ANTICANCER RESEARCH *41*: 4101-4115 (2021)
doi:10.21873/anticanres.15213

Efficacy of Intranodal Neoantigen Peptide-pulsed Dendritic Cell Vaccine Monotherapy in Patients With Advanced Solid Tumors: A Retrospective Analysis

TAKASHI MORISAKI[1], TAKAFUMI MORISAKI[1,2], MAKOTO KUBO[2], HIDEYA ONISHI[3], TATSUYA HIRANO[4], SHINJI MORISAKI[1,3,5], MASATOSHI ETO[6], KEISUKE MONJI[6], ARIO TAKEUCHI[6], SHINICHIRO NAKAGAWA[1], HIROTO TANAKA[1], NORIHIRO KOYA[1], MASAYO UMEBAYASHI[1], KENTA TSUJIMURA[1], POH YIN YEW[7], SACHIKO YOSHIMURA[7], KAZUMA KIYOTANI[8] and YUSUKE NAKAMURA[8]

[1]Fukuoka General Cancer Clinic, Fukuoka, Japan;
[2]Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;
[3]Department of Cancer Therapy and Research, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;
[4]Department of Surgery, Sada Hospital, Fukuoka, Japan;
[5]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;
[6]Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;
[7]Cancer Precision Medicine Inc., Kawasaki, Japan;
[8]Cancer Precision Medicine Center, Japanese Foundation for Cancer Research, Tokyo, Japan

Abstract. *Background/Aim: Neoantigens are tumor-specific antigens that emerge due to gene mutations in tumor cells, and are highly antigenic epitopes that escape central immune tolerance in the thymus, making cancer vaccine therapy a desirable option. Patients and Methods: Tumor neoantigens were predicted in 17 patients with advanced cancer. They were resistant to the standard treatment regime, and their synthetic peptides were pulsed to the patient's monocyte-derived dendritic cells (DCs), and administered to the patient's lymph nodes via ultrasound. Results: Some patients showed sustained tumor shrinkage after this treatment, while some did not respond, showing no ELISpot reaction. Although the number of mutations and the predicted neoantigen epitopes differed between patients, the clinical effect depended more on the presence or absence of an immune response after vaccination rather than the number of neoantigens. Conclusion: Intranodal neoantigen peptide-pulsed DC vaccine administration therapy has clinical and immunological efficacy and safety.*

Correspondence to: Takashi Morisaki, Fukuoka General Cancer Clinic, Fukuoka, Japan. Tel: +81 922827696, Fax: +81 924056376, e-mail: tmorisaki@cancer-clinic.jp

Key Words: Neoantigen, dendritic cells, peptide, vaccine, intranodal.

Cancer vaccine therapy induces its effects by amplifying the antitumor activity of cancer-associated antigen-reactive T lymphocytes (1). To date, various attempts have been made to identify cancer-related antigens and administer them as vaccines (2, 3). In recent years, the ability to perform detailed genetic analysis of individual patients' tumors has brought about a major paradigm shift in cancer vaccine therapy (4). Neoantigens are ideal antigens based on amino acid substitutions due to gene mutations in tumors, and are true tumor-specific antigens (4, 5).

The first evidence for T cell recognition of mutant peptide in tumor was provided by Monach and Schleiber *et al*., who found that a single amino acid substitution through a single nucleotide variant was a T-cell reactive tumor specific antigen (6). Many studies have revealed that somatic acquired genetic changes in tumor cells can be tumor-specific antigens, such as single-nucleotide variants, insertions and deletions (indels), and structural variants such as fusion genes (5, 7, 8).

However, the development of immune checkpoint inhibitors (ICIs) has determined the importance of immunopharmaceutical therapy that targets immune checkpoint molecules such as PD-1, PD-L1, and CTLA-4 in cancer treatment (9). Many reports have been published on the association between the antitumor effect of ICIs and neoantigen-specific cytotoxic T lymphocytes (CTL) (10-13). Thus, both cancer vaccine therapy and ICIs involve neoantigens produced by genetic mutations, which are

Table I. *Baseline clinical characteristics of patients.*

Case	Age/Gender	Diagnosis	Diagnosis date	Prior therapies	Involved organs	Vaccine starting
1	71/M	Lymphoma	May-07	Sur, Chem	Stomach	Feb-19
2	62/M	Lymphoma	Jan-14	Chem, Rad	LNs	Dec-18
3	53/F	Thymoma	Jan-06	Surg, Chem, Rad	Pleura, lung	Jan-19
4	47/M	Thymic Ca	Mar-11	Surg, Chem, Rad	Pleura, lung	Nov-18
5	43/F	Renal cell Ca	Apr-18	Surr, Rad	Bone, lungs	Nov-19
6	74/M	Renal cell Ca	Jan-17	Surg, Chem	Lung	Jan-19
7	79/F	Pancreas Ca	Jan-16	Surg, Chem	LNs, lung	Sep-19
8	64/F	Urothelial Ca	Feb-14	Surg, Chem	Lung	May-19
9	57/F	Colon Ca	May-17	Surg	Peritoneum	Apr-19
10	73/F	Colon Ca	Jan-18	Surg, Chem	Peritoneum, liver	Jul-19
11	39/F	Adenoid cystic Ca	Jun-05	Surg	Lung, LNs, subcutaneous	Jan-19
12	58/F	Uterine sarcoma	Aug-18	Surg, Chem	Liver, peritoneum, lung	Aug-19
13	44/F	Uterine sarcoma	Jan-19	Surg, Chem	Lung, peritoneum, pleura, liver	Apr-20
14	57/M	Gastric Ca	Jul-05	Surg, Chem, Rad	Bone, perineum, LNs, liver	Jul-20
15	72/F	Ovarian Ca	Aug-16	Surg, Chem	Peritoneum, pleura	Jun-18
16	59/M	Granular tumor	May-19	Surg, Chem	Lung, LNs	Jun-20
17	68/M	Liposarcoma	Aug-11	Surg	Thoracic wall	Aug-20

LNs: Lymph nodes; Age: age at the present (April 2021); M: male; F: female; Ca: cancer; prior therapies: therapies previously experienced by the patients were surgery (Surg), chemotherapy (Chem), and radiation therapy (Rad).

true tumor-specific antigens found only in tumor cells not affected by central immune tolerance in the thymus. The neoantigen vaccine is expected to elicit a strong antitumor immune response by CTL. The safety and efficacy of this vaccine have been reported in the first clinical trial by Carreno *et al*. (14), and have also been proven through phases Ib trials (15-17).

We have reported a prediction pipeline for neoantigen (18). Neoantigen epitopes were predicted based on amino acid substitution *via* single base mutation by whole-exome DNA comparison test of patient's tumor cells and peripheral blood mononuclear cells. After the prediction, the predicted neoantigen peptides were analyzed for their RNA expression and HLA Class I affinity. Based on these results, the patient's unique neoantigen vaccine candidate was determined, and the synthetic peptide could be pulsed on patients' monocyte derived dendritic cells.

Cancer vaccine therapy depends on the source of the tumor antigen and its administration with adjuvant or antigens-pulsed dendritic cells. Considering that the principle of presentation of tumor antigens to T lymphocytes in the lymphoid tissue is represented by antigenic peptide delivery *via* dendritic cells, it seems that the most reliable method is neoantigens-pulsed DCs, as reaffirmed in a recent review (19). Due to the importance of dendritic cell vaccine therapy, including the antigen sources or route of administration of these cells, it has been extensively studied. For the DCs administration routes, we adopted the direct intranodal injection method.

We have previously reported DC vaccine therapy for cancer treatment (20-22). Most recently, we reported the first patient who was treated with neoantigen peptide pulsed-DC vaccine monotherapy based on the Act on securement of Safety of Regenerative Medicine in Japan (23). Subsequently, we treated patients with advanced or chemo-refractory tumors with the same DC vaccine therapy. For the vaccine protocol, the vaccine cells were directly administered into the patient's lymph nodes (LNs) after pulsing the synthetic peptides into the patients' dendritic cells. To date, tumor shrinkage effects with few adverse events have been confirmed in cases in which the induction of CTL to neoantigens could be inferred from the IFN-γ ELISpot reaction after vaccination.

In the present report, we described the results of a retrospective study on the usefulness and clinical application of intranodal vaccination therapy with neoantigen-pulsed DCs, and discussed the future development, potential, and hurdles to overcome.

Patients and Methods

Patients. Neoantigen peptides pulsed-DC vaccine therapy was administered to 17 patients with various advanced cancer types from December 2018 to October 2020. Clinical profiles are shown in Table I. Seventeen cases treated with intranodal administration monotherapy with neoantigen peptide pulsed dendritic cells are shown for each cancer type: 2 cases of malignant lymphoma, 2 cases of renal cell carcinoma, 2 cases of colon cancer, 4 cases of sarcoma, and 1 case of each different type of cancer with metastatic disease resistant to standard treatment.

