SUPPLEMENTAL MATERIAL

Phase I clinical trial evaluating the safety and efficacy of ADP-A2M10 SPEAR T cells in patients with MAGE-A10+ advanced non-small cell lung cancer

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HLA and MAGE-A10 testing

Patients had to be positive for HLA-A*02:01 and/or HLA-A*02:06 alleles and negative for HLA-A*02:05, HLA-B*15:01, and/or HLA-B*46:01 as determined by central testing by the Histocompatibility and Molecular Genetics Laboratory, American Red Cross (Philadelphia, Pennsylvania, USA). In addition, tumor (either an archival specimen or a fresh biopsy) was pathologically reviewed and MAGE-A10 IHC staining performed by QualTek Molecular Laboratories (Goleta, California, USA). Using an analytically validated and Clinical Laboratory Improvement Amendments–certified MAGE-A10 clinical trial assay, IHC positivity was determined by a pathologist on the basis of both percentage of positive tumor cells and intensity of expression, as determined by P-score of IHC staining. A P-score ≥10% of tumor cells that were ≥1^+ intensity by IHC was used to define positivity. During the trial, the expression threshold was changed to ≥30% of tumor cells that were 2^+ and/or 3^+ intensity by IHC. Tumor MAGE-A10 expression for eligibility was derived by calculating the P-score and presented for translational correlative studies using the H-score. The H-score was derived from the P-score by 1 × (% of 1+ cells) + 2 × (% of 2+ cells) + 3 × (% of 3+ cells). Tumor biopsies of all treated patients met the expression requirements at the time of screening.

T-cell manufacturing

ADP-A2M10 were manufactured at Minaris Regenerative Medicine, LLC (formerly Hitachi Chemical Advanced Therapeutics Solutions, LLC; Allendale, New Jersey, USA) using vectors manufactured and supplied by City of Hope (Duarte, California, USA), Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio, USA), or Lentigen Technologies Inc. (Gaithersburg, Maryland, USA). Final cell product release was contingent on several different specifications including $CD3^+$ ≥80% of cells, ≥10% transduced cells, vector copy number ≤5.0, and cell viability

≥70%. The final ADP-A2M10 product was cryopreserved in 5% dimethyl sulfoxide and thawed prior to intravenous administration.

Gene therapy–related delayed AEs, molecular replication–competent lentivirus testing, and insertional oncogenesis

Gene therapy–related delayed AEs included: new malignancies, new incidence or exacerbation of a pre-existing neurological disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new hematologic disorder, opportunistic or serious infections, or unanticipated illness and/or hospitalization deemed related to gene-modified cell therapy. The gene therapy–related delayed AEs, molecular replication–competent lentivirus testing (qPCR for the vesicular stomatitis virus-G DNA sequence), and, if necessary, insertional oncogenesis were assessed at 3, 6, and 12 months during year 1, then every 6 months from year 2 to year 5, then annually up to year 15.[1-3]

Translational studies

RNAish

RNAish for ADP-A2M10 TCR was performed using the RNAscope2.5 LS Red kit and RNAscope probes specific to ADP-A2M10 TCR according to the manufacturer's instructions. Briefly, 4-µm formalin-fixed, paraffin-embedded tissue sections were pre-treated with heat and protease prior to hybridization with the target oligo probes. Pre-amplifier, amplifier, and AP-labeled oligos were then hybridized sequentially. RNAish assay was followed by CD3 chromogenic precipitate IHC (anti-CD3 [2GV6], rabbit monoclonal primary antibody; Roche Diagnostics, Indianapolis, Indiana, USA) using the DISCOVERY Teal HRP detection kit (Roche Diagnostics). Each sample was quality controlled for RNA integrity with an RNAscope probe specific to PPIB RNA (Hs-PPIB; Advanced Cell Diagnostics, Newark, California). Specific RNA staining signal was identified as red punctate dots and CD3 by the Teal signal. Samples were counterstained with Hematoxylin. The whole slides were scanned using AxioScan.Z1

microscope slide scanner and were analyzed using HALO image analysis software (Indica Labs, Albuquerque, New Mexico, USA). Images were annotated and analyzed using Indica Labs—ISH v3.3.9 algorithm with CD3 staining as the nuclear stain, resulting in CD3 positive cell count and ADP-A2M10 TCR percentage positivity.

