


Immunological evaluation of peptide vaccination for cancer patients with the HLA -A11⁺ or -A33⁺ allele

Shinjiro Sakamoto,^{1,2,3}  Satoko Matsueda,² Shinzo Takamori,⁴ Uhi Toh,⁴ Masanori Noguchi,¹ Shigeru Yutani,²  Akira Yamada,¹ Shigeki Shichijo,² Teppei Yamada,⁵ Shigetaka Suekane,⁶ Kouichiro Kawano,⁷ Masayasu Naitou,² Tetsuro Sasada,^{2,8}  Noboru Hattori,³ Nobuoki Kohno⁹ and Kyogo Itoh²

¹Research Center for Innovative Cancer Therapy, Kurume University, Kurume; ²Cancer Vaccine Center, Kurume University, Kurume; ³Department of Molecular and Internal Medicine School of Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima; ⁴Department of Surgery, Kurume University School of Medicine, Kurume; ⁵Department of Gastroenterological Surgery, Fukuoka University School of Medicine, Fukuoka; ⁶Department of Urology, Kurume University School of Medicine, Kurume; ⁷Department of Obstetrics and Gynecology, Kurume University School of Medicine, Kurume; ⁸Kanagawa Cancer Center Research Institute, Yokohama; ⁹Hiroshima Cosmopolitan University, Hiroshima, Japan

Key words

cytotoxic T-Lymphocytes, HLA-A11, HLA-A33, IgG, peptide vaccine

Correspondence

Shinjiro Sakamoto, MD, Research Center for Innovative Cancer Therapy, Kurume University, 67 Asahi-machi, Kurume 830-0011, Japan. Tel: +81-942-31-7989; E-mail: sakamoto_shinjiro@kurume-u.ac.jp

Funding Information

Japan Agency for Medical Research and Development, Regional Innovation Cluster Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan

Clinical Trial Number: UMIN Clinical Trials Registry UMIN no. 1482, 1844, 1856, 1883, 2282, 2908, 2984, 2987, 3028, 3081, 6249, 6295, 6493, 7493, 10290 and 11230

Received October 17, 2016; Revised January 25, 2017; Accepted February 4, 2017

Cancer Sci 108 (2017) 598–603

doi: 10.1111/cas.13189

Although many clinical trials of peptide-based cancer vaccines for HLA-A2⁺ and -A24⁺ cancer patients have been conducted in the past two decades, cancer patients with the other alleles, including the HLA-A11 and -A33 allele, were excluded from the clinical trials because of the relatively lower expression of these alleles worldwide.^(1–8) We identified short peptides capable of inducing CTLs in HLA-A11⁺ or -A33⁺ cancer patients.^(5,6) Some of them were provided for clinical trials to advanced cancer patients refractory or intolerant to standard therapy under PPV regimens in the past 7 years. The HLA-A11 or -A33 allele is found in approximately 18.2% or 9.9% of the Asian population (10.4% or 7.7% in the Japanese, 9.4% or 14.9% in the Korean, 20.3% or 3.4% in the North Han, and 32.5% or 13.6% in the Thai population), 5.9% or 2.2% of the European population (5.5% and 1.0% in the US Caucasian, 6.2% or 3.1% in the French, 4.6% or 3.1% in the German, and 7.3% or 1.7% in the British population), 7.9% or 3.9% of the Spanish population (7.0% or 2.8% in the Spanish, and 8.8% or 5.0% in the Gypsy population), and 1.1% and 8.7% of the Black population (4.2% or 8.0% in the North African, 0.0% or 1.5% in the South African, 0.0 or 15.2 of the

The HLA-A11 or -A33 allele is found in approximately 18% or 10% of the Asian population, respectively, but each of which is a minor allele worldwide, and therefore no clinical trials were previously conducted. To develop a therapeutic peptide vaccine for each of them, we investigated immunological responses of advanced cancer patients with the HLA-A11⁺/A11⁺ (*n* = 18) or -A33⁺/A33⁺ (*n* = 13) allele to personalized peptide vaccine (PPV) regimens. The primary sites of HLA-A11⁺/A11⁺ or -A33⁺/A33⁺ patients were the colon (*n* = 4 or 2), stomach (2 or 3), breast (3 or 2), lung and pancreas (2 or 2), and so on. For PPV, a maximum of four peptides were selected from nine different peptides capable of binding to HLA-A11 and -A33 molecules based on the pre-existing peptide-specific IgG responses. There were no severe adverse events related to PPV. At the end of the first cycle, peptide-specific CTL responses were augmented in 4/12 or 2/9 of HLA-A11⁺/A11⁺ or -A33⁺/A33⁺ patients, while peptide-specific IgG responses were augmented in 6/14 or 4/10 patients, respectively. Clinical responses consisted of four stable diseases and 14 progressive diseases in HLA-A11⁺/A11⁺ patients, versus seven and six in -A33⁺/A33⁺ patients, respectively. Further clinical study of PPV could be recommended because of the safety and positive immunological responses.