Ethics. Cell processing, neoantigen examination, immunotherapy procedures, and immunological analysis were approved by the ethics committee of our institution (Fukuoka General Cancer Clinic)

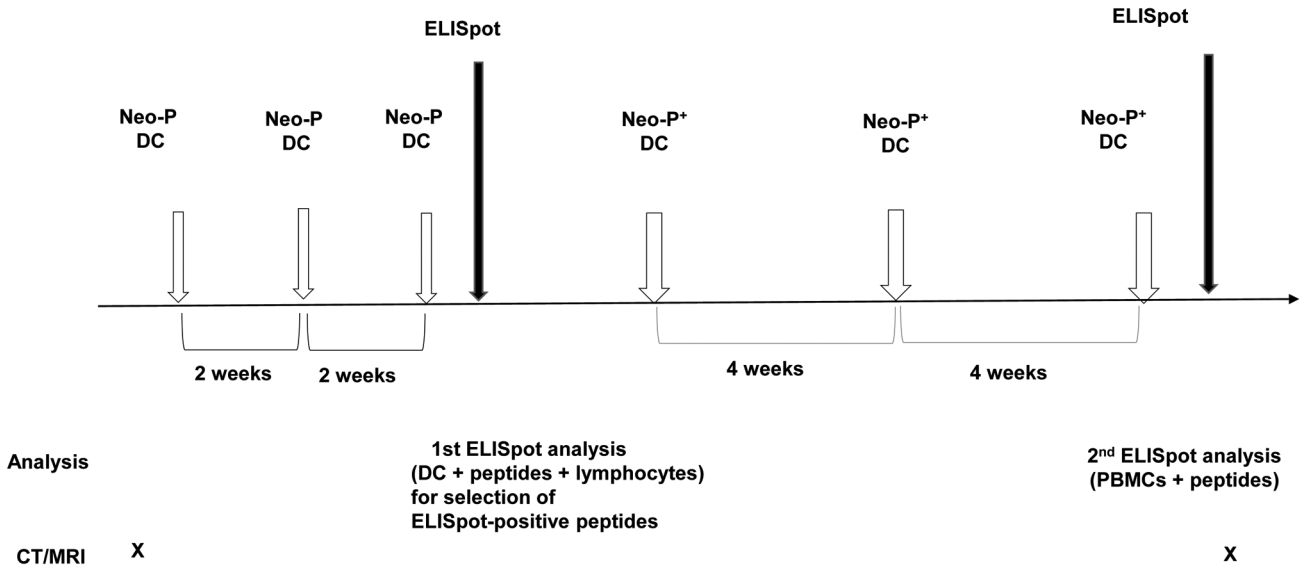

Figure 1. *Schedule of neoantigen peptides-pulsed DC vaccine. Before starting the vaccines, patients underwent leukapheresis, and the PBMCs were cryopreserved before use. The patients received the neoantigen peptide DC vaccine (Neo-DC) on day 1 (priming), day 14 (boost), and day 28 (2nd boost), and lymphocytes were obtained from all patients on day 35 for ELISpot analysis for selection of T cell reactive peptides. T cell reactivity against each neoantigen peptides was analyzed in peptide-pulsed DCs co-incubated with the lymphocytes. After four vaccines, ELISpot-reactive peptides-pulsed DCs (Neo-P+ DC) were used for therapies. After 6 cycles of DC vaccines, ELISpot analysis was performed again using peptide-pulsed PBMCs obtained after the 6th vaccine.*

with the patient's written informed consent for the procedure, based on the Act on Securement of Safety of Regenerative Medicine in Japan.

Treatment protocol. For neoantigen analysis, fresh tumor specimens or formalin-fixed paraffin-embedded (FFPE) samples were used. All neoantigen prediction pipeline procedures were outsourced to Cancer Precision Medicine, which handles neoantigen analysis and synthesis of neoantigen peptides. The treatment protocol for the neoantigen peptide pulsed dendritic cell vaccine is shown in Figure 1. For dendritic cells, peripheral blood mononuclear leukocytes (PBMCs) obtained by leukaphelesis were collected and cryopreserved until use. First, priming DC vaccinations were performed, and boost DC vaccinations were performed at 14 and 28 days after vaccination. After three rounds of vaccinations, the immune response of lymphocytes to neoantigens-pulsed DCs was determined by IFN-γ ELISpot, which was compared to the lymphocyte response before vaccination. In the latter half of the protocol, the vaccination was administered three times using peptides that were positive for the ELISpot reaction. Before and after one course therapies (six cycles of vaccinations), computed tomography (CT) or magnetic resonance imaging (MRI) were performed to determine the antitumor effect.

Whole-exome and RNA sequencing and prediction of neoantigens. Neoantigen predictions were made as previously reported (18). Briefly, both genomic DNA and total RNA were extracted from the patient's tumor tissues stored in RNAlater using an AllPrep DNA/RNA mini kit (Qiagen, Venlo, Netherlands), while normal control genomic DNA was extracted from the patient's PBMCs. In the case for FFPE samples, only genomic DNA was extracted using GeneRead DNA FFPE kit (Qiagen). Whole-exome libraries were prepared from genomic DNA using SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. RNAseq libraries were prepared using a TruSeq Stranded mRNA Library Prep kit (Illumina, San Diego, CA, USA). The prepared whole-exome and RNAseq libraries were sequenced using100 bp paired-end reads on a HiSeq sequencer or 150 bp paired-end reads on a NovaSeq (Illumina).

Mutation calling was performed as described previously (24) using the following parameters: (i) base quality of ≥15; (ii) sequence depth of ≥10; (iii) variant depth of ≥4; (iv) variant frequency in tumor of ≥10%; (v) variant frequency in normal samples of <2%; and (vi) Fisher *p*-value of <0.05.

HLA class I genotypes of the patients were predicted from normal whole-exome sequencing data using the OptiType algorithm (25). Neoantigens were predicted for each non-synonymous mutation-peptides with single nucleotide variant SNV), and the binding affinities of all possible 8- to 11-mer peptides to HLA class I molecules (HLA-A, -B, and -C) were examined using NetMHC v3.4 software and NetMHCpan v2.8, as described previously (18). Candidate neoantigen peptides with predicted binding affinity IC50 values of less than or equal to 500 nM were selected for further analysis. Detection of mutated mRNA by RNAseq data was also considered for the selection of potential neoantigen candidates. Since RNAseq was not performed for FFPE samples, gene expression data from TCGA database was referred.

Predicted neoantigen peptides (4-9 peptides per patient) were synthesized and their quality was confirmed by High Performance Liquid Chromatography (HPLC) analysis.

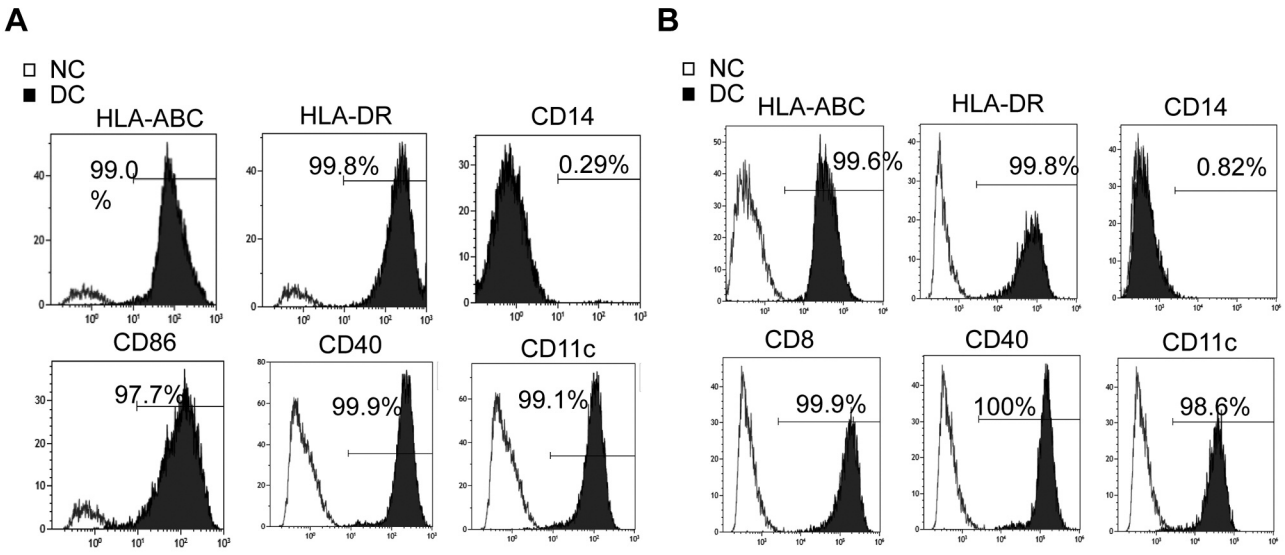

Figure 2. *Phenotype of mature dendritic cells (DCs). Flow cytometry analysis of DCs matured with TNF α and IFN-γ for the expression of DC-related surface markers. DCs were analyzed by staining separately for all markers (CD40, CD86, HLA-Class I, HLA-DR, CD14, and CD11c). DCs were highly positive for CD86, a co-stimulatory molecule. Two examples (A; Case 2) and (B; Case 6) are shown. NC: negative control antibodies against DCs. DC: Histogram of specific antibodies against dendritic cells.*

Generation of DC vaccine and phenotypical analysis. PBMCs from each patient were obtained using a leukapheresis procedure performed using White Blood Cell Collection SET according to the manufacturer's instructions (Haemonetics, Braintree, MA, USA). The product was diluted with RPMI-1640 (Kojin-Bio Inc., Saitama, Japan) for isolation using Ficoll-Hypaque (GE Healthcare, Upsala, Sweden). Afterwards, the cells were washed three times with RPMI and cryopreserved until use.

DCs were generated from frozen the PBMCs from each patient. PBMCs were thawed and then cultured in 6-well plates (FALCON, Franklin Lake, NJ, USA) in complete medium containing 1% autologous serum for 30 min. After removing the floating cells and washing with RPMI, adherent cells were cultured in complete DC medium containing GM-CSF (Primmune Inc., Kobe, Japan) and interleukin-4 (Primmune). On day 6, the cells were stimulated with a maturation cytokine cocktail containing TNF-α (Pepro Tech Inc., Rocky Hill, NJ, USA) and interferon-γ (Dainippon Pharma, Osaka, Japan) for 18-24 h.

Phenotypic DC changes were monitored by light microscopy, and flow cytometry analysis was performed as previously described (23). DCs used for therapy are expected to express high levels of HLA class I, HLA-DR, CD40, CD11c, and CD86, and be negative for CD14. All cultures were inspected for safety by examining contamination with endotoxin, β-glucan and peptide-glycan with Toxinometer ET-6000 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) following the Food and Drug Administration guidelines. Mycoplasma contamination was inspected by MycoAlert (Lonza Rockland Inc., Rockland, ME, USA).