CD3, MHCI, and PD-L1 IHC

CD3 and MHCI levels were detected using 2GV6 rabbit monoclonal antibody (Ventana) and EMR8-5 mouse monoclonal antibody, respectively. PD-L1 expression was determined using PD-L1 IHC 22C3 pharmDx (Agilent Technologies, Santa Clara, California, USA). Tumor proportion score and combined positive score for PD-L1 expression were assessed as recommended by the manufacturer.

HLA-A*02:01 LOH

HLA-A*0201 LOH assessment was performed using whole exome sequencing data generated from tumor and matched normal samples. DNA extraction from tumor samples, DNA library preparation, and sequencing were done at Q squared solutions (Morrisville, North Carolina, USA). Tumor tissue was macro-dissected from 5-µm sections of formalin-fixed, paraffin-embedded tissues and genomic DNA was extracted using the AllPrep DNA/RNA Kit (Qiagen, Hilden, Germany). Matched normal DNA was isolated from PBMC at BioAgilytix (Boston, Massachusetts, USA) using QIAamp DNA Blood Mini Kit (Qiagen). DNA libraries were generated using SureSelect V6 Exome kit (Agilent Technologies) and run on Illumina platform sequencer with paired-end 100 base pairs reads with average coverage of 100X and 200X for normal and tumor respectively. DNA extraction from tumor samples, DNA library preparation, and sequencing were done at Q squared solutions (Morrisville). Fastq files were aligned using Sentieon bioinformatic tools (https://www.sentieon.com/). LOH was determined using VarScan2 somatic caller utilized within the ArrayStudio toolset using default parameters. In addition, OptiType was used to determine the number of reads mapping to each predicted allele of HLA-

A for both tumor and matched normal samples.[4] Visual inspection of predicted LOH was done using Integrated Genomic Viewer.[5]

Transcriptomic analysis

Transcriptomic analyses were performed at CellCarta (Antwerp, Belgium) as previously described,[6] with the following modifications:

Background gene count levels were determined for each sample using the mean plus two standard deviations of the included negative control counts. For data normalization, background-subtracted counts were then scaled using geometric means of 27 housekeeping genes shared between the two gene panels. The following gene signatures were used: APM,[7] $CD3^+$ T cells, $CD8^+$ T cells[8] (NanoString Technologies, Seattle, Washington, USA), and T-cell exhaustion.[9] The heatmap represents scores standardized per gene. The scores were generated as number of standard deviations away from the mean (z-score).

SUPPLEMENTAL RESULTS

Patient characteristics

A total of 2810 patients with NSCLC were HLA screened under the APD-0000-001, A Screening Protocol to Determine Tumor Antigen Expression and HLA Sub-Type for Eligibility Determination for Clinical Trials Evaluating the Safety and Efficacy of Autologous T Cells Expressing Enhanced TCRs in Subjects with Solid or Hematological Malignancies (NCT02636855). Of those, 1127 patients including 884 with AC, 175 with SCC, 12 with adenosquamous, and 56 with other histologieshad HLA alleles that matched the eligibility criteria for ADP-A2M10. Of those, 736 patients (577 AC, 113 SCC, 10 adenosquamous, and 36 other) were screened for the MAGE-A10 antigen and 78 patients (48 AC, 24 SCC, two adenosquamous, and four other) had MAGE-$A10^+$ tumors (supplemental table 1). Twenty-seven patients with the appropriate HLA phenotype and tumor MAGE-A10 expression met all other eligibility characteristics, signed informed consent, and underwent leukaphereses. From July

2017 to December 2019, 11 patients underwent lymphodepletion and were treated with ADP-A2M10. One patient received a second ADP-A2M10 infusion. Of the 16 patients who did not receive lymphodepletion chemotherapy or ADP-A2M10, six died prior to treatment, four did not meet eligibility criteria prior to lymphodepletion, one was discontinued by the investigator, and five were not treated when it was decided to conclude the trial.