West Africa, and 0.0 or 10.0% in the Senegalese population), respectively.^(7,8) To develop peptide-based cancer vaccines for cancer patients in these minor populations, we conducted clinical studies of PPV for advanced cancer patients with the HLA-A11⁺/A11⁺ or -A33⁺/A33⁺ allele in the past 7 years, and report herein the safety and immune responses of these clinical trials. Although we also performed the same PPV regimens for HLA-A11 or -A33 cancer patients who co-expressed the other HLA-A, including the -A2, -A24, or -A3 supertype, or -A26, those results were not included in this retrospective study.

Patients and Methods

Peptides. Nine different CTL epitope peptides—CypB_{129–138} KLKHYGPGWV, Lck_{90–99} ILEQSGEWWK, Lck_{422–430} DVWSFGILL, Lck_{449–458} VIQNLGERYR, WHSC2_{103–111} ASLSDPWV, SART3_{109–118} VYDYNCHVDL, SART3_{511–519} WLEYYNLER, SART3_{734–742} QIRPFSNR and PAP_{248–257} GIHKQKEKSR—were used in this study. These peptides were previously shown to be capable of inducing peptide-specific and tumor-reactive CTL activity in HLA -A11⁺

and -A33⁺ cancer patients.^(5,6) These peptides were prepared under the conditions of Good Manufacturing Practice by the PolyPeptide Laboratories (San Diego, CA, USA) and American Peptide Company (Vista, CA, USA) as reported previously.⁽¹⁻⁶⁾

Patients. This is a retrospective analysis with cancer patients with HLA-A11⁺/A11⁺ or -A33⁺/A33⁺, who were enrolled in phase II studies of PPV with different regimens for different types of cancers. 18 HLA-A11⁺/A11⁺ patients, including four colon, three breast, two pancreas, two lung, two prostate, two stomach, two biliary tract, and each of bone, uterus and ovary patients, and 13 HLA-A33⁺/A33⁺ patients, including three stomach, two pancreas, two breast, two lung, two colon, and each of prostate and bone patients, were enrolled. They had to show positive IgG responses to at least two of the nine peptide candidates applicable for HLA-A11⁺ and -A33⁺ cancer patients.^(5,6) Other inclusion criteria were as follows: age between 20 and 80 years; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 at the time of first visit; life expectancy of at least 12 weeks; and adequate hematologic, hepatic, and renal function. Exclusion criteria included pulmonary, cardiac, or other systemic diseases; an acute infection; a history of severe allergic reactions; pregnancy or nursing; and other inappropriate conditions for enrollment as judged by clinicians. These studies were approved by the Kurume University Ethical Committee and registered in the UMIN Clinical Trials Registry (UMIN no. 1482, 1844, 1856, 1883, 2282, 2908, 2984, 2987, 3028, 3081, 6249, 6295, 6493, 7493, 10290 and 11230). All patients were given a full explanation of the protocol and provided their informed consent before enrollment.

Clinical protocol. The main objective of these phase II studies was to evaluate the safety and immunological responses. The secondary endpoints were clinical benefits from the viewpoint of overall survival (OS) and biomarker analysis. Peptides for vaccination to individual patients were selected in consideration of the pre-existing host immunity before vaccination, as assessed by the titers of IgG specific to each of the nine different vaccine candidates.⁽³⁻⁶⁾ A maximum of four peptides (3 mg/each peptide), which were selected based on the results of HLA typing and peptide-specific IgG titers, were subcutaneously administered with incomplete Freund's adjuvant (Montanide ISA51; Seppic, Paris, France). There are three different administration interval regimens based on the different protocols for different sites of primary cancers (Fig. 1). Administration interval of 1st cycle were six times weekly (UMIN no. 1482, 1844, 1856, 1883, 2282, 6249, 6295, 6493, 7493 and 10290), four times weekly followed by four times every 2 weeks (UMIN no. 2908, 2984, 2987, 3028 and 3081), or