Vaccine administration. Before use, neoantigen peptides were dissolved in DMSO-containing sterile water, filtered through 0.22 μm Millipore syringe (Millipore, Mosheim, France), and then tested for endotoxin, β-glucan, and mycoplasma. DCs were pulsed with neoantigen peptides for 4 h at 37˚C, then washed three times with saline and resuspended in a total volume of 0.2-0.5 ml saline according to LNs size (0.5 ml for LN size ≥5 mm, 0.2 ml for LN size <5 mm), in a 1 ml disposable syringe. The antigen-loaded DCs were immediately administered to the patient *via* intranodal injection under Ultra Sonograpy (US) by a skilled medical doctor. In detail, using a superficial vascular US probe, DCs in 0.2 to 0.5 ml saline were injected targeting the lymph node cortex with a 25G needle after local anesthesia with 0.5% xylocaine The vaccination procedure was well-tolerated by each patient, without any treatment-associated adverse events.

ELISPOT assay, Tetramer assay. ELISpot assay was performed using a Human IFN-γ ELISpot^{plus} kit (MABTECH, Cincinnati, OH, USA) according to the manufacturer' instructions. Briefly, 96-well plates with nitrocellulose membranes precoated with primary anti-IFN-γ antibody MABTECH) were pretreated with RPMI medium containing 10% autologous serum at 4˚C overnight. A total of at 5×103 autologous immature DCs were added to each well, and DC maturation cocktail was added to each well and incubated overnight. Then, each neoantigen peptide (25 μg/ml) or their mixtures was added to each well and incubated for 4 h. After washing three times with RPMI medium, 1.5×10^5 autologous lymphocytes were added to each well and then incubated for 48 h. The plate was washed three times with PBS, and a secondary antibody was added to each well and incubated for 2 h. The plates were then incubated with the HRP-reagent and stained with TNB (MABTECH). The positivity of the neoantigen-specific T cell response was quantitatively defined as a specific spot. Spots were captured and analyzed using an automated ELISpot reader 08 classic (AID GmbH, Strassberg, Germany). In some experiments using the most responsive peptide,

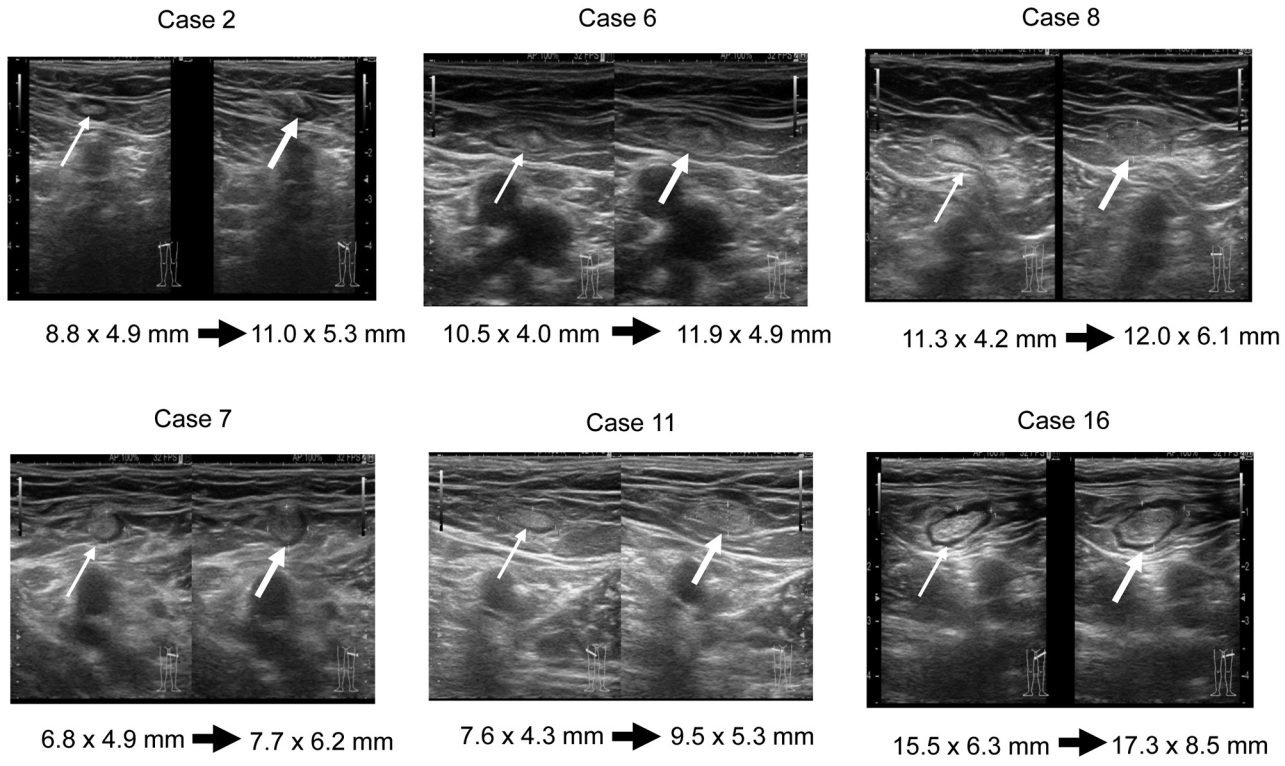

Figure 3. *Intranodal administration of DC vaccine. Examples of images of lymph nodes (LNs) before and after intranodal DC vaccine administration. Intranodal administration of DC vaccine into groin LNs using direct ultrasound guidance was provided by a skilled doctor. In each panel, the left side shows LNs before DC vaccine administration, and the right side shows the same LNs after DC injection. Thin-white arrows indicate LNs before vaccine injection. Bold white arrows indicate the LNs after vaccination.*

we performed a peptide-dose-dependent assay for the lymphocytes IFN-γ response using PBMCs obtained at six time points after initiation of vaccine therapy.

In an experiment, we performed a neoantigen peptide-MHC Class-I tetramer assay using peripheral blood lymphocytes from a patient after six neoantigen DC vaccines. Briefly HLA-A 2402-binding BCL2 R129H-1 peptide tetramers were constructed using QuickSwitch Quant HLA-A2402 Tetramer kit (MBL, Nagoya, Japan). Cells were incubated with PE-conjugated tetramers for 30 min at 4˚C, and then FITC-conjugated anti-CD8 (Beckman Coulter, Brea, CA, USA) were added and incubated for an additional 30min at 4˚C. After washing with PBS, the cells were analyzed by flow cytometry (Nabios EX: Beckman Coulter).

Safety. Adverse events were determined according to the RESIST criteria.

Statistical analyses. Data are presented as mean±standard deviation (SD). Student's *t*-test was used to compare continuous variables between the two groups. Statistical significance was set at $p<0.05$.

Results

Intranodal vaccination of neoantigen peptides-pulsed matured DCs and safety. Neoantigen prediction, peptides synthesis, and matured DC culture were successfully performed in all cases. Neoantigen peptide-pulsed DCs were defined as CD40+, CD86+, CD14-, CD11c+, HLA-Class I +, and HLA-DR+, as shown in Figure 2 (examples from cases 2 and 6). The most important procedure for rapid and precise stimulation of neoantigen-reactive T cells was the delivery of neoantigens to professional antigen-presenting cells (dendritic cells) in lymphoid tissue. Therefore, we directly administered neoantigen peptide-pulsed DCs into the lymph nodes under ultrasonographic guidance by a skilled doctor. After DC injection, the lymph nodes appeared enlarged, as shown in Figure 3 (examples from 6 cases). Diameter changes of the lymph nodes before and after intranodal injection of the dendritic cell vaccine are depicted in six cases. As shown in the upper left panel in Figure 3, the length and width of the lymph nodes increased by 1-2 mm after DC vaccine injection.

Vaccination therapy was well-tolerated by patients without any treatment-associated severe adverse events.

Clinical and immunological responses. Table II summarizes the total mutation number, class-I restricted neoepitopes, number of used peptides, number of T cell-reactive (ELISpot-

Table II. *Profiles of neoantigens, immunological responses, and clinical responses.*

Case	Number of mutation	Predicted HLA Class I neoantigens peptide	Number of selected neoantigen	ELISPOT positive peptide spot (30%)	ELISPOT positive peptide activity (30%)	Measurable lesions	Response (M: months)	AEs
1	152	673	4	0	1	Gastric mucosa	SD (>20M)	(–)
2	82	70	7	4	2	LNs	PR (>24)	(–)
3	11	65	6	1	1	Pleural tumor	PR (6M)	(–)
4	118	241	4	1	1	Lung	SD (>12M)	Fever
5	19	32	6	1	0	Lung	SD (8M)	(–)
6	75	285	6	2	2	Lung	CR (>23M)	Fatigue
7	141	47	5	2	2	LNs	SD (12M)	(–)
8	67	138	9	1	1	Lung	PR (6M)	(–)
9	85	27	7	2	2	(–)	SD (>18M)	(–)
10	129	388	9	1	1	Liver	SD (6M)	(–)
11	13	66	7	2	1	Soft tissue	SD (>12M)	(–)
12	185	267	9	1	0	Lung	PD	(–)
13	34	106	10	0	0	Lung	PD	(–)
14	27	67	9	0	2	Bone	SD (3M)	(–)
15	25	57	4	3	3	(–)	SD	(–)
16	20	42	9	0	1	Lung	SD (6M)	(–)
17	45	180	9	0	5	Soft tissue	SD (4M)	(–)

AEs: Adverse events; PR: partial response; SD: stable disease; CR: complete response; LNs: lymph nodes.

positive) peptides, best clinical response, and adverse reactions. Candidate neoantigen epitope peptides in each patient were selected using the following methods: binding affinity IC_{50} values of less than or equal to 500 nM and mRNA expression of the neoantigen candidate epitopes by referring to the RNAseq data. Neoantigen epitope candidates were selected as highly potential four to nine peptides derived from non-synonymous mutations in each patient, as shown in Table II. For monitoring vaccine-induced immune response, ELISpot assays were performed to detect neoantigen-specific T cell responses in the presence of peptide-loaded dendritic cells after three rounds of vaccination. Table II also shows the number of T cell-reactive peptides in the ELISpot analysis. Further, ELISpot analysis was performed for the IFN-γ response in lymphocyte samples obtained following six vaccinations after therapy initiation. In the ELISpot analysis, we applied two methods for detecting positive T-cell responses to neoantigen peptides: spot number and activity. Activity refers to the data of multiplying each spot by each density and dividing by 1,000, which is considered for both spot number and spot intensity (data were provided by AID GmbH, Penzberg, Bayern, Germany). We confirmed that activity data were more relevant for IFN-γ ELISA than spot numbers (data not shown).