AEs of special interest

To evaluate the possible role of ADP-A2M10 in the development of aplastic anemia in patient 9, total RNA and BMMC were isolated from the patient's bone marrow aspirate for interrogation of target antigen expression, transduced T-cell infiltration, and T-cell activation analysis. It was not possible to perform target antigen expression and T-cell activation analysis owing to insufficient total RNA and BMMC numbers, respectively, very likely due to ongoing pancytopenia and hypocellularity of the marrow at the time of sample collection. However, quantification of ADP-A2M10 infiltration analysis by qPCR was performed from BMMC (supplemental figure 1A). In parallel, the same assay was performed on genomic DNA extracted from PBMC to evaluate persistence of ADP-A2M10 in the patient's bloodstream. Although ADP-A2M10 were detected in the BMMC of patient 9, no evidence of enrichment of ADP-A2M10 in the marrow was observed. BMMC from patient 9 (collected at week 8) had 15 269 vector copies/microgram genomic DNA, whereas DNA from PBMC collected at the same timepoint had 67 068 vector copies/microgram genomic DNA. Comparisons of persistence between the first infusion and second infusion received by patient 9 (supplemental figure 1A) demonstrated that although the vector copy numbers were higher after the second infusion, the ADP-A2M10 persistence values during the first 8 weeks were comparable between the first and second infusions. This observation may be attributable to the ongoing pancytopenia after the second infusion.

Translational data

MAGE-A10 expression in screening and baseline biopsies of patients 2, 3, 6, and 8

Differences in MAGE-A10 expression were observed in screening biopsies of patients 2, 3, 6, and 8 collected from 3 to 10 months before infusion and baseline biopsies taken within 3 weeks before infusion (figure 3A and supplemental figure 2A,B). Although the screening biopsies of patients 2, 3, and 6 had levels of MAGE-A10 expression to allow entry to the trial, baseline samples from these patients were below the threshold for trial entry (figure 3A and supplemental figure 2A,B). The baseline sample of patient 8 had significantly higher MAGE-A10 expression (P-score=100) compared with the screening sample (P-score=50) (supplemental figure 3A). These differences in MAGE-A10 levels may be explained by inter- and intralesional tumor heterogeneity.[10]

Supplemental table 1 Subjects Screened for HLA and MAGE-A10 Under ADP-0022-003 and Under Screening Protocol ADP-0000-001

NSCLC	HLA Screened Under Screening Protocol for Multiple Studies[1]	Subjects with HLA Alleles Matching ADP-A2M10 Eligibility Criteria	MAGE-A10 Antigen Screened	MAGE-A10 Antigen Positive[2]	MAGE-A10 Antigen Negative[2]	MAGE-A10 Antigen Not Evaluable[3]
Adenocarcinoma	2232	884	577	48	474	55
Adenosquamous	30	12	10	2	8	0
Squamous	420	175	113	24	78	11
Other	128	56	36	4	28	4
Total	2810	1127	736	78	588	70

[1] ADP-0000-001 (NCT02636855) screened subjects for ADP-0022-003 and other studies throughout this time period.
[2] Mage-A10 positive and negative staining by IHC was determined by the cut-off at the time of testing.
[3] Not Evaluable defined as: tumor content insufficient, ie, < 100 tumor cells, no tumor present, ineligible sample type, or ineligible cancer indication/histology.

Supplemental table 2 All AEs related to ADP-A2M10 of grade ≥3 following first infusion (mITT population)

	Number of patients with AEs related* to T cells (n=11)		
Preferred term	**Any grade**	**Grade 3**	**Grade 4**
Patients with any AEs	8	5	2
CRS	3	0	1
Lymphopenia/lymphocyte count decreased	3	2	0
Leukopenia/WBC decreased	2	2	0
Thrombocytopenia/platelet count decreased	2	2	0
Alanine aminotransferase increased	1	1	0
Aspartate aminotransferase increased	1	1	0
Neutropenia/neutrophil count decreased	1	0	1

