Immune responses of patients without cancer recurrence after a cancer vaccine over a long term

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Abstract. The present study aimed to clarify the humoral and cellular immune responses of patients with cancer who experienced no recurrence over a long term after receiving a cancer vaccine. The immune kinetics were investigated in response to a personalized peptide vaccination (PPV) among 44 Japanese patients without an active tumor at entry to the vaccination: Lung adenocarcinoma (n=11); colon (n=18); and breast cancer (n=15) (9, 10, 12, 8 and 5 patients with stage I, II, III and IV recurrences, respectively). The patients' immunoglobulin G (IgG) and cytotoxic T lymphocyte (CTL) activities were measured using a multiplexed Luminex assay and an interferon-γ release assay, respectively. There were no severe adverse events related to the PPV other than a grade III injection site reaction. A potent boost in IgG or CTL at the end of the 1st vaccination cycle was observed in 77% of the patients (n=84). The IgG levels were sustained throughout the follow-up period, whereas the CTL levels declined and were transient. A total of 37 of the 44 patients (84%) had no recurrence, with a median follow-up of 67.6 months (interquartile range, 45.6-82.8 months). Overall, the PPV induced long-term humoral immunity with transient cellular immunity in the majority of patients with cancer without an active tumor at their entry to the PPV.

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Abbreviations: CTL, cytotoxic T lymphocyte; ELISPOT, Enzyme-Linked ImmunoSpot; FIU, fluorescence intensity unit; HER2, human epidermal growth factor receptor 2; HLA, human leukocyte antigen; IgG, immunoglobulin G; IFN, interferon; OS, overall survival; PPV, personalized peptide vaccination

Key words: recurrence prevention, lung adenocarcinoma, colon cancer, breast cancer, personized peptide vaccine, immune kinetics

Introduction

Large-scaled randomized clinical studies of active specific immunizations to prevent the recurrence of lung cancer with melanoma-associated antigen 3 antigen (1), colon cancer with irradiated autologous tumor cells (2), and breast cancers with human epidermal growth factor receptor 2 (HER2)-derived peptides (3,4) have been conducted since the 1980s. None of those studies identified sufficient clinical benefits for the immunizations' approval, although benefits were observed in some of the patients. In addition, the immunological mechanisms involved in the immunizations' insufficient clinical benefits were not fully investigated. Although remarkable advances in cancer therapy were achieved in the field of immune checkpoint inhibitors over the past decade (5,6), the advances do not negate the need to develop vaccines that could prevent the recurrence of cancer, because active specific immunity is pivotal to cancer control.

The robust humoral immunity induced by prophylactic vaccines against human papilloma virus is responsible for the prevention of human papilloma virus-related cancers (7). Humoral immunity has also been suggested to play an important role in the prevention of the recurrence of several cancers (8-10). It is thus worthwhile to investigate whether robust humoral immunity is observed in cancer patients who have received a peptide-based cancer vaccine, as such vaccines have been considered a promising preventive or therapeutic option since the 1990s (11,12). We conducted the present study to investigate the immune kinetics of personalized peptide vaccination (PPV)-induced immunity in patients with non-advanced patients and no active tumor at their entry to the PPV. For the PPV examined herein, four peptides chosen from a set of 31 warehouse peptides were vaccinated to individual patients based on the patients' human leukocyte antigen (HLA) type and their pre-existing immunity as shown by their peptide-specific immunoglobulin G (IgG) levels (13-17).

Patients and methods

Patients and protocols. From November 2008 to March 2019, phase II studies of a PPV were conducted for 44 Japanese patients with cancers of different histologies at the Kurume University Cancer Vaccine Center or Kurume University Hospital. They were diagnosed as having no active tumor

at the time of their entry for the PPV: lung adenocarcinoma (n=11), colon cancer (n=18), and breast cancer (n=15). The eligibility criteria were a pathologically confirmed diagnosis of lung adenocarcinoma, colon cancer, or breast cancer; positive IgG responses [≥10 fluorescence intensity units (FIU)] for ≥2 of the 31 warehouse peptides in their prevaccination plasma (Table SI); positive status for HLA-A2, -A24, -A3 supertypes (HLA-A3, -A11, -A31, or -A33) or -A26; age ≥20 years; Eastern Cooperative Oncology Group performance status of 0-2; a life expectancy of ≥12 weeks; and adequate bone marrow, hepatic, and renal functions.