The number of neoantigen peptides used for dendritic cell vaccine therapy and those that tested positive for ELISpot response after treatment were depicted for each case (Figure 4). In cases with durable CR or PR (Cases 2 and 6), multiple T cell-reactivities to the peptide were confirmed by ELISpot analysis, whereas in cases with PD (Cases 13 and 14), the number of T cell-reactive peptides was very small. Although the T cell-reactive peptide was limited in some cases (Cases 3 and 8) with PR, the magnitude of ELISpot response to the neoantigen was moderately high. These results suggest that the number of T cell-reactive neoantigen epitopes used and tumor shrinkage were particularly relevant, and that the effect of neoantigen peptide-pulsed dendritic cell vaccine therapy was more related to the reactivity of T cells that respond to the neoantigen peptides used than to the number of neoantigens.

Case report of a patient with metastatic lung cancer who showed durable complete responses. The patient (case 6) was a 74-year-old man who underwent surgery for renal cell carcinoma in July 2018 after preoperative chemotherapy. In September 2018, he developed multiple lung metastases. Due to pulmonary venous thrombus, the molecular-targeted drugs were not indicated, and neoantigens were predicted using fresh frozen renal tumor specimens. Six neoantigen epitope peptides were predicted, as shown in Table III, and the neoantigen peptide-pulsed DC vaccine therapy was initiated in January 2019. Imaging tests after the third vaccination resulted in PR, six rounds of vaccination resulted in near CR in May 2019, and CT examination in August 2020 confirmed durable CR (Figure 5A). For monitoring vaccine-induced immune response, ELISpot assays were performed to detect neoantigen-specific T cell responses after three rounds of

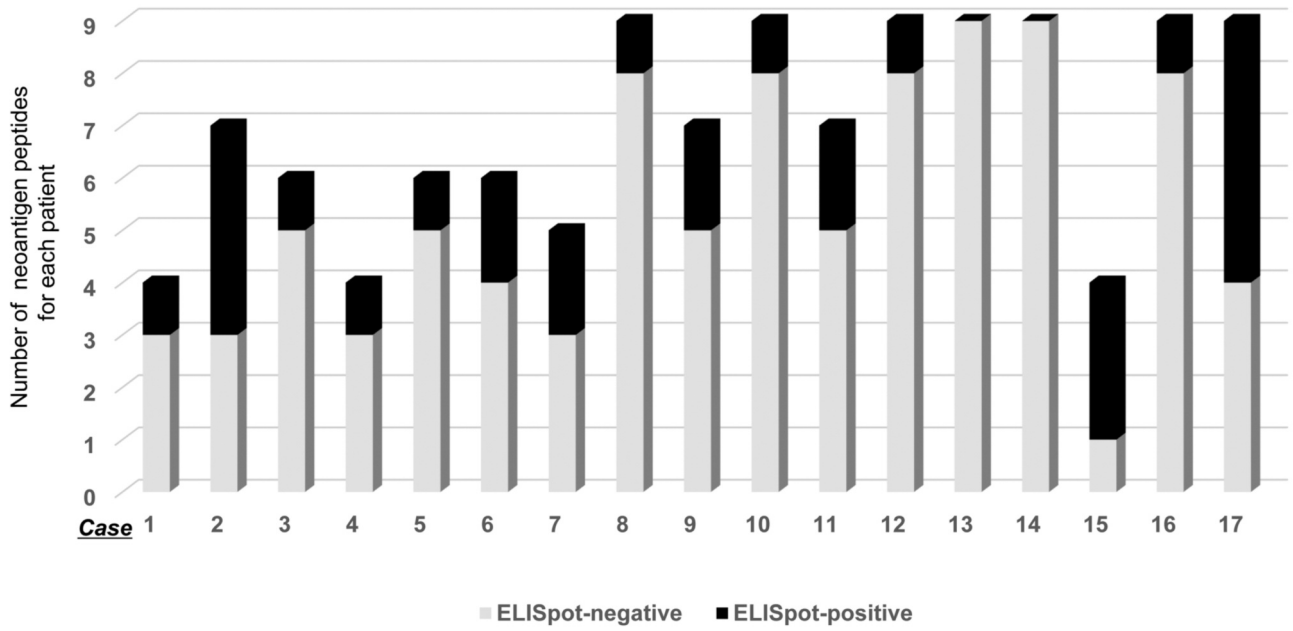

Figure 4. *The number of neoantigen peptides used in each case and the number of peptides that are positive for T cell response after vaccine. The horizontal axis shows the case number, and the vertical axis shows the number of peptides used for the vaccine in each case. The black box on each vertical axis is an ELISpot-positive peptide, and the gray box indicates the number of ELISpot-negative peptides.*

Table III. *The expression of neoantigen candidate epitopes by referring to the RNAseq data in case 6.*

Number	Gene	Amino-acid change	Amino-acid length	Mutation position	Mutated peptide sequence	Affinity of mutated peptide to HLA (nM)	Wild-type peptide sequence	Affinity of wild-type peptide to HLA (nM)	HLA Class-I	Mutated peptide mRNA	Tumor (Variant/ Total reads)	Normal (Variant/ Total reads)
1	*TMEM66*	F158I	8	0	KQHGFASI	20	KQHGFASF	88	HLA-A02:06	124	8/44	0/42
2	*TBC1D9B*	I744V	8	0	KQSVSPPV	28	KQSVSPPI	88	HLA-A02:06	10	7/49	0/31
3	*SCCPDH*	T24S	10	3	FTGQFVSEEV	34	FTGQFVTEEV	33	HLA-A02:06	13	9/49	0/39
4	*PURA*	E214V	10	0	LIDDYGVEEV	36	LIDDYGVEEE	10,411	HLA-A02:06	16	39/299	0/223
5	*BTBD2*	D428Y	10	4	QIIHTYSNTV	87	QIIHTDSNTV	206	HLA-A02:06	26	12/56	0/58
6	*ITGA1*	I775T	8	1	FQDSVRTT	120	FQDSVRIT	415	HLA-A02:06	1	15/46	0/43

vaccination. In the ELISpot analysis, four of the six types of neoantigen peptides used showed a moderately positive response, which showed an increase of 30% or more compared to control (Figure 5B). After that, the ELISpot reaction was performed regularly, and it was confirmed that the CTL reaction to the three neoantigens persisted (data not shown). In particular, PBMCs obtained after six rounds of neoantigens DC vaccine showed a dose-dependent ELISpot response to the neoantigen based on the Purine Rich Element Binding Protein A (PURA) gene mutation-derived peptides (Figure 5C). He was tumor-free in March 2021.

Case report of a patient who showed durable partial responses with follicular lymphoma. In May 2014, a 55-year-old man was diagnosed with follicular lymphoma. Treatment with rituximab, radiation, and chemo-radiation was performed, but tumor progression was observed in April 2019. The tumor had spread to the neck, axilla, abdomen, and groin lymph nodes. Lymphoid specimens in the inguinal region were collected and subjected to neoantigens predictive genetic testing. As a result, seven HLA Class-I restrictive neoantigens peptides were selected. Most tumors, which were apparent at the start of vaccine