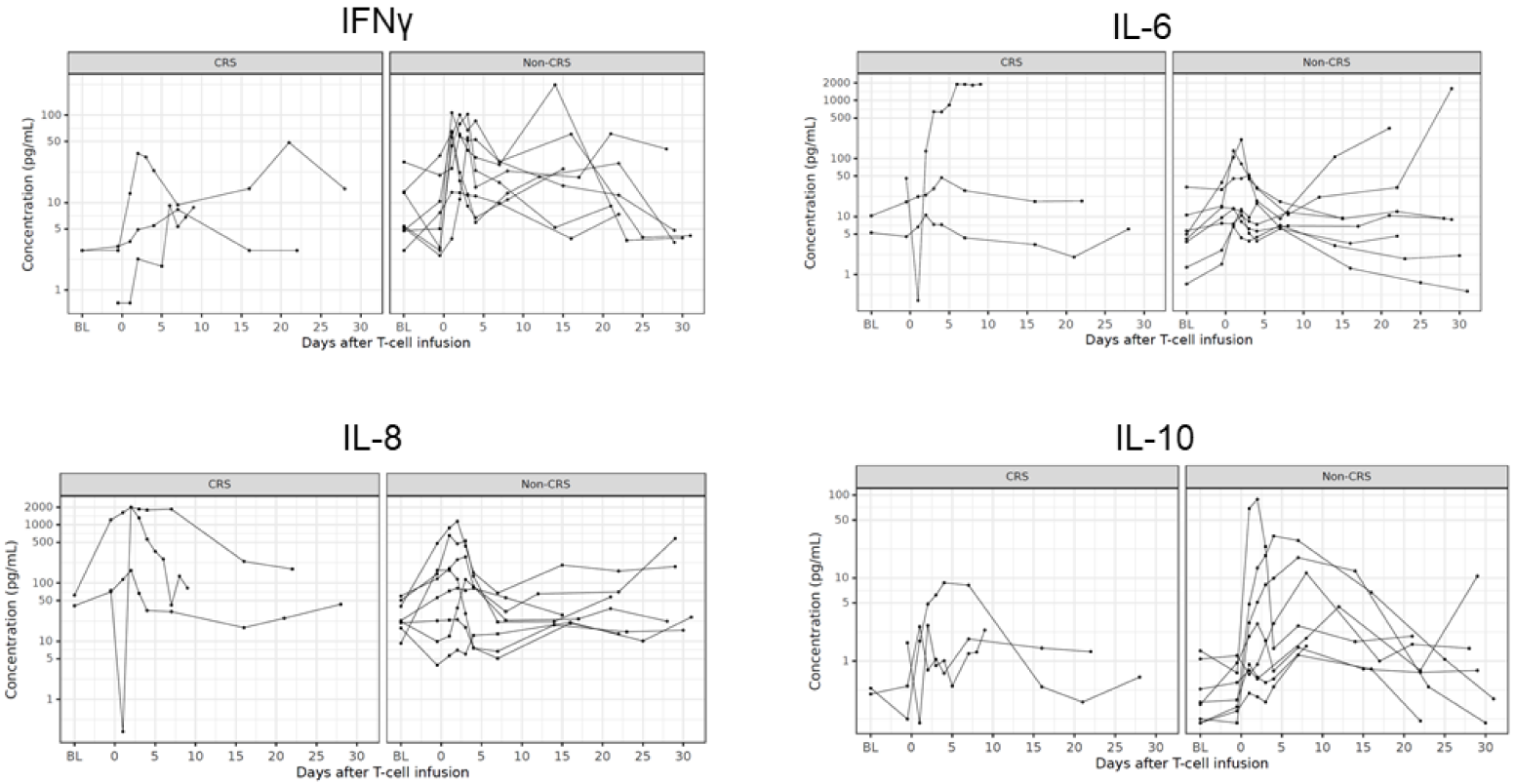
*Related AEs include definitely, probably, or possibly related events.

AE, adverse event; CRS, cytokine release syndrome; mITT, modified intent-to-treat; WBC, white blood cell.