The exclusion criteria were acute infection, a history of severe allergic reactions, or other systemic diseases as described (13-17). The 31 warehouse peptides consisted of peptides matched for 12 HLA-A2-positive, 14 for HLA-A24-positive, and nine for HLA-A3 supertype-positive patients, and four peptides for HLA-A26-positive cancer patients, respectively (Table SI). The peptides were prepared under Good Manufacturing Practice conditions with the use of a multiple peptide system (Multiple Peptide System, Inc.). The patients were vaccinated in a subcutaneous region with two to four peptides based on their HLA type and on their pre-existing immunity represented by their peptide-specific IgG levels as described (13-17).

The protocols were as follows: patients with lung cancer (under the protocol with UMIN registration no. 00002984), breast cancer (no. 000003081), or colon cancer (no. 000002987) received vaccinations consisting of a 1.5-ml emulsion (3 mg/each peptide) of 2-4 peptides across three cycles as follows: four visits at 1-week intervals, followed by four visits at 2-week intervals (the 1st cycle); four visits at 2-week intervals followed by four visits at 4-week intervals (the 2nd cycle); and eight visits at 4-week intervals (the 3rd cycle).

In addition, certain colon cancer patients from northern Japan or outside of Japan were entered in the protocols with UMIN registration nos. 000006927, 000011230 and 000001482. They received vaccinations consisting of a 3.0-ml emulsion (6 mg/each peptide) of two to four peptides at each visit (half the dose was injected into either side of the body) across three cycles as follows: four visits at 4-week intervals (the 1st cycle), followed by four visits at 4-to-8-week intervals (the 2nd cycle), and finally four visits at 8- to 12-week intervals (the 3rd cycle).

After the 3rd cycle, all patients who wished to continue received the vaccination at 4- to 8-week intervals until withdrawal of consent or unacceptable toxicity. All protocols were approved by the ethical committee of Kurume University and by the regional ethical committee [Fukuoka Clinical Research Board (no. 718004)] and then registered in the UMIN Clinical Trials Registry of the Japanese government. All of these studies were in accordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines and were conducted in an outpatient setting. Before their inclusion in the study, all participants gave written informed consent to participate in the clinical trial and to have their data used for research and publication purposes.

Toxicity and general conditions were monitored at the time of each visit. Toxicity was evaluated using the Common Terminology Criteria for Adverse Events ver. 4.0.

Immune responses. The patients' peripheral blood was collected at pre-treatment and at the end of the 1st, 2nd and 3rd cycles, and then at each visit after ≥3 months (range 3-45 months). Their IgG titers and cytotoxic T lymphocyte (CTL) activity specific to the peptide in plasma or peripheral blood mononuclear cells (PBMCs) were evaluated by a beadbased multiplexed Luminex assay (Luminex Platform LHC6003M; Invitrogen; Thermo Fisher Scientific, Inc.) and by an interferon (IFN)-γ ELISPOT (Immunocyte IFN-γ ELISPOT kit; Medical and Biological Laboratories), respectively (17). Pre-vaccination peptide-specific IgG levels with a cut-off level of 10 FIU were taken as detecTable levels of IgG. Patients were considered to have a positive IgG boost when the total sum of their post-vaccination IgG levels against the vaccinated peptides (2-4 peptides) was >5,000 FIUs compared to that of the pre-vaccination level.