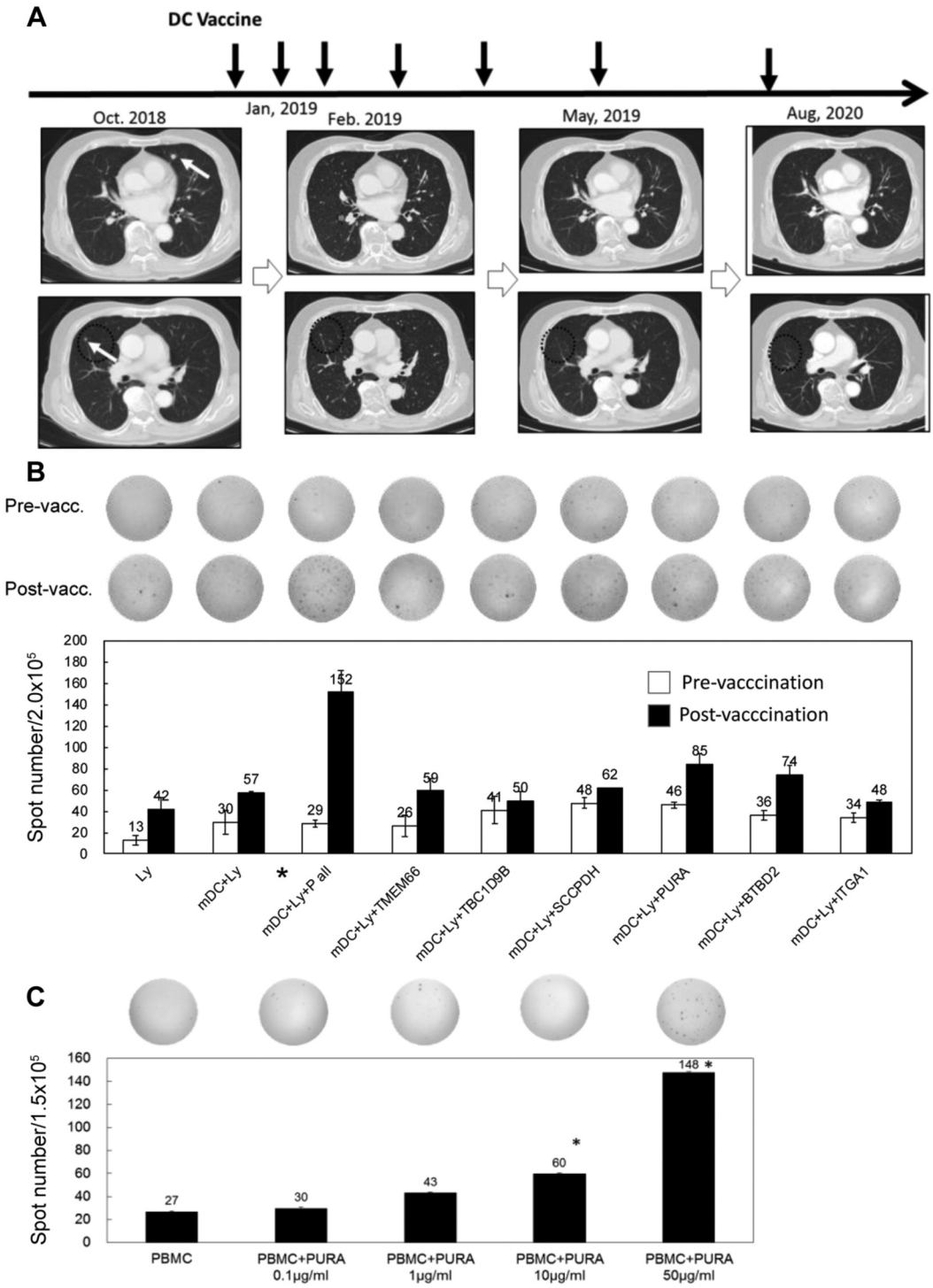

Figure 5. *Antitumor and immunological effects by neoantigen peptides-pulsed DC vaccine monotherapy in Case 6. A) Timeline of DC vaccines and changes in CT images of lung metastases before and after treatment: white arrows in the images indicate tumors at baseline. It showed a partial response after three vaccine doses, and the tumor disappeared completely after six vaccinations in May 2019. B) IFN-γ ELISpot response to each neoantigen of peripheral blood lymphocytes after three doses of vaccine is shown. A slight reaction to mutant PURA (Purine rich element binding protein A) and mutant BTBD2 (BTB domain containing 2) was observed, and when all six types of peptides (*) were allowed to act, an ELISpot reaction was observed more than three times. Ly: Lymphocytes; mDC: matured dendritic cells; C) IFN-γ ELISpot when mutant PURA peptide is added to the PBMCs obtained after the 6th vaccine. Dose-dependent IFN-γ secretion by lymphocytes incubated with peptides is shown. Data represent the mean of triplicate assays. *Significant compared without peptides.*

therapy in May 2019, were confirmed to shrunk in December 2019 (Figure 6A). After three rounds of the vaccine, the ELISpot response to four out of seven neoantigens was confirmed (Figure 6B). The reaction has continued to date. Mutant peptide BCL2 (R129H), which showed the highest positive reaction, was added to PBMCs after eight vaccinations, and an ELISpot analysis was performed. As a result, the dose-dependency was confirmed (data not shown). CD8+ T cells that were reactive for mutant epitope (BCL2 R129H) were also detected by HLA tetramer assay (Figure 6C).

Case report of a patient who showed partial responses with multiple lung metastasis from urothelial cancer. A 64-year-old woman, who underwent surgery with a renal pelvis carcinoma diagnosis in 2014, was found to have metastases in both lungs in 2015 and received until 2nd line chemotherapy. At one point, the tumor shrank; however, both lung metastases increased in August 2018, and ICIs therapy with pembrolizumab was started. In April 2019, she developed autoimmune pituitary inflammation and had increased metastases to both lungs; therefore, treatment was discontinued, and palliative care was started. Simultaneously, genetic tests aimed at predicting neoantigens were performed on FFPE specimens of surgical specimens. Predicted Class I binding neoantigen peptide epitopes were pulsed into monocyte-derived dendritic cells of the patient, and vaccine therapy with intranodal infusion of neoantigen peptide-pulsed DCs was initiated in May 2019. CT in July showed a marked reduction in lung metastases (Figure 7A). ELISpot testing after three rounds of vaccine therapy showed a strong lymphocyte response to one of the nine peptides (Figure 7B). In November 2019, pembrolizumab therapy with replenishing cortisol was resumed after the end of six vaccine therapies. As of March 2021, the patient maintained stable disease with good performance status.

Case report of a patient who showed partial responses with pleural metastasis from malignant thymoma. A 53-year-old woman who was diagnosed with malignant thymoma in 2004 underwent surgery, but had repeated recurrences in the chest wall and pleura. Each time, reoperation and radiation therapy were performed; however, pain due to a recurrent tumor of 6 cm in size appeared on the left chest wall in October 2018. A core needle biopsy under US guidance was performed, and neoantigens were predicted. The neoantigen peptide DC vaccine therapy was started in December 2018. CT examination in May 2019 confirmed a marked reduction of the tumor at the same site (Figure 8A). The ELISpot test performed at the same time also confirmed a positive T-cell reactivity to one of six neoantigen epitopes (Figure 8B).

Discussion

Neoantigen peptide-pulsed DC vaccine therapies were successfully completed in 17 patients with refractory advanced tumors, and a retrospective analysis was performed. Multiple HLA-Class I-affinity peptides were selected using the neoantigen prediction pipeline, and peptides were synthesized under good quality control. These were administered intranodally, under US guidance. There were no serious adverse events, and several patients had durable CR, PR, or SD. In particular, it was meaningful that this monotherapy alone induced continuous CR and T-cell response against neoantigen peptides in a patient with lung metastases from renal cell carcinoma.

Tumor proteins/lysates or tumor-related antigen peptides were originally used as tumor vaccine (26, 27). Usually, tumor lysates were contaminated with non-tumor proteins. Tumor peptides used for vaccine are almost all shared antigens and therefore, are not tumor-specific. On the other hand, neoantigens are highly tumor-specific and immunogenic due to their absence in normal cells, and they bypass central thymic tolerance. Recently, neoantigen-based personalized immunotherapy has been used in various clinical trials, revealing its efficacy and safety (14-17). Moreover, immunotherapy using the patient's own immune cells is a form of personalized therapy. Therefore, neoantigen peptide-pulsed DC vaccines are the ultimate personalized immunotherapy.

There are many ways to administer a dendritic cell vaccine, each with its own weaknesses and strengths. We have traditionally used the US-guided method of administering the vaccine into the lymph nodes, and we believe it is a more reliable and faster method to obtain positive results. For example, in intradermal administration of dendritic cells, which is the most widely used method, only a small percent of the number of administered DCs reach the lymph nodes (28). Furthermore, studies comparing intradermal and intranodal administration of the dendritic cell vaccine have reported that the latter was superior (29). It has also been shown that intranodal administration of tumor-RNA alone or tumor-RNA-pulsed DCs resulted in strong anti-tumor immune responses (30, 31). It has also been reported that mature DCs play an important role in lymph node expansion (32). From the above, it can be said that intranodal injection of mature dendritic cells is a rational method for the administration of dendritic cells.

In the present retrospective study, the antitumor effect did not appear to correlate with the expected number of neoantigens or neoantigen peptides used. ICIs have been reported to be more effective as the number of neoantigens increases, but the effectiveness of neoantigen vaccine therapy, even with a smaller number of neoantigens, has been shown in brain tumors and leukemia (33, 34). Meanwhile, the degree