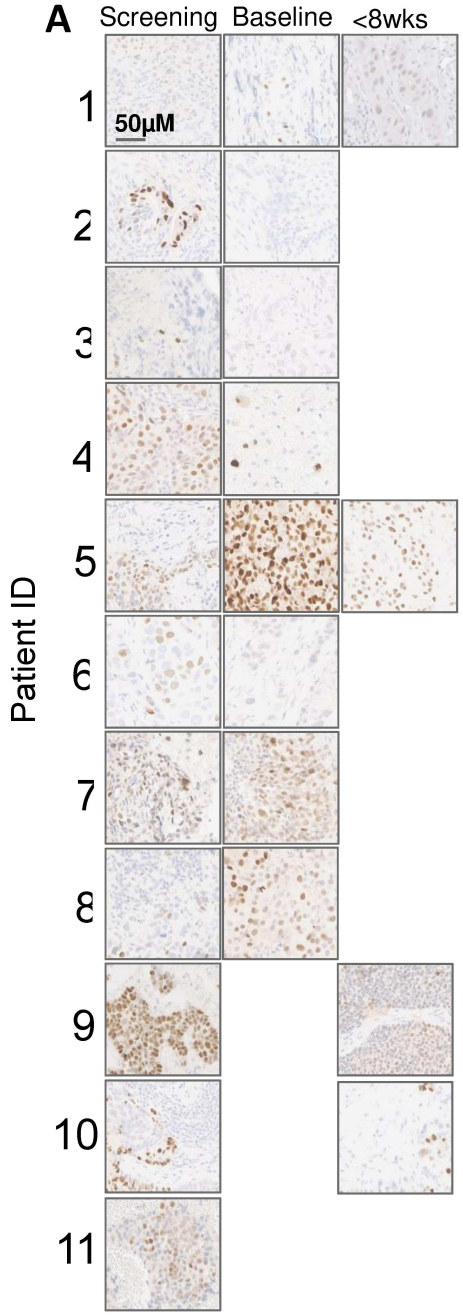
Supplemental table 3 Peak persistence (copies/microgram of DNA) following first infusion by dose group (mITT population)

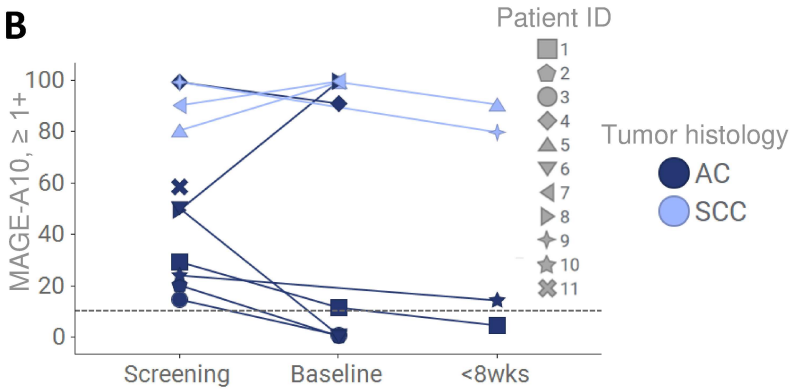
	Dose group 1 (n=5)	**Dose group 2 (n=3)**	**Dose group 3 (n=2)**	**Expansion group (n=1)**	**Overall (N=11)**
Peak persistence					
Mean	952.1	13 319.3	57 769.3	159 591.8	29 077.2
Median	825.4	12 640.5	57 769.3	159 591.8	5289.4
Min, max	592.7, 1797.2	5289.4, 22 028.1	44 181.3, 71 357.3	159 591.8, 159 591.8	592.7, 159 591.8
Time to peak persistence (days)					
Mean	2.0	7.7	6.0	2.0	4.3
Median	2.0	8.0	6.0	2.0	2.0
Min, max	2, 2	2, 13	4, 8	2, 2	2, 13

Max, maximum; min, minimum; mITT, modified intent-to-treat.



Supplemental figure 1 Elevation of selected serum cytokines after infusion. Serum IFNγ, IL-6, IL-8, and IL-10 were measured via a multiplexed electrochemiluminescence-based immunoassay (Meso Scale Diagnostics) at the indicated days pre and post infusion. Patients with (left panels) and without (right panels) cytokine release syndrome (CRS) events are shown separately. BL, baseline; IFNγ, interferon gamma; IL, interleuki