The spreading of IgG boosting to non-vaccinated peptides was quantified by measuring the total sum of FIU in response to non-vaccinated peptides. The HLA-matched peptide-specific CTL activity in PBMCs was evaluated by the IFN-γ ELISPOT assay. Positive CTL boosting was defined as a >5-fold increase in the total sum of HLA-matched peptide-specific IFN-γ spots compared to the pre-vaccination level or >50 IFN-γ spots if the pre-vaccination CTL activity was undetectable. The CEF peptide pool (Mabtech, Cincinnati, OH) consisting of 23 HLA-class I-restricted peptides from human influenza virus, cytomegalovirus, and Epstein Barr virus was used as a control peptide.

The target cells used for the assay were T2 cells for HLA-A2 or HMy2.CIR (CRL-1993; ATCC) cells transfected with the HLA- A11, -A24, -A26, -A31, or -A33 gene as described (13-17). We calculated the spreading of CTL activity to non-vaccinated peptides by subtracting the sum of IFN-γ spots in response to all 31 peptide-mixtures from the sum of IFN-γ spots in response to the vaccinated peptides. Pre-vaccination samples at the screening time (14 days before the first vaccination) were provided for the measurement of blood cell counts and C-reactive protein (CRP) as described (17).

The corresponding author had full access to all of the study data and had final responsibility for the decision to submit this report for publication.

Statistical analyses. The Kaplan-Meier method was used for the statistical analyses. Recurrence was determined by the new appearance of tumor based on a radiological diagnosis or the increase of a tumor marker. Recurrence-free survival (RFS) and overall survival (OS) were calculated as the time in months from the day of the 1st vaccination (for events) or to the date of last contact. Mixed model analysis using Kenward-Roger F test was used for the patients' IgG level values after peptide vaccination. All statistical analyses were performed using JMP version 16.0 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics and clinical outcome. Table I summarizes the patients' characteristics and clinical outcomes. The 44 patients without an active tumor consisted of 11 patients with lung adenocarcinoma, 18 with colon

Table I. Characteristics of the enrolled patients without active tumor at entry.

Characteristics	Characteristics of all patients (n=44)	Patients with lung adenocarcinoma (n=11)	Patients with colon cancer (n=18)	Patients with breast cancer (n=15)
Median age, years (range)	58 (36-83)	66 (54-80)	56 (36-83)	53 (37-69)
Male:Female	17:27	7:4	10:8	0:15
Performance status (0/1)	43/1	10/1	18/0	15/0
HLA (A24/A2/A3family/A26)	30/16/26/9	7/4/2/6	15/5/12/1	8/7/12/2
Stage at entry (I/II/III/IV recurrence)	9/10/12/8/5	4/2/3/1/1	0/2/6/6/4	5/6/3/1/0
Chemotherapy/hormone therapy/ targeted therapy	7/6/1	0/0/0	6/0/0	1/6/1
Median number of vaccinations (range)	20 (4-65)	24 (8-65)	16 (4-47)	19 (5-56)
Median follow-up, months (range)	68 (13-113)	70 (13-113)	51 (25-96)	74 (22-96)
Recurrence free patients	37/44	10/11	14/18	13/15
IgG boosted at end of 1st cycle	36/43	10/11	15/18	11/14
CTL boosted at end of 1st cycle	23/30	1/1	11/15	11/14

HLA, human leukocyte antigen.