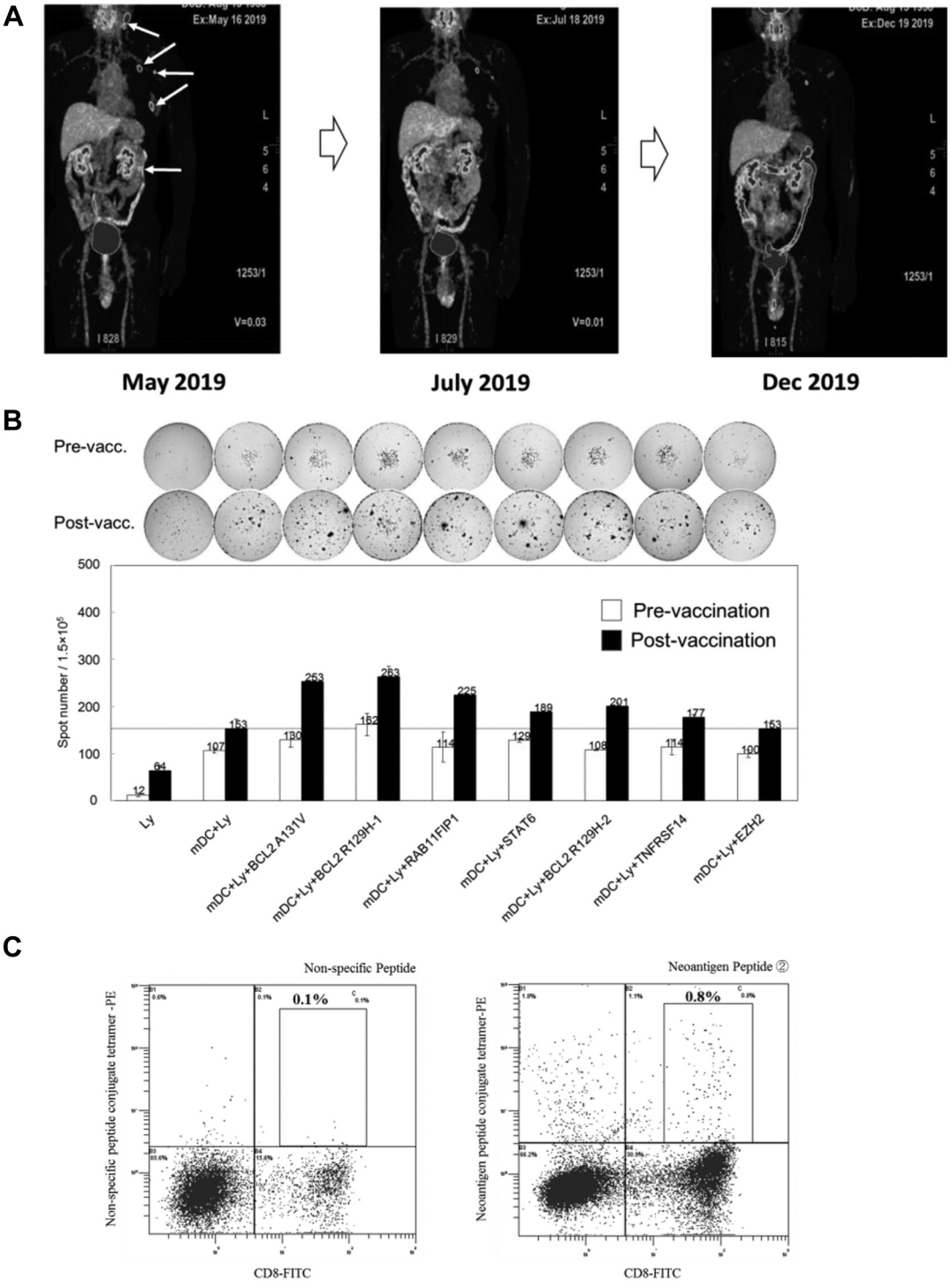

Figure 6. *Antitumor and immunological effects of neoantigen peptides DCs vaccine in a patient with chemo-refractory follicular lymphoma. A) Clinical response using positron emission tomography imaging. White arrows indicate several Fluorodeoxyglucose (FDG)-avid lesions at baseline (before vaccine initiation). Almost all lesions decreased in size after vaccination. B) Results of epitope-specific IFN-γ-secreting cells detected by ELISpot assays. The preserved lymphocytes (1.5×10^5 cells/well) before or after three rounds of vaccination were co-incubated with or without seven different types of mutant peptide-loaded autologous matured dendritic cells (5×10^3 cells/well) for 48 h, and IFN-γ secretion spots were detected using an ELISpot reader. Data indicate the means of duplicate assays. C) Frequency of CD8+ T cells against neo-epitopes in lymphocytes post-vaccination detected by HLA2402-tetramer (BCL R129H-1peptides). Lymphocytes obtained after 6 vaccinations were incubated with PE-conjugated tetramer and FITC-conjugated anti-CD8 antibody, and analyzed by FACS. Control (left), tetramer (right).*

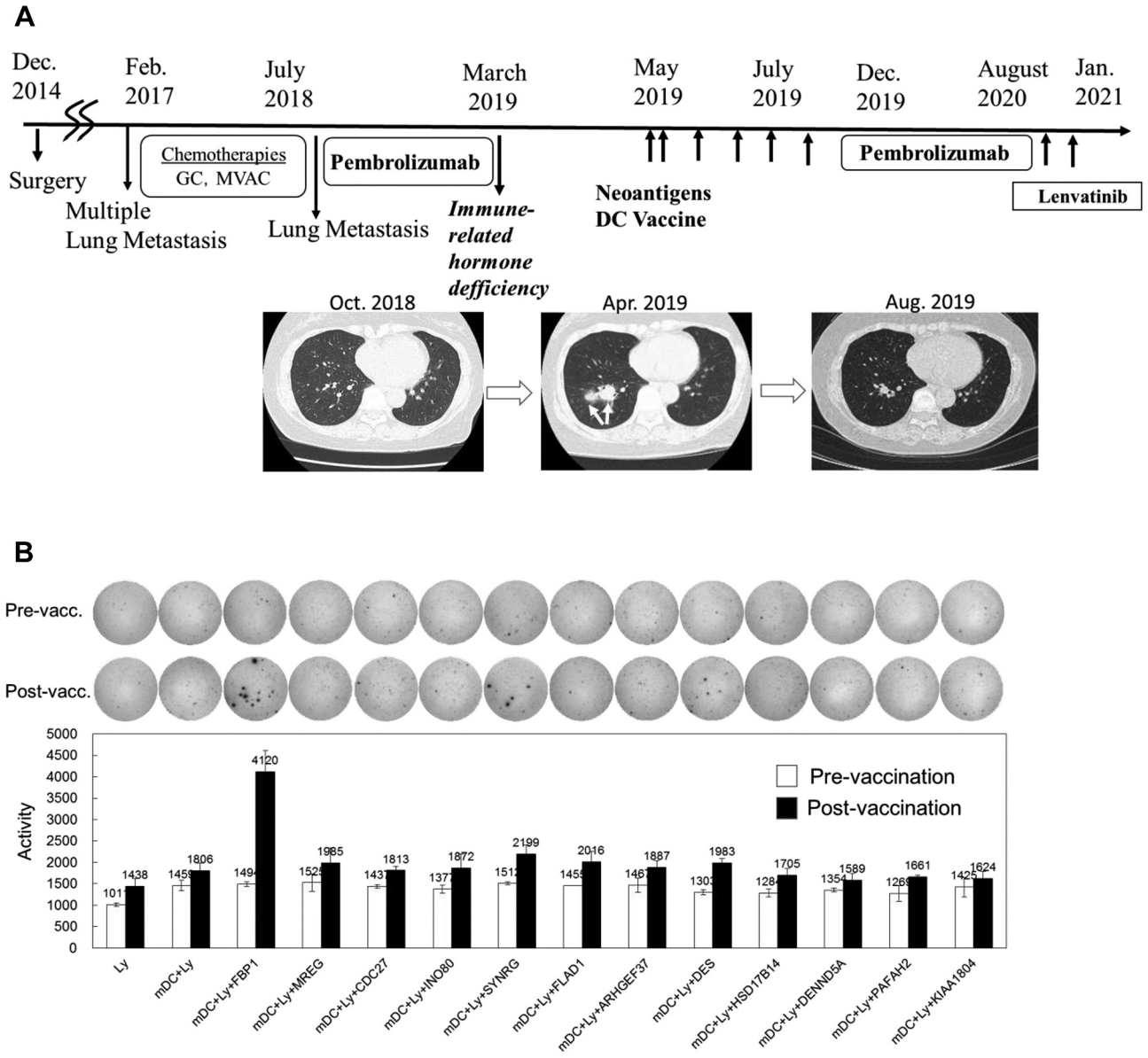

Figure 7. *Antitumor and immunological effects in Case 8. A) Timeline of the treatment course and computed tomography of the metastatic lung tumor. The white arrow indicates the tumor. After pembrolizumab (July 2018) therapy, the lung tumor progressed (April 2019), and immune-related hormone deficiency occurred. Neoantigen DC vaccine therapy was initiated in May 2019. After six cycles of DC vaccines, the size of the tumor was remarkably reduced (August, 2019). B) T cell reactivity against each neoantigen peptide-pulsed DCs using lymphocytes from before and after three vaccinations by ELISpot analysis. Only one peptide showed a prominent ELISpot T cell response.*

of T lymphocyte response to neoantigens after vaccination appeared to be related to clinical efficacy, as shown in this study. For example, in Case 6, which showed persistent CR, the number of gene mutations was 75 and the number of vaccine-suitable neoantigen peptides was 6, and only two of them were positive for specific T-cell response.

To induce tumor-specific cytotoxicity by T cells, MHC Class I-affinity-high neoantigens targeting CD8 T cells will be important (35). In the present study, we used HLA Class I-affinity neoantigen peptides. However, recent studies have reported that Class II-affinity neoantigen peptides were also involved in the effects of neoantigen vaccine therapy (36). It has also been reported that mutant peptides by insertion/deletion (INDEL) frameshift mutations can be powerful neoantigens (37, 38). At present, we have initiated a prediction pipeline for neoantigens that were compatible