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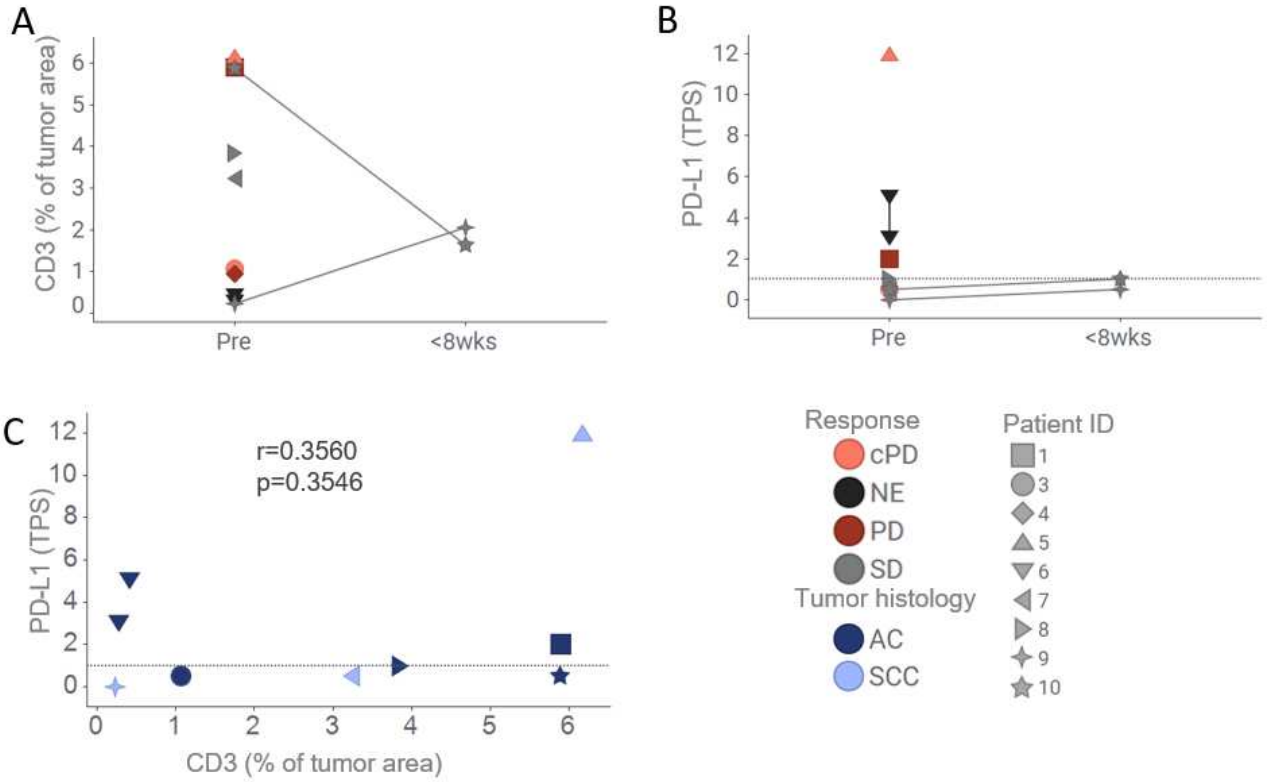
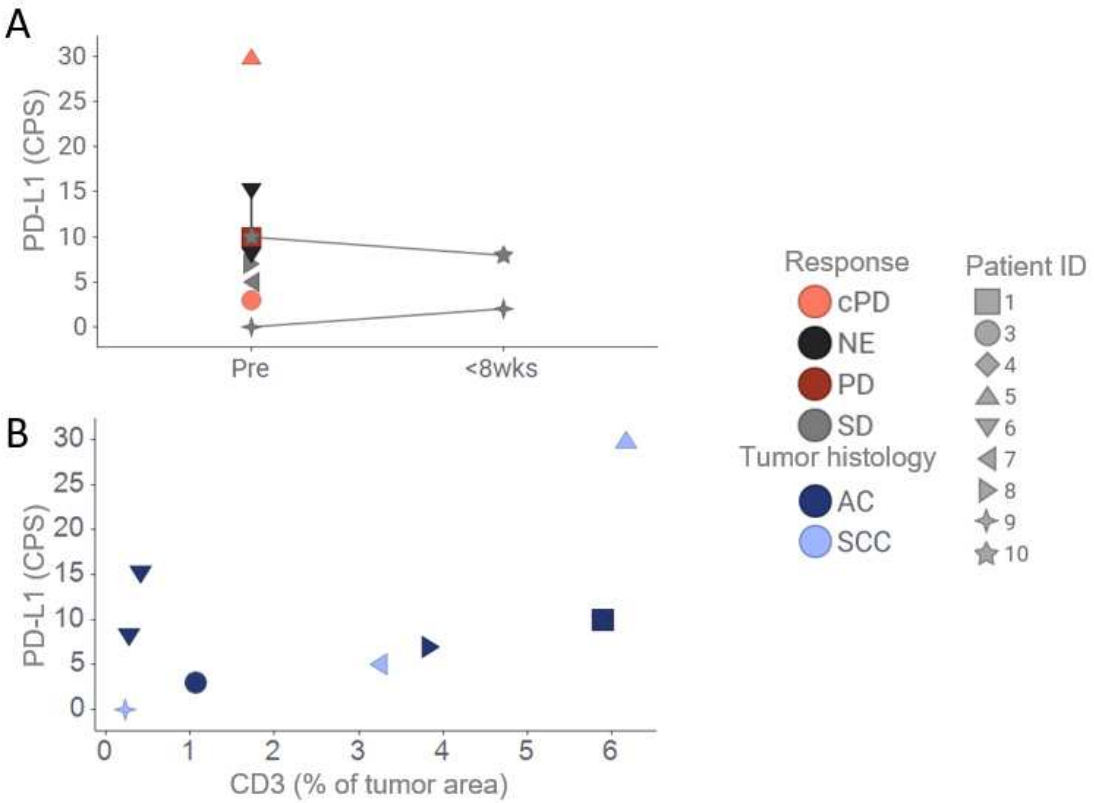
Patient ID	Dose group, Response	Visit	LOH HLA-A*0201
3	1, cPD	Pre-infusion	Not detected
6	2, NE	Pre-infusion	Detected
8	2, SD	Pre-infusion	Not detected
10	3, SD	< 8 weeks	Detected

