnicity



MAGE-A4

- In the 2729 HLA-eligible patients, 1543 had tumor samples evaluable for MAGE-A4 across sites in the US, Canada, and Spain; among these, 313 patients were MAGE-A4+.
- Most tumor samples (93%) were collected within 4 years of testing.
- Overall, MAGE-A4+ rate remained stable within 4 years of tissue archival (**Figure 3**). Effect of storage time beyond 4 years on MAGE-A4 detection cannot be concluded due to small sample size and other variables.
- In some indications (eg, non-small cell lung cancer [NSCLC]), MAGE-A4+ rate decreased sharply in samples collected more than 4 years before testing (**Figure 3**), which may reflect differences in tumor biology (eg, histology), small sample size, or compromised MAGE-A4 stability in these indications.

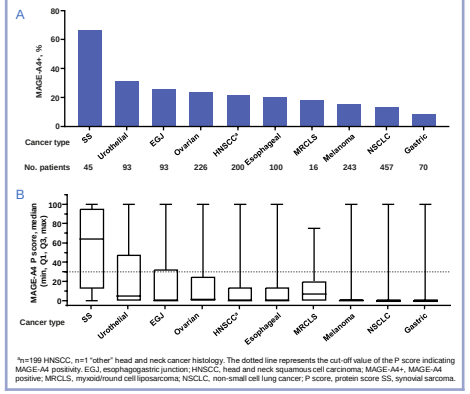
Figure 3. Effect of Formalin-Fixed, Paraffin-Embedded Tumor Sample Storage Time on MAGE-A4 Positivity Across Cancer Types



[a]n=199 HNSCC, n=1 "other" head and neck cancer histology. [b]Defined as SCC/(AC+SCC). AC, adenocarcinoma; EGJ, esophagogastric junction cancer; HNSCC, head and neck squamous cell carcinoma; MAGE-A4+, MAGE-A4 positive; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; SS, synovial sarcoma.

- The MAGE-A4+ rate (%) was highest in synovial sarcoma (SS) and lowest in gastric cancer (**Figure 4A**). MAGE-A4 expression level (P score) was highest, on average, in SS and lowest in melanoma, NSCLC, and gastric cancer (**Figure 4B**).

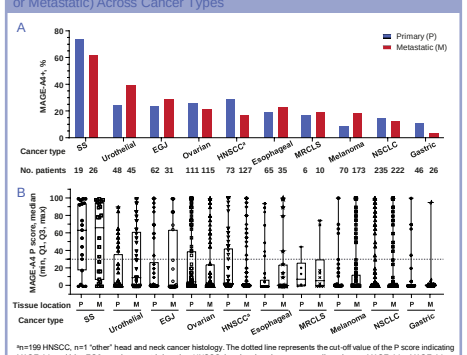
Figure 4. MAGE-A4 Positivity and Expression by Cancer Type



[a]n=199 HNSCC, n=1 "other" head and neck cancer histology. The dotted line represents the cut-off value of the P score indicating MAGE-A4 positivity. EGJ, esophagogastric junction; HNSCC, head and neck squamous cell carcinoma; MAGE-A4+, MAGE-A4 positive; MRCLS, myxoid/round cell liposarcoma; NSCLC, non-small cell lung cancer; P score, protein score SS, synovial sarcoma.

- MAGE-A4+ rate (%) was higher in (non-paired) primary vs metastatic samples in gastric cancer and head and neck squamous cell carcinoma[b] (HNSCC), but the reverse was observed in melanoma and urothelial cancer (**Figure 5A**).
- MAGE-A4+ rate (%) and MAGE-A4 expression level (P score) were similar between primary and metastatic samples in SS, esophageal cancer, esophagogastric junction (EGJ) cancer, myxoid/round cell liposarcoma, NSCLC, and ovarian cancer, whereas melanoma metastatic samples showed higher MAGE-A4 expression level than primary samples (P=0.02) (**Figure 5**).

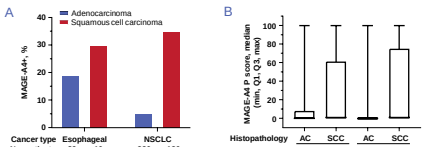
Figure 5. MAGE-A4 Positivity and Expression by Tissue Location (Primary or Metastatic) Across Cancer Types



[a]n=199 HNSCC, n=1 "other" head and neck cancer histology. The dotted line represents the cut-off value of the P score indicating MAGE-A4 positivity. EGJ, esophagogastric junction; HNSCC, head and neck squamous cell carcinoma; MAGE-A4+, MAGE-A4 positive; M, metastatic; MRCLS, myxoid/round cell liposarcoma; NSCLC, non-small cell lung cancer; P, primary; P score, protein score; SS, synovial sarcoma.