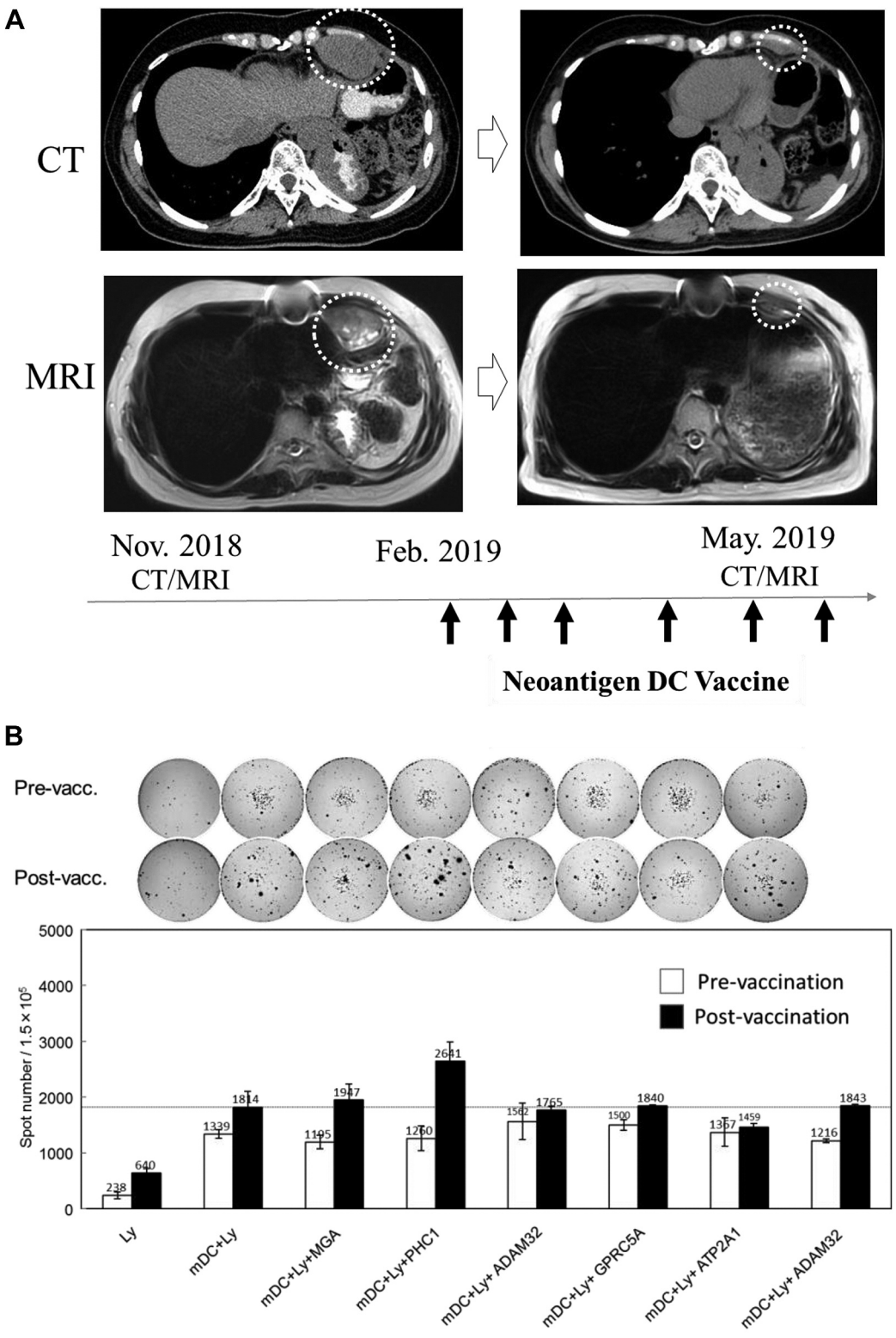

Figure 8. *Antitumor and immunological effects of Case 3. A) Computed tomography (CT) scan and magnetic resonance imaging (MRI) scans were performed at baseline (November 2018) and 6 months after vaccination (May 2019). The dotted circles indicate the target tumor lesions. Before CT (left upper) and MRI (left lower) and after CT (right upper) and MRI (right lower). B) Ex vivo IFN-γ ELISpot of lymphocytes stimulated with neoantigen peptide-pulsed DCs was performed before and 3 times after DC vaccine. Mutant PHC1 peptide showed strong density and spots.*

with both HLA Class I and Class II, including neoantigens based on single nucleotide substitutions as well as neoantigens based on insertion/deletion frameshift mutations, and are preparing to use vaccines with DCs pulsed with these peptides.

For neoantigens vaccine therapy, various methods using neoantigen mRNA or using peptides with adjuvants have been reported (39). Although none of the reports have compared neoantigen-specific mRNA/peptide with adjuvants with neoantigen mRNA/peptide-pulsed DCs, it has been reported that the method of using neoantigen peptide-pulsed dendritic cells was superior by comparing the method of administering the neoantigen peptides together with adjuvant treatment in a murine model (40). Since there are no confirmed methods for selecting the types of neoantigens and delivering them to antigen-presenting cells, we are currently continuing the method of intranodal neoantigen peptide-pulsed dendritic cell administration because of its safety and efficacy.

A recent clinical study showed the clinical efficacy of a combination of a neoantigen vaccine with ICIs (41). Theoretically, the combination of neoantigen vaccine and ICIs is very meaningful. However, from the point of view of adverse effects, neoantigen vaccines can also be combined with other therapies, such as molecular targeting drugs. The combination of neoantigen vaccines with other therapies is under clinical study in many ways.

In the future, the neoantigen vaccine can be used to prevent postoperative recurrence. In addition, the neoantigens DC vaccine monotherapy can be expected to be effective in cases with a small amount of tumor. However, combination therapies with molecular-targeted drugs or ICIs may be more effective in cases with rapid progression or large amounts of tumor. In addition, patients with PD in this retrospective study had a large number of tumors and had progressed before the immune response was induced. Therefore, it was considered that control was difficult with this therapy alone. Hence, it is necessary to clarify this medically through large clinical trials. However, it is also necessary to study immunological and oncological aspects of each case as a practical medical treatment.

In conclusion, our retrospective study indicated the clinical and immunological efficacy and safety of intranodal neoantigen peptide-pulsed DC vaccine administration therapy. However, further profound immunological and clinical studies regarding this type of new therapy are warranted.

Conflicts of Interest

Poh Yin Yew and Sachiko Yoshimura are employees of Cancer Precision Medicine, Inc. The remaining Authors declare no conflicts of interest.

Authors' Contributions

Takashi Morisaki conceived and designed the study. Takafumi Morisaki, S Nakagawa, H Tanaka, N Koya, M Umebayashi, K Tsujimura, and Takashi Morisaki performed the experiments: Takafumi Morisaki, M Kubo, H Onishi, S Morisaki, S Nakagawa, N Koya, H Tanaka, M Umebayashi, PY Yew, S Yoshimura, and Takashi Morisaki analyzed the data; Takashi Morisaki drafted the manuscript; T Hirano, M Eto, K Monji, A Takeuchi, K Kiyotani, and Yusuke Nakamura provided intellectual input.

Acknowledgements

The Authors would like to thank Editage (www. Editage.com) for English language editing.

References

1 Palucka K, Ueno H and Banchereau J: Recent developments in cancer vaccines. J Immunol *186(3)*: 1325-1331, 2011. PMID: 21248270. DOI: 10.4049/jimmunol.0902539

2 Melief CJ, van Hall T, Arens R, Ossendorp F and van der Burg SH: Therapeutic cancer vaccines. J Clin Invest *125(9)*: 3401-3412, 2015. PMID: 26214521. DOI: 10.1172/JCI80009

3 Heemskerk B, Kvistborg P and Schumacher TN: The cancer antigenome. EMBO J *32(2)*: 194-203, 2013. PMID: 23258224. DOI: 10.1038/emboj.2012.333

4 Schumacher TN and Schreiber RD: Neoantigens in cancer immunotherapy. Science *348(6230)*: 69-74, 2015. PMID: 25838375. DOI: 10.1126/science.aaa4971

5 Schumacher TN, Scheper W and Kvistborg P: Cancer neoantigens. Annu Rev Immunol *37*: 173-200, 2019. PMID: 30550719. DOI: 10.1146/annurev-immunol-042617-053402

6 Monach PA, Meredith SC, Siegel CT and Schreiber H: A unique tumor antigen produced by a single amino acid substitution. Immunity *2(1)*: 45-59, 1995. PMID: 7600302. DOI: 10.1016/1074-7613(95)90078-0

7 Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E and Rosenberg SA: A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med *183(3)*: 1185-1192, 1996. PMID: 8642260. DOI: 10.1084/jem.183.3.1185

8 Brändle D, Brasseur F, Weynants P, Boon T and Van den Eynde B: A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. J Exp Med *183(6)*: 2501-2508, 1996. PMID: 8676070. DOI: 10.1084/jem.183.6.2501

9 Topalian SL, Drake CG and Pardoll DM: Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell *27(4)*: 450-461, 2015. PMID: 25858804. DOI: 10.1016/j.ccell.2015.03.001

10 McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, Watkins TB, Shafi S, Murugaesu N, Mitter R, Akarca AU, Linares J, Marafioti T, Henry JY, Van Allen EM, Miao D, Schilling B, Schadendorf D, Garraway LA, Makarov V, Rizvi NA, Snyder A, Hellmann MD, Merghoub T, Wolchok JD, Shukla SA, Wu CJ, Peggs KS, Chan TA, Hadrup SR, Quezada SA and Swanton C: Clonal neoantigens elicit T cell

immunoreactivity and sensitivity to immune checkpoint blockade. Science *351(6280)*: 1463-1469, 2016. PMID: 26940869. DOI: 10.1126/science.aaf1490

11 Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN and Schreiber RD: Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature *515(7528)*: 577-581, 2014. PMID: 25428507. DOI: 10.1038/nature13988

12 Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Foppen MHG, Goldinger SM, Utikal J, Hassel JC, Weide B, Kaehler KC, Loquai C, Mohr P, Gutzmer R, Dummer R, Gabriel S, Wu CJ, Schadendorf D and Garraway LA: Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science *350(6257)*: 207-211, 2015. PMID: 26359337. DOI: 10.1126/science.aad0095

13 Keenan TE, Burke KP and Van Allen EM: Genomic correlates of response to immune checkpoint blockade. Nat Med *25(3)*: 389-402, 2019. PMID: 30842677. DOI: 10.1038/s41591-019-0382-x

14 Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER and Linette GP: Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science *348(6236)*: 803-808, 2015. PMID: 25837513. DOI: 10.1126/science.aaa3828

15 Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, Li S, Lieb DJ, Eisenhaure T, Gjini E, Stevens J, Lane WJ, Javeri I, Nellaiappan K, Salazar AM, Daley H, Seaman M, Buchbinder EI, Yoon CH, Harden M, Lennon N, Gabriel S, Rodig SJ, Barouch DH, Aster JC, Getz G, Wucherpfennig K, Neuberg D, Ritz J, Lander ES, Fritsch EF, Hacohen N and Wu CJ: An immunogenic personal neoantigen vaccine for patients with melanoma. Nature *547(7662)*: 217-221, 2017. PMID: 28678778. DOI: 10.1038/nature22991

16 Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Löwer M, Bukur V, Tadmor AD, Luxemburger U, Schrörs B, Omokoko T, Vormehr M, Albrecht C, Paruzynski A, Kuhn AN, Buck J, Heesch S, Schreeb KH, Müller F, Ortseifer I, Vogler I, Godehardt E, Attig S, Rae R, Breitkreuz A, Tolliver C, Suchan M, Martic G, Hohberger A, Sorn P, Diekmann J, Ciesla J, Waksmann O, Brück AK, Witt M, Zillgen M, Rothermel A, Kasemann B, Langer D, Bolte S, Diken M, Kreiter S, Nemecek R, Gebhardt C, Grabbe S, Höller C, Utikal J, Huber C, Loquai C and Türeci Ö: Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature *547(7662)*: 222-226, 2017. PMID: 28678784. DOI: 10.1038/nature23003