four times every 4 weeks (UMIN no. 11230), respectively. After the first cycle of vaccinations, up to four antigen peptides were re-selected according to the titers of peptide-specific IgG. Administration interval of 2nd cycle were six times every 2 weeks (UMIN no. 1482, 1844, 1856, 1883, 2282, 6249, 6295, 6493, 7493 and 10290), eight times every 2 weeks (UMIN no. 2908, 2984, 2987, 3028 and 3081), or four times every 4 weeks (UMIN no. 11230). During the PPV, patients were allowed to receive combination therapies, including chemotherapies such as target therapy, hormone therapy, or radiotherapy. Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI-CTC Ver.-4.0). Complete blood counts and serum biochemistry tests were performed before and after each cycle of vaccinations. Tumor assessments by computed tomography (CT) or magnetic resonance imaging (MRI) scans were carried out before and after PPV, and were evaluated according to the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.

Laboratory markers. Humoral immune responses specific to the vaccine peptides were determined by peptide-specific IgG levels using a bead-based multiplex assay with the Luminex system (Luminex, Austin, TX, USA) as previously reported.⁽³⁻⁶⁾ If the titers of peptide-specific IgG to at least one of the vaccinated peptides at the end of the 1st cycle were more than two-fold higher than those in the pre-vaccination plasma, the changes were considered to be significant as previously reported.⁽³⁻⁶⁾ Cytotoxic T lymphocyte (CTL) responses specific to the vaccinated peptides were evaluated by capacity of interferon (IFN)- γ production with ELISPOT assays (MBL, Nagoya, Japan) using peripheral blood mononuclear cells (PBMCs) before and at the end of the first and second cycle as previously reported.^(3-6,9) As non-vaccinated peptides, a mixture of virus-derived CTL epitopes (CEF peptides; Mabtech) was provided for the assay. In the CTL assay, if the spot numbers in response to at least one of the vaccine peptides in the post-vaccination PBMC were more than two-fold higher than those in the pre-vaccination PBMC, the changes were considered significant. Moreover, in order to evaluate the strength of the peptide-specific IgG response, we evaluated the rate of changes of peptide-specific IgG titers between before and after the first or second cycle of vaccination. In this evaluation, IgG titers before PPV were set to 1.0. We also assessed the significance of strength of the peptide-specific IgG response after the second cycle of vaccination. Patients who finished the second cycle of PPV were divided into two subgroups according to the median value of the rate of changes of peptide-specific IgG titers between before and after the second cycle of vaccination. Regarding the cytokines, the levels of granulocyte

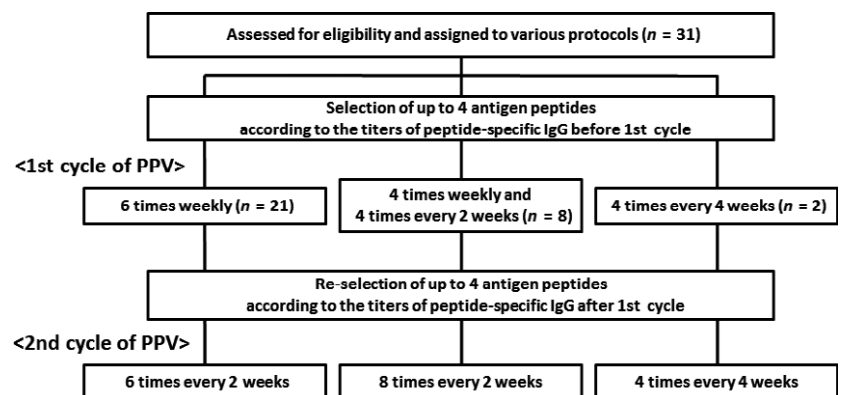


Fig. 1. Personalized peptide vaccine's flow and three kinds of administration interval.

macrophage colony-stimulating factor (GM-CSF), interleukin (IL)1 β , IL2, IL4, IL5, IL6, IL8, IL10 and tumor necrosis factor (TNF)- α in plasma before and after one cycle of vaccination were measured with a Luminex system (Luminex) using kits from Thermo Fisher Scientific K.K. (Yokohama, Japan), as reported previously.^(4,9)

Statistical analyses. All data were analyzed retrospectively. Comparison of quantitative variables was carried out by Welch's *t*-test or Mann–Whitney *U*-test. Comparison of categorical variables was carried out by the chi-square test. The survival curves were estimated by the Kaplan–Meier method, and differences in survival functions were compared using the log-rank test. OS was calculated from the 1st day of peptide vaccination until the date of death or the last date when the patient was known to be alive using the log-rank test. Values of *P* < 0.05 were considered statistically significant. Statistical analyses were conducted using JMP version software, version 12 (SAS Institute Inc., Cary, NC, USA).