- In both esophageal cancer and NSCLC, MAGE-A4+ rate (%) was higher in squamous cell carcinoma samples, compared with adenocarcinoma samples. MAGE-A4 expression level (P score) was also higher in squamous cell carcinoma samples (**Figure 6**).

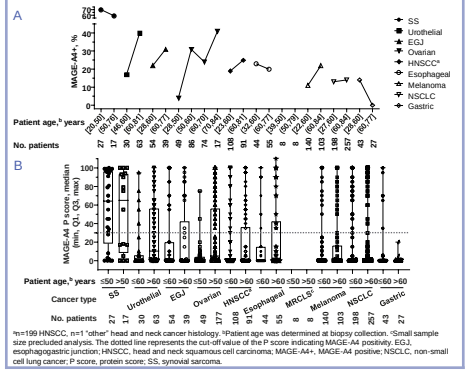
Figure 6. MAGE-A4 Positivity and Expression by Histopathology



AC, adenocarcinoma; MAGE-A4+, MAGE-A4 positive; NSCLC, non-small cell lung cancer; P score, protein score; SCC, squamous cell carcinoma.

- MAGE-A4+ rate (%) was not correlated with patient age in esophageal cancer, EGJ cancer, HNSCC, NSCLC, or SS.
- A negative correlation of MAGE-A4 positivity with patient age was observed in gastric cancer, while a positive correlation was observed in melanoma, ovarian cancer, and urothelial cancer (**Figure 7**). Further confirmatory studies may be warranted to investigate potential confounding factors.

Figure 7. Effect of Patient Age on MAGE-A4 Positivity and Expression Across Cancer Types



[a]n=199 HNSCC, n=1 "other" head and neck cancer histology. [b]Patient age was determined at biopsy collection. [c]Small sample size precluded analysis. The dotted line represents the cut-off value of the P score indicating MAGE-A4 positivity. EGJ, esophagogastric junction; HNSCC, head and neck squamous cell carcinoma; MAGE-A4+, MAGE-A4 positive; NSCLC, non-small cell lung cancer; P score, protein score; SS, synovial sarcoma.

Conclusions

- HLA-A genotype and MAGE-A4 tumor expression are key biomarkers determining SPEAR T-cell therapy eligibility, which may be affected by demographics, cancer type, histopathology, tissue location, and duration of tissue storage:
  - Inclusion of A*02:02P, A*02:03P, and A*02:06P increased the proportion of eligible patients across Asian, Hispanic or Latino, and Black or African American populations.
  - MAGE-A4 expression may be reliably assessed in fresh biopsy or archival tissues (up to 4 years old). The effect of storage time on MAGE-A4 expression beyond 4 years needs further investigation.
  - MAGE-A4+ rate and expression level were highest in SS and lowest in melanoma, NSCLC, and gastric cancer.
  - MAGE-A4 expression level varied widely, with most indications having samples with P scores up to 100.
  - MAGE-A4+ rate was higher in primary vs metastatic samples in gastric cancer and HNSCC, whereas the reverse was observed in melanoma and urothelial cancer.
  - MAGE-A4+ rate and expression level were higher in squamous cell carcinoma than in adenocarcinoma.

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Footnotes and abbreviations used in text

[a]P score was initially defined as % cell staining at ≥1+ with 10% cut-off for patient screening/enrollment but was later changed to the definition presented here upon which analyses of MAGE-A4+ are based. [b]n=199 HNSCC, n=1 "other" head and neck cancer histology. EGJ, esophagogastric junction; HLA, human leukocyte antigen; HNSCC, head and neck squamous cell carcinoma; MAGE-A4, melanoma-associated antigen A4; MAGE-A4+, MAGE-A4 positive; NSCLC, non-small cell lung cancer; P score, protein score; SPEAR, specific peptide enhanced affinity receptor; SS, synovial sarcoma; TCR, T-cell receptor.

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- **Tianjiao Wang (email – Tianjiao.Wang@adaptimmune.com):** Employee of Adaptimmune and holds stock/stock options in Adaptimmune.