17 Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, Shukla SA, Hu Z, Li L, Le PM, Allesøe RL, Richman AR, Kowalczyk MS, Abdelrahman S, Geduldig JE, Charbonneau S, Pelton K, Iorgulescu JB, Elagina L, Zhang W, Olive O, McCluskey C, Olsen LR, Stevens J, Lane WJ, Salazar AM, Daley H, Wen PY, Chiocca EA, Harden M, Lennon NJ, Gabriel S, Getz G, Lander ES, Regev A, Ritz J, Neuberg D, Rodig SJ, Ligon KL, Suvà ML, Wucherpfennig KW, Hacohen N, Fritsch EF, Livak KJ, Ott PA, Wu CJ and Reardon DA: Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature *565(7738)*: 234-239, 2019. PMID: 30568305. DOI: 10.1038/s41586-018-0792-9

18 Kiyotani K, Chan HT and Nakamura Y: Immunopharmacogenomics towards personalized cancer immunotherapy targeting neoantigens. Cancer Sci *109(3)*: 542-549, 2018. PMID: 29288513. DOI: 10.1111/cas.13498

19 Saxena M and Bhardwaj N: Re-emergence of dendritic cell vaccines for cancer treatment. Trends Cancer *4(2)*: 119-137, 2018. PMID: 29458962. DOI: 10.1016/j.trecan.2017.12.007

20 Morisaki T, Matsumoto K, Onishi H, Kuroki H, Baba E, Tasaki A, Kubo M, Nakamura M, Inaba S, Yamaguchi K, Tanaka M and Katano M: Dendritic cell-based combined immunotherapy with autologous tumor-pulsed dendritic cell vaccine and activated T cells for cancer patients: rationale, current progress, and perspectives. Hum Cell *16(4)*: 175-182, 2003. PMID: 15147037. DOI: 10.1111/j.1749-0774.2003.tb00151.x

21 Katano M, Morisaki T, Koga K, Nakamura M, Onishi H, Matsumoto K, Tasaki A, Nakashima H, Akiyoshi T and Nakamura M: Combination therapy with tumor cell-pulsed dendritic cells and activated lymphocytes for patients with disseminated carcinomas. Anticancer Res *25(6A)*: 3771-3776, 2005. PMID: 16302738.

22 Onishi H, Morisaki T, Baba E, Nakamura M, Inaba S, Kuroki H, Matsumoto K and Katano M: Long-term vaccine therapy with autologous whole tumor cell-pulsed dendritic cells for a patient with recurrent rectal carcinoma. Anticancer Res *31(11)*: 3995-4005, 2011. PMID: 22110233.

23 Morisaki T, Hikichi T, Onishi H, Morisaki T, Kubo M, Hirano T, Yoshimura S, Kiyotani K and Nakamura Y: Intranodal administration of neoantigen peptide-loaded dendritic cell vaccine elicits epitope-specific T cell responses and clinical effects in a patient with chemorefractory ovarian cancer with malignant ascites. Immunol Invest: 1-18, 2020. PMID: 32660279. DOI: 10.1080/08820139.2020.1778721

24 Kiyotani K, Park JH, Inoue H, Husain A, Olugbile S, Zewde M, Nakamura Y and Vigneswaran WT: Integrated analysis of somatic mutations and immune microenvironment in malignant pleural mesothelioma. Oncoimmunology *6(2)*: e1278330, 2017. PMID: 28344893. DOI: 10.1080/2162402X.2016.1278330

25 Szolek A, Schubert B, Mohr C, Sturm M, Feldhahn M and Kohlbacher O: OptiType: precision HLA typing from next-generation sequencing data. Bioinformatics *30(23)*: 3310-3316, 2014. PMID: 25143287. DOI: 10.1093/bioinformatics/btu548

26 Garg AD, Vara Perez M, Schaaf M, Agostinis P, Zitvogel L, Kroemer G and Galluzzi L: Trial watch: Dendritic cell-based anticancer immunotherapy. Oncoimmunology *6(7)*: e1328341, 2017. PMID: 28811970. DOI: 10.1080/2162402X.2017.1328341

27 Aranda F, Vacchelli E, Eggermont A, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G and Galluzzi L: Trial Watch: Peptide vaccines in cancer therapy. Oncoimmunology *2(12)*: e26621, 2013. PMID: 24498550. DOI: 10.4161/onci.26621

28 Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D and Lyerly HK: Migration of human dendritic cells after injection in patients with metastatic malignancies. Cancer Res *59(1)*: 56-58, 1999. PMID: 9892184.

29 Bedrosian I, Mick R, Xu S, Nisenbaum H, Faries M, Zhang P, Cohen PA, Koski G and Czerniecki BJ: Intranodal administration of peptide-pulsed mature dendritic cell vaccines

results in superior CD8+ T-cell function in melanoma patients. J Clin Oncol *21(20)*: 3826-3835, 2003. PMID: 14551301. DOI: 10.1200/JCO.2003.04.042

30 Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci O and Sahin U: Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. Cancer Res *70(22)*: 9031-9040, 2010. PMID: 21045153. DOI: 10.1158/0008-5472.CAN-10-0699

31 Aarntzen EH, Schreibelt G, Bol K, Lesterhuis WJ, Croockewit AJ, de Wilt JH, van Rossum MM, Blokx WA, Jacobs JF, Duiveman-de Boer T, Schuurhuis DH, Mus R, Thielemans K, de Vries IJ, Figdor CG, Punt CJ and Adema GJ: Vaccination with mRNA-electroporated dendritic cells induces robust tumor antigen-specific CD4+ and CD8+ T cells responses in stage III and IV melanoma patients. Clin Cancer Res *18(19)*: 5460-5470, 2012. PMID: 22896657. DOI: 10.1158/1078-0432.CCR-11-3368

32 Acton SE, Farrugia AJ, Astarita JL, Mourão-Sá D, Jenkins RP, Nye E, Hooper S, van Blijswijk J, Rogers NC, Snelgrove KJ, Rosewell I, Moita LF, Stamp G, Turley SJ, Sahai E and Reis e Sousa C: Dendritic cells control fibroblastic reticular network tension and lymph node expansion. Nature *514(7523)*: 498-502, 2014. PMID: 25341788. DOI: 10.1038/nature13814

33 Blaeschke F, Paul MC, Schuhmann MU, Rabsteyn A, Schroeder C, Casadei N, Matthes J, Mohr C, Lotfi R, Wagner B, Kaeuferle T, Feucht J, Willier S, Handgretinger R, StevanoviĆ S, Lang P and Feuchtinger T: Low mutational load in pediatric medulloblastoma still translates into neoantigens as targets for specific T-cell immunotherapy. Cytotherapy *21(9)*: 973-986, 2019. PMID: 31351799. DOI: 10.1016/j.jcyt.2019.06.009

34 Zamora AE, Crawford JC, Allen EK, Guo XJ, Bakke J, Carter RA, Abdelsamed HA, Moustaki A, Li Y, Chang TC, Awad W, Dallas MH, Mullighan CG, Downing JR, Geiger TL, Chen T, Green DR, Youngblood BA, Zhang J and Thomas PG: Pediatric patients with acute lymphoblastic leukemia generate abundant and functional neoantigen-specific CD8$^+$ T cell responses. Sci Transl Med *11(498)*: eaat8549, 2019. PMID: 31243155. DOI: 10.1126/scitranslmed.aat8549

35 Durgeau A, Virk Y, Corgnac S and Mami-Chouaib F: Recent advances in targeting CD8 T-cell immunity for more effective cancer immunotherapy. Front Immunol *9*: 14, 2018. PMID: 29403496. DOI: 10.3389/fimmu.2018.00014

36 Brightman SE, Naradikian MS, Miller AM and Schoenberger SP: Harnessing neoantigen specific CD4 T cells for cancer immunotherapy. J Leukoc Biol *107(4)*: 625-633, 2020. PMID: 32170883. DOI: 10.1002/JLB.5RI0220-603RR

37 Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A and von Knebel Doeberitz M: Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology *134(4)*: 988-997, 2008. PMID: 18395080. DOI: 10.1053/j.gastro.2008.01.015

38 Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, Chambers T, Salgado R, Savas P, Loi S, Birkbak NJ, Sansregret L, Gore M, Larkin J, Quezada SA and Swanton C: Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. Lancet Oncol *18(8)*: 1009-1021, 2017. PMID: 28694034. DOI: 10.1016/S1470-2045(17)30516-8

39 Blass E and Ott PA: Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. Nat Rev Clin Oncol *18(4)*: 215-229, 2021. PMID: 33473220. DOI: 10.1038/s41571-020-00460-2

40 Zhang R, Yuan F, Shu Y, Tian Y, Zhou B, Yi L, Zhang X, Ding Z, Xu H and Yang L: Personalized neoantigen-pulsed dendritic cell vaccines show superior immunogenicity to neoantigen-adjuvant vaccines in mouse tumor models. Cancer Immunol Immunother *69(1)*: 135-145, 2020. PMID: 31807878. DOI: 10.1007/s00262-019-02448-z

41 Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, Friedlander T, Bushway ME, Balogh KN, Sciuto TE, Kohler V, Turnbull SJ, Besada R, Curran RR, Trapp B, Scherer J, Poran A, Harjanto D, Barthelme D, Ting YS, Dong JZ, Ware Y, Huang Y, Huang Z, Wanamaker A, Cleary LD, Moles MA, Manson K, Greshock J, Khondker ZS, Fritsch E, Rooney MS, DeMario M, Gaynor RB and Srinivasan L: A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell *183(2)*: 347-362.e24, 2020. PMID: 33064988. DOI: 10.1016/j.cell.2020.08.053

Received May 22, 2021
Revised June 12, 2021
Accepted June 14, 2021