Results

Patients' characteristics. Between January 2009 and November 2015, 31 advanced cancer patients with HLA-A11⁺/A11⁺ (*n* = 18) or -A33⁺/A33⁺ (*n* = 13) were enrolled in this study. Their genotypes were all HLA-A11:01 or HLA-A33:03, respectively. Details of the patients' characteristics are shown in Table 1. The median age of HLA-A11⁺/A11⁺ patients was 59 years with 7 males and 11 females. Performance status was 0 in 13 and 1 in 15 patients. The sites of primary cancers were the colon (*n* = 4), breast (3), pancreas (2), lung (2), prostate

(2), stomach (2), biliary tract (2), and each of bone, uterus and ovary. All patients had failed (*n* = 15) or proven intolerant to (*n* = 3) standard chemotherapy before entry to PPV. Eleven patients received PPV combined with systemic therapies, whereas the remaining seven patients received PPV alone since of intolerance to any combined systemic therapies. The median vaccination time was 4.6 months (0.0–29.0).

The median age of HLA-A33⁺/A33⁺ patients was 58 years with nine males and four females. Performance status was 0 in 10 and 1 in three patients. The sites of primary cancers were the stomach (*n* = 3), pancreas (2), breast (2), lung (2), colon (2), and each of prostate and bone. All patients failed (*n* = 10) or proved intolerant to (*n* = 3) standard chemotherapy before entry to PPV. Nine patients received PPV combined with systemic therapies, whereas the remaining four patients received PPV alone since of intolerance to any combined systemic therapies. The median vaccination time was 3.8 months (1.4–31.9).

Adverse events. Grade I (*n* = 21) or II (*n* = 6) injection site skin reactions were the most frequently observed adverse events during PPV (Table S1). There were 18 cases of grade 3 severe adverse events (SAEs), and one case of grade 4 SAE (leukopenia). All of the SAEs were concluded to be not directly associated with the PPV, but with the disease progression or combined therapy according to the evaluation by an independent safety evaluation committee.

Immune responses. Both peptide-specific CTL and IgG responses were analyzed in blood samples before and after the first or second cycle of vaccination. CTL responses to the vaccinated peptides before vaccination were detected in only 1 of 12 HLA-A11⁺/A11⁺ or 0 of 9 HLA-A33⁺/A33⁺ patients tested, respectively (Tables S2 and S3). In contrast, those to the non-vaccinated control CEF peptides were observed in nine of 12 or four of nine patients, respectively. CTL boosting in response to the vaccinated peptides at the end of the first cycle was observed in four of 12 HLA-A11⁺/A11⁺ or two of nine HLA-A33⁺/A33⁺ patients tested, and that at the end of the second cycle was observed in three of six or one of seven, respectively. In contrast, IFN- γ spots of CTL activity in response to the non-vaccinated control CEF peptides were not significantly different throughout the PPV (Tables S2 and S3).

IgG responses before vaccination were well observed in all patients. Increase of IgG responses specific to at least one of the vaccinated peptides after the 1st and 2nd cycles were observed in six of 14 and seven of seven patients tested with the HLA-A11⁺/A11⁺, and in four of 10 and seven of nine patients tested with the -A33⁺/A33⁺ (Tables S2 and S3). Regarding the rate of changes of peptide-specific IgG titers, a 25- or 127-fold increase of IgG response was observed in the samples at the end of the first or second cycle when the titers of pre-vaccinations were set as 1.0 in HLA-A11⁺/A11⁺ patients (*P* = 0.08 or *P* = 0.002), respectively (Fig. 2a). Similarly, a 4- or 41-fold increase of IgG response was observed in HLA-A33⁺/A33⁺ patients (*P* = 0.11 or *P* = 0.01, respectively). Moreover, regarding the significance of strength of the peptide-specific IgG response after 2nd cycle of vaccination, the patients with higher rate of changes of peptide-specific IgG titers after 2nd cycle of vaccination showed longer prognosis than those with lower rate (*P* = 0.03; Fig. 2b).