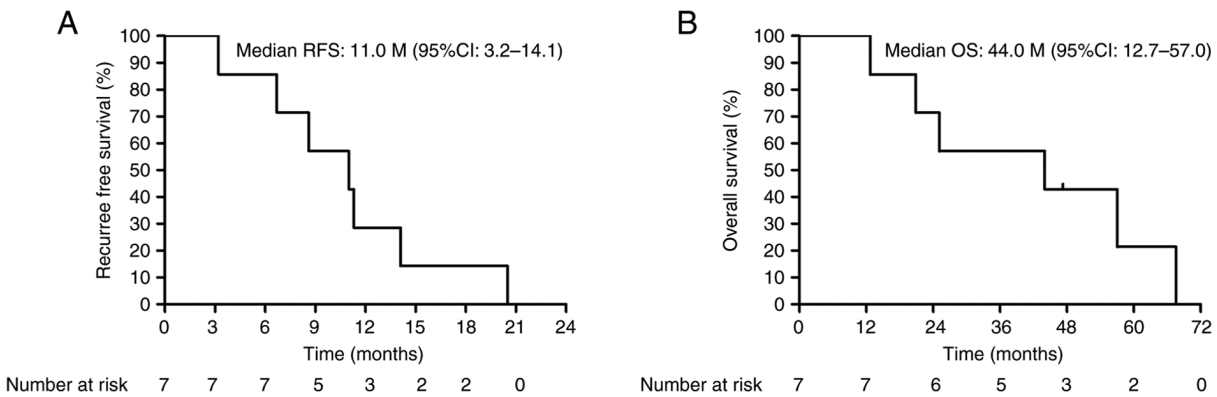

Figure 1. (A) RFS and (B) OS of the seven patients with cancer recurrence among the 44 patients without an active tumor. RFS, recurrence-free survival; OS, overall survival; CI, confidence intervals; M, months.

cancer (origin of primary cancers: 13 rectal, two sigmoid colon, one each of cecum, ascending, and transverse colon); and 15 with breast cancer (eight luminal, two HER2+, four triple-negative types, and one apocrine cancer). Systemic therapies were combined with PPV in none of the patients with lung cancer, six of the patients with colon cancer (chemotherapy in six cases), and eight of the patients with breast cancer (chemotherapy and target therapy in one case each, and hormone therapy in six cases) (Table I). The median number of vaccinations was 20. The median follow-up time was 67.6 months (interquartile range (IQR): 45.6-82.8). Under these circumstances, 37 of the 44 patients (84%) had no recurrence. The remaining seven patients (one stage III lung cancer, two stage IV and two recurrent colon cancers, and two stage II breast cancers) had recurrence, and their median RFS and OS rates were 11.0 months [95% confidence interval (CI): 3.2-14.1] and 44.0 months (95% CI: 12.7-57.0), respectively (Fig. 1A and B).

The detailed information for each of the 44 enrolled patients from their first cancer surgery to the end of the clinical study (Table II) helps clarify the intervals from the surgery to the vaccination effect to the outcome of PPV-induced cancer prevention. The median period from surgery to the first vaccination was 14.5 months (IQR: 6.4-27.5 months). The median periods of the seven patients with recurrence from their surgery to their first vaccination were as follows: 1.5 months (stage IV colon cancer), 2.5 months (stage II triple-negative breast cancer), 6.5 months (stage III colon cancer), 13 months (stage III lung cancer), 17.5 months (stage II luminal type breast cancer), 17.5 months (stage II colon cancer), and 90 months (stage I colon cancer). These results indicate that the intervals from surgery to the first vaccination in the entire series of 44 patients were not very different from the intervals of the seven recurrent patients.

Adverse events. The majority of patients had grade I or II skin reactions at the injection sites. There were no PPV-related severe adverse events other than grade III injection site reactions. Details are given in Table SII.

Table II. Detailed information of 44 enrolled patients: From first operation to end of following-up observation.