Supplemental figure 2 Variability of MAGE-A10 expression in tumor cells across the trial patients and detection of HLA-A*0201 LOH in pre- and post-infusion tumors. (A-C) Pre-infusion biopsies (screening and baseline) and post-infusion biopsies collected within 8 weeks after infusion were used for MAGE-A10 expression evaluation and HLA-A*0201 LOH detection. The assays were completed based on sample availability. For patient 9, only the data obtained before and after the first infusion are shown. (A, B) MAGE-A10 expression was assessed by MAGE-A10 IHC. (A) Representative fields of MAGE-A10 IHC stain. (B) MAGE-A10 IHC data were plotted as percentage of tumor cells with 1^+, 2^+, and 3^+ intensities. Horizontal lines designate the cut-off of 10% of tumor with $\geq 1^+$ intensity of staining. Data points are colored by tumor histology. Patient IDs are indicated by shape. (C) Result table reporting the detection of HLA-A*0201 LOH in two of four pre- or post-infusion tumor samples. AC, adenocarcinoma; cPD, clinical PD; HLA, human leukocyte antigen; ID, identifier; IHC, immunohistochemistry; LOH, loss of heterozygosity; MAGE-A10, melanoma-associated antigen A10; NE, not evaluable; PD, progressive disease; SCC, squamous cell carcinoma; SD, stable disease; wks, weeks.