Clinical response and biomarkers. Best clinical responses were evaluated according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 based on tumor assessments with computed tomography (CT) or magnetic resonance imaging (MRI) scans before and after PPV. There were no

Table 1. Patients' characteristics

| | HLA-A11 ⁺ /A11 ⁺ (<i>n</i> = 18) | HLA-A33 ⁺ /A33 ⁺ (<i>n</i> = 13) | <i>P</i> -value† |
|---------------------------------------|--|--|------------------|
| Median age (range) | 59 (20–71) | 58 (23–84) | 0.99 |
| Sex | | | |
| Male/Female | 7/11 | 9/4 | 0.07 |
| Performance status | | | |
| 0/1 | 13/15 | 10/3 | 0.83 |
| Primary tumor site | | | |
| Pancreas | 2 | 2 | 0.35 |
| Breast | 3 | 2 | |
| Colon | 4 | 2 | |
| Lung | 2 | 2 | |
| Prostate | 2 | 1 | |
| Stomach | | 3 | |
| Bone | 1 | 1 | |
| Liver | | | |
| Biliary tract | 2 | | |
| Uterus | 1 | | |
| Ovarian | 1 | | |
| Stage at diagnosis | | | |
| II/III/IV/Recurrence | 0/1/7/10 | 2/1/6/4 | 0.16 |
| Median lymphocyte count of enrollment | 1372 (911–2055) | 1411 (1071–2656) | 0.79 |
| Previous chemotherapy | | | |
| +/- | 3/15 | 3/10 | 0.61 |
| Combined chemotherapy with PPV | | | |
| +/- | 7/11 | 4/9 | 0.72 |

PPV, personalized peptide vaccine. †Welch test or the χ^2 test was performed to examine *P*-values for continuous values or categorical values.

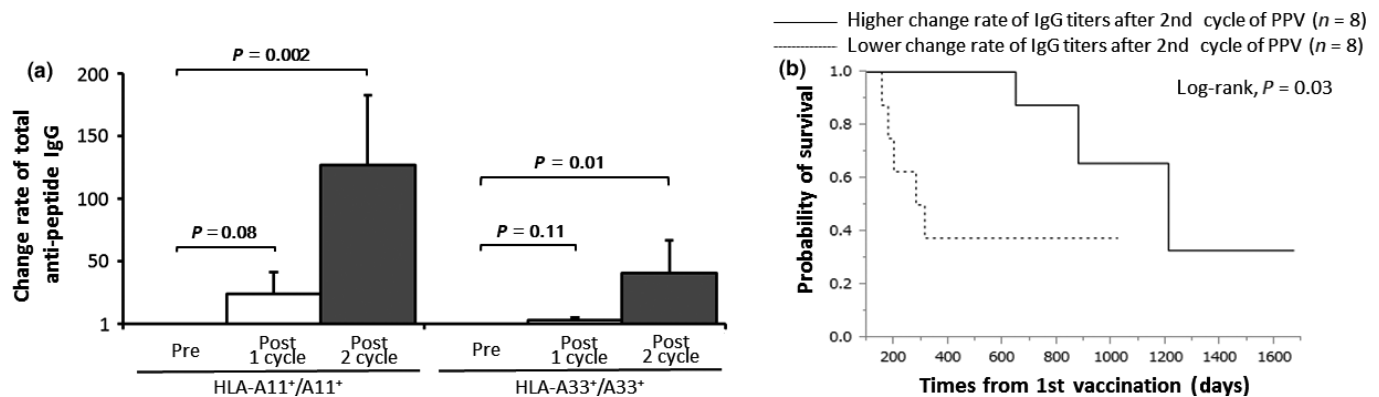


Fig. 2. Immune responses. (a) Rates of changes of peptide-specific IgG titers. A 25- or 127-fold increase of IgG titers was observed when the IgG titers of pre-vaccinations were set as 1.0 in HLA-A11⁺/A11⁺ patients ($P = 0.08$ or $P = 0.002$), respectively. Similarly, a 4- or 41-fold increase was observed in HLA-A33⁺/A33⁺ patients ($P = 0.11$ or $P = 0.01$). Fig. 2(b): To examine the significance of strength of the peptide-specific IgG response, when 16 HLA-A11⁺/A11⁺ or -A33⁺/A33⁺ patients who finished 2nd cycle of PPV were divided into two subgroups according to the median value of the rate of changes of peptide-specific IgG titers between before and after 2nd cycle of vaccination, the patients with higher rate of changes of peptide-specific IgG titers after 2nd cycle of vaccination showed longer prognosis than those with lower rate ($P = 0.03$).

complete or partial responses. There were four cases of stable disease and 14 of progressive disease in HLA-A11⁺/A11⁺ patients with a median overall survival (MST) of 332 days, while these numbers were seven and six in -A31⁺/A31⁺ patients with an MST of 177 days, respectively. Most patients received PPV combined with chemotherapy, hormone therapy, or radiotherapy, and therefore it was difficult to evaluate the true contribution of the PPV to SD. However, one HLA-A31⁺/A31⁺ lung cancer patient was treated with PPV mono-therapy, and he showed a long SD term of 26.7 months (Fig. 3).