Patients	Origin/histology/ stage	Operation to first vaccine, months	Age, years	Stage at first vaccine	Number of vaccinations	OS from first vaccine, months
AMA-001	BC/Lum/II	2.0	69	II	8	94.6
AMA-003	BC/Lum/I	39.5	41	I	5	78.5
AMA-004	BC/apocrine Ca/II	6.0	64	IV	16	95.6
AMA-005	BC/TN/I	4.5	61	I	48	91.3
AMA-006[a]	BC/Lum/II	17.5	42	II	19	66.7
AMA-007	BC/TN/III	16.0	37	III	24	87.6
AMA-010	BC/Her2/I	6.8	63	I	49	89.1
AMA-012	BC/Lum/III	12.0	48	III	9	70.2
AMA-013	BC/Lum/II	150.0	51	II	56	82.9
AMA-014[a]	BC/TN/II	2.5	55	II	16	24.7
AMA-015	BC/Her2/I	20.0	65	I	43	79.1
AMA-025	BC/Lum/III	76.0	52	III	24	41.4
AMA-026	BC/TN/IIA	16.5	38	II	11	80.2
AMA-042	BC/Lum/II	26.0	54	II	16	36.0
AMA-053	BC/Lum/I	7.6	50	I	24	49.0
ALU-001	LC/Ad/ II	11.5	66	II	18	98.5
ALU-004	LC/Ad/II	6.0	65	II	24	95.7
ALU-005	LC/Ad/I	6.3	54	I	65	113.0
ALU-006	LC/Ad/III	6.2	61	III	8	93.9
ALU-014	LC/Ad/II	33.0	58	Recurrence	24	86.4
ALU-047	LC/Ad/I	114.0	68	I	23	62.1
ALU-059	LC/Ad/IV	11.0	70	IV	23	67.1
ALU-080	LC/Ad/III	4.0	67	III	24	52.7
ALU-082	LC/Ad/I	11.6	72	I	52	69.8
ALU-112	LC/Ad/I	64.0	65	I	20	27.8
ALU-119[a]	LC/Ad/III	13.0	80	III	8	12.6
F2-037	CC/II	3.5	52	II	6	81.3
F2-047	CC/III	45.0	83	III	8	72.5
F-141	CC/III	12.5	50	IV	47	95.7
F2-GAS-048	CC/III	2.0	72	III	4	45.6
F2-GAS-052	CC/III	9.0	36	III	4	35.0
F2-GAS-062	CC/III	19.0	42	III	4	25.0
ACO-002[a]	CC/IV	1.5	51	IV	45	43.4
ACO-040	CC/III	10.5	59	IV	24	75.5
ACO-054	CC/III	26.5	48	III	22	72.4
ACO-086[a]	CC/I	90.0	72	II	21	20.6
ACO-091	CC/IV	55.0	40	IV	40	64.7
ACO-093	CC/III	22.0	65	IV	8	61.3
ACO-094[a]	CC/III	6.5	60	III	11	7.0
ACO-106[a]	CC/II	17.5	53	IV	11	56.2
ACO-134	CC/III	19.5	62	Recurrence	16	46.7
ACO-137	CC/II	27.6	73	Recurrence	16	45.1
ACO-145	CC/IV	30.5	43	Recurrence	27	43.5
ACO-149	CC/IV	27.0	50	IV	30	41.5

[a]Indicates the seven recurrent patients. BC, breast cancer; LC, lung cancer; CC, colon cancer.

IgG responses. The kinetics throughout the study for the patients without an active tumor are depicted in Fig. 2 (lung adenocarcinoma, n=11), (colon cancer, n=18), and (breast cancer, n=14), respectively. The plotted points of IgG levels (FIU) were pre-vaccination, the end of the 1st cycle (2-3 months later), the end of the 2nd cycle (7-9 months later), the end