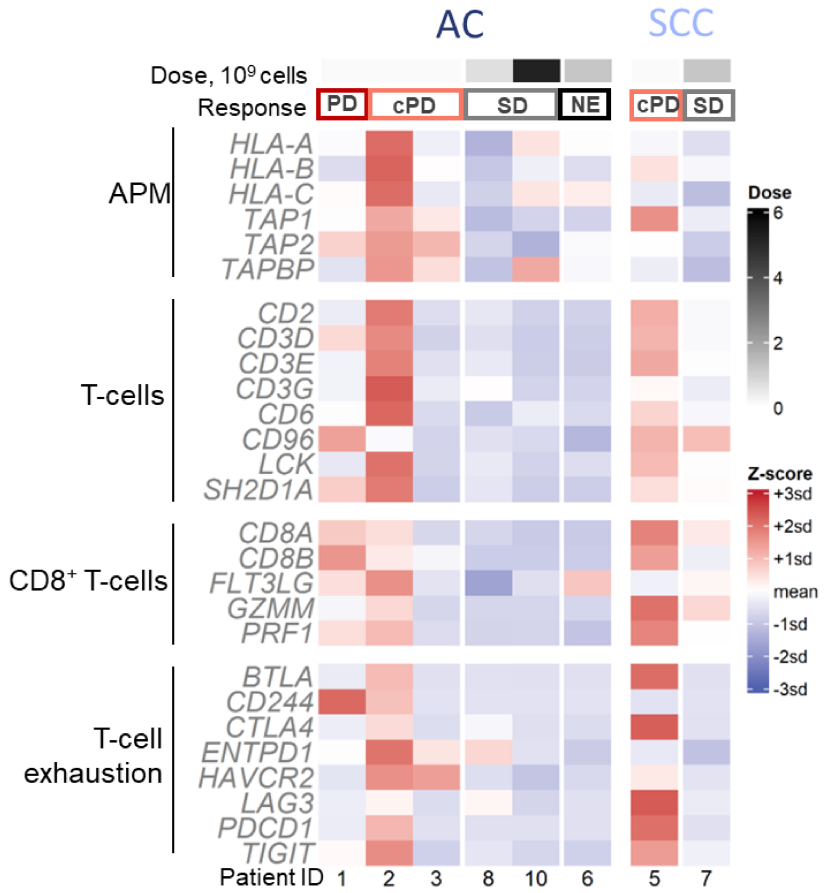
Supplemental figure 3 Variability of CD3⁺ and PD-L1 expression in tumor tissue across the trial patients. Pre-infusion biopsies (screening and baseline) (A–C) and post-infusion biopsies collected within 8 weeks after infusion (A, B) were used for CD3⁺ and PD-L1 expression evaluation. For patient 9, only the data obtained before and after the first infusion are shown. (A, C) CD3⁺ was assessed by IHC and plotted as percentage of CD3⁺ cell area in tumor area. (B, C) PD-L1 expression was assessed using PD-L1 IHC 22C3 pharmDx assay and plotted as tumor proportion score (TPS). (B, C) Horizontal lines mark PD-L1 TPS=1. Data points are colored by

response (A, B) or by tumor histology (C). Patient IDs are indicated by shape. AC, adenocarcinoma; cPD, clinical PD; ID, identifier; NE, not evaluable; PD, progressive disease; PD-L1, programmed death-ligand 1; Pre, pre infusion; SCC, squamous cell carcinoma; SD, stable disease; wks, weeks.



Supplemental figure 4 Variability of PD-L1 expression in tumor tissue across the trial patients. Pre-infusion biopsies (screening and baseline) (A, B) and post-infusion biopsies collected within 8 weeks after infusion (B) were used for PD-L1 expression evaluation. PD-L1 expression was assessed using PD-L1 IHC 22C3 pharmDx assay and plotted as combined positive score (CPS) per visit code (A) or per percentage of $CD3^+$ cell area in tumor area (B). Data points are colored by response (A) or by tumor histology (B). For patient 9, only the data obtained before and after the first infusion are shown. Patient IDs are indicated by shape. AC, adenocarcinoma; cPD,

clinical PD; ID, identifier; NE, not evaluable; PD, progressive disease; PD-L1, programmed death-ligand 1; Pre, pre infusion; SCC, squamous cell carcinoma; SD, stable disease; wks, weeks.



Supplemental figure 5 Variability of antigen-processing machinery (APM), CD3⁺ T-cell, CD8⁺ T-cell, and T-cell exhaustion gene signature expression in pre-infusion tumor tissue available for the study. Gene expression analysis was performed using the NanoString nCounter system and presented as heatmap of scores standardized per gene. The scores were obtained as the number

of standard deviations away from the mean (Z-score). AC, adenocarcinoma; cPD, clinical PD; ID, identifier; NE, not evaluable; PD, progressive disease; SCC, squamous cell carcinoma; SD, stable disease.

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