There were no significant differences in MST between the 4 HLA-A11⁺/A11⁺ patients with increased IgG responses at the end of the first cycle and the remaining seven HLA-A11⁺/A11⁺ patients without it. None of the cytokine levels in pre- or post-vaccination samples were predictive of overall survival. However, when the HLA-A11⁺/A11⁺ patients were divided into two groups according to whether their various cytokine levels increased or decreased after PPV, those with increased IL4 or IL6 levels, but not those with increases in any other cytokines tested, showed worse prognosis than those with decreased IL4 ($P = 0.01$; Fig. 4a) or IL6 ($P = 0.07$, Fig. 4b) levels, respectively. Since the number of HLA-A31⁺/A31⁺ patients ($n = 7$) was so small, it was difficult to reach any definitive conclusions in regard to these biomarkers.

Discussion

Although large numbers of clinical trials of peptide-based cancer vaccine have been conducted in the past two decades, as far as we know, this is the first report of a clinical trial of peptide-based cancer vaccines for HLA-A11⁺ or -A31⁺ cancer patients. This delay could be primarily due to the lower allele frequency (around 5%) in European, Spanish and Black populations. However, the frequencies of both alleles are relatively high in the Asian population (10% to 20%). In addition, HLA-A11 or -A33 shares cross-reactive binding affinity and thus they are classified as members of the HLA-A3 supertype. Indeed, five of nine peptides used in PPV have confirmed binding to the HLA-A3 supertype allele, IgG responses and CTL responses.^(10–12) In addition, remaining four peptides were tested for the capability of CTL responses.^(13–16) These results suggest that the shared peptides could be applicable for both HLA-A11⁺ and -A31⁺ patients. These several advantages

allowed us to carry out a clinical trial of peptide-based cancer vaccines for 18 HLA-A11⁺/A11⁺ or 13 -A31⁺/A31⁺ cancer patients under the PPV over the past 7 years. Although we also performed the same PPV regimens for HLA-A11 or -A33 cancer patients who co-expressed the other HLA-A, including the -A2, -A24, or -A3 supertype alleles, or -A26, those results were not included in this retrospective study in order to avoid any bias in terms of the safety evaluation, immune responses, or clinical benefits, primarily because the nine peptides used in this study exhibit cross-reactivity to the other HLA-A molecules, including a member of the HLA-A3 supertype.

From the viewpoint of adverse events, grade 1 or 2 injection site reaction was the primary event, observed in 68% or 19% of the patients, respectively. Grade 3 or 4 SAEs were observed in 18 cases or in one case. However, in all these cases the SAEs were concluded to be not directly associated with the PPV, but rather with the disease progression or combined therapy. These results indicated that PPV was safely conducted in HLA-A11⁺/A11⁺ and -A31⁺/A31⁺ cancer patients, which is consistent with the results on PPV for the HLA-A24⁺ or -A2⁺ cancer patients reported previously.^(3–6,9)

Successful boosting of HLA-A11 or -A33 restricted peptide-specific CTL responses after the PPV was observed in only a portion of the patients tested. This may have been partly due to the deeply depressed anti-tumor cellular responses of these patients, since CTL responses to the vaccinated peptides in pre-vaccination PBMCs, but not CTL responses to non-vaccinated CEF peptides reactive to virus, were undetectable in these patients (i.e., they were detectable in only one of 12 HLA-A11 or zero of nine -A33 patients). In contrast, peptide-specific IgG responses were well observed in pre-vaccination samples in all patients tested. In addition, the successful boosting was observed in nearly half of all patients tested at the end of the first or second cycles, respectively. There were several possible explanations for this discrepancy between the rate of successful CTL and IgG boosting. CD8⁺ T cell numbers and their functions, but not the numbers and functions of CD4⁺ T cells, are generally depressed in advanced cancer patients who become refractory to chemotherapy. The other explanation could be deletion or depressed expression of HLA-class IA molecules on the surface of cancer cells from HLA-A11⁺/A11⁺ or -A31⁺/A31⁺ cancer patients. Cancer cells with a homogenous HLA-class I A allele might more easily become

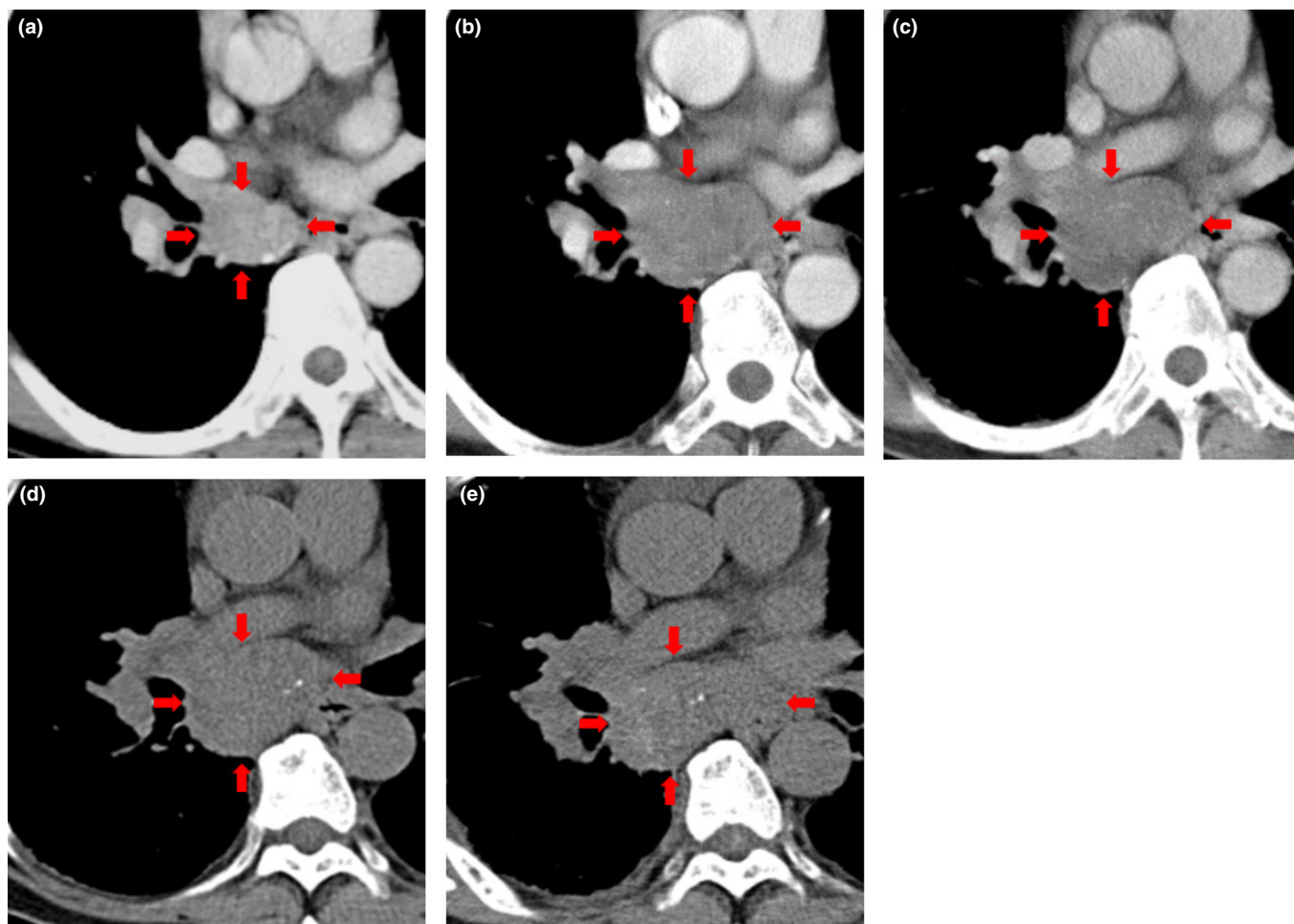


Fig. 3. A case of long stable disease (SD). Computed tomography findings of an HLA-A31⁺/A31⁺ non-small cell lung cancer patient (adenocarcinoma) with PPV monotherapy throughout PPV. (a) At diagnosis of lung cancer at stage 3a (8 months before PPV; tumor size: 37 × 22 mm); (b) At the time of 1st vaccination of PPV (tumor size: 50 × 35 mm); (c) At 3 months after PPV (52 × 33 mm); (d) At 12 months after PPV (53 × 36 mm); and (e) At 26 months after PPV (64 × 35 mm). An interval of SD was judged as 26.7 months. Red arrows indicate main tumor site.

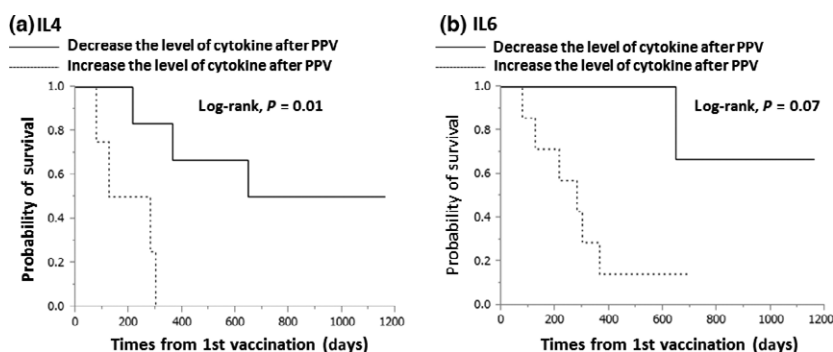


Fig. 4. Biomarker study. When 10 HLA-A11⁺/A11⁺ patients were divided into two groups according to the increase ($n = 7$) or decrease ($n = 3$) in each cytokine level from pre-vaccination to the end of the first cycle, the patients with increased IL4 or IL6 levels showed worse prognosis than those with decreased levels in IL4 ($P = 0.01$; Fig. 3a) or IL6, respectively ($P = 0.07$; Fig. 3b).

resistant to CTL-mediated tumor cell killing as compared to those with a heterogeneous allele, which might in turn be partly responsible for the decrease of memory/activated CTLs in circulation. Therefore, from a clinical point of view, if HLA-A11 or -A33 cancer patients co-expressed the HLA-A2 and -A24 allele, it might be appropriate to use both peptides matched to the HLA-A3 supertype and those matched to HLA-A2 or -A24. In fact, we have been doing this in PPV regimens for some time now, and have achieved high rates of successful boosting of peptide-specific CTL responses, as reported

previously.^(3-6,9) Alternatively, the numbers of pooled peptides may have been too small to correspond to antigenic heterogeneity of tumor cells and also the diverse immune responses among cancer patients.

Clinical benefit was not the main objective of this small-scale study for cancer patients with different histological types. Nonetheless, the clinical-outcome data are of interest. Clinical responses consisted of four cases of stable disease and 14 of progressive disease in HLA-A11⁺/A11⁺ patients with an MST of 332 days, versus seven and six cases in -A33⁺/A33⁺ patients

with an MST of 177 days, respectively. Notably, a longer period of SD of 26.7 months was observed in one HLA-A33⁺/A33⁺ lung cancer patient under PPV alone. Despite these limited data, we considered it worthwhile to analyze the ability of the various biomarkers to predict overall survival. The results indicated that an increase in IL4 or IL6 levels after PPV was an unfavorable biomarker in HLA-A11⁺/A11⁺ cancer patients. This was expected, since we previously reported that an increase of inflammatory cytokines (IL6, IL8 and others) was an unfavorable biomarker in advanced cancer patients under PPV. Although we previously reported that an increase in peptide-specific immune responses, particularly IgG responses, was a favorable biomarker,^(9,17,18) it was not achieved in this study primarily due to the fact that successful IgG boosting was obtained in the vast majority of patients (all seven HLA-A11⁺/A11⁺ and seven of nine -A33⁺/A33⁺ patients) at the end of the second cycle. However, patients with more successful IgG boosting survived longer than those without it suggesting that peptide-specific IgG responses could also be a favorable biomarker for the overall survival of these patients. This issue, however, will be addressed in the next clinical study with a larger number of patients.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Adverse events during the personalized peptide vaccine.

Table S2. Immune responses of HLA-A11⁺/A11⁺ patients after 1st and 2nd cycle of PPV.

Table S3. Immune responses of HLA-A33⁺/A33⁺ patients after 1st and 2nd cycle of PPV.

Collectively, the results in this study suggest that, PPVs with these nine CTL epitope peptides exhibit a good safety profile and positive immunological responses in HLA-A11⁺ or -A33⁺ cancer patients. Further clinical trials are warranted.

Acknowledgments

This study was supported in part by the Japan Agency for Medical Research and development (AMED); by a research program of the Regional Innovation Cluster Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan; and by a grant from the Sendai Kousei Hospital.

Disclosure Statement

Akira Yamada is a board member of the Green Peptide Co., Ltd. Kyogo Itoh and Akira Yamada hold stock in the Green Peptide Co., Ltd. Kyogo Itoh received research funds from Taiho Pharmaceutical Co., Ltd. No potential conflicts of interest were declared by the other authors.

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