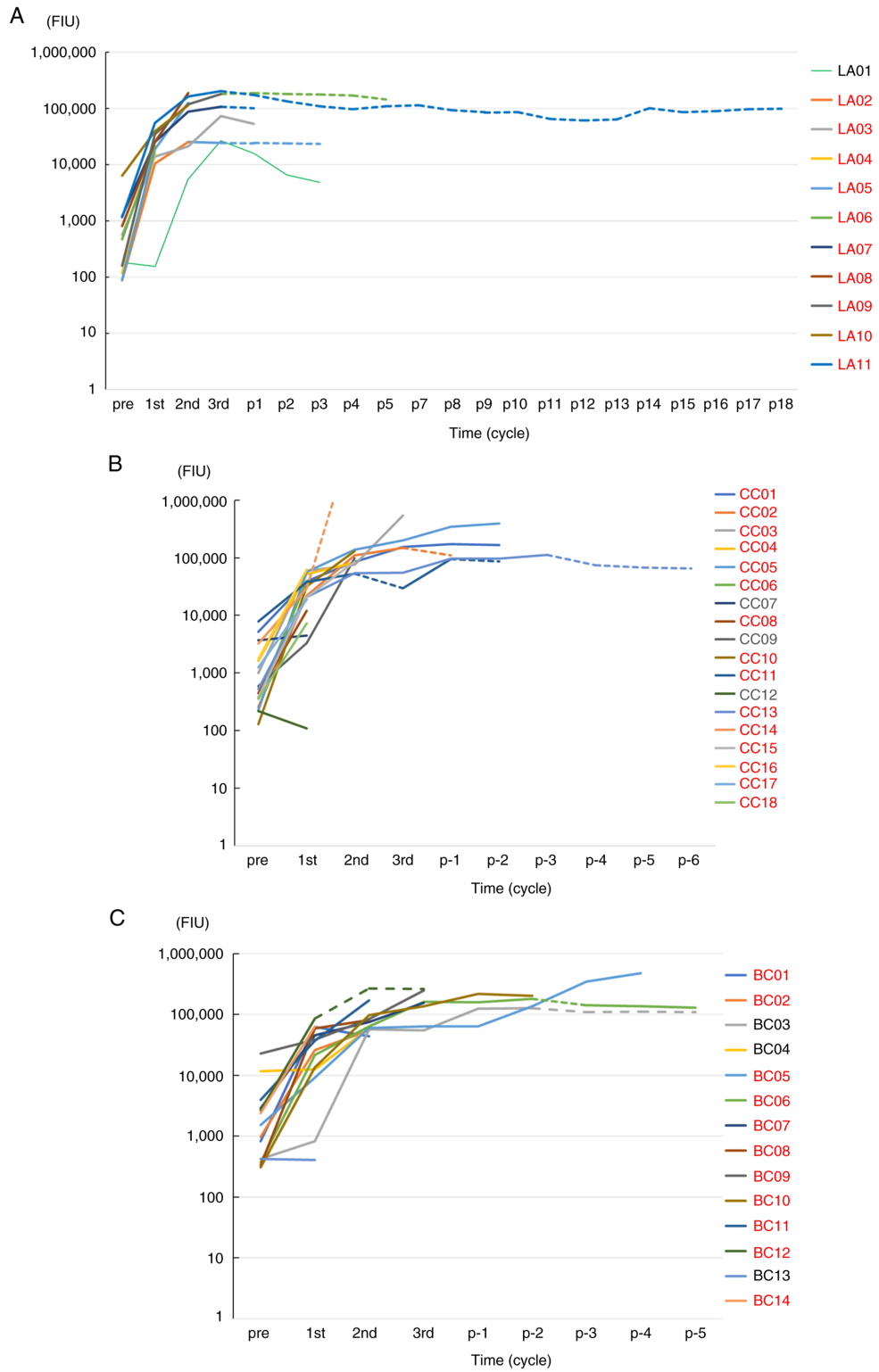

Figure 2. Immune kinetics of IgG boosting in the patients with (A) lung adenocarcinoma (n=11), (B) colon cancer (n=18) and (C) breast cancer (n=14). IgG levels (FIU) are plotted at pre-vaccination, the end of the 1st cycle (2-3 months later), the end of the 2nd cycle (7-9 months later), the end of the 3rd cycle (15-17 months later) and thereafter every cycle (cycle duration range, 3-34 months) during the follow-up. Dotted lines indicate the no-vaccination periods. Patient numbers in red indicate the patients who showed IgG boosting (>5,000 FIU compared to the pre-vaccination level). FIU, fluorescence intensity unit; IgG, immunoglobulin G.

of the 3rd cycle (15-17 months later), and thereafter (every 3-34 months) during the follow-up. IgG boosting was observed in 36 of the 43 patients tested at the end of the 1st cycle, followed by an increase in IgG thereafter. In all 12 patients tested after the vaccine's termination, the IgG boosting levels were maintained at levels ranging from 23,165 FIU at 34 months to 1,601,706 FIU at 63 months post-termination.

Collectively, among the 44 patients without an active tumor, IgG boosting against the vaccinated peptides at the end of the 1st and 2nd cycles of PPV was observed in 36 of 43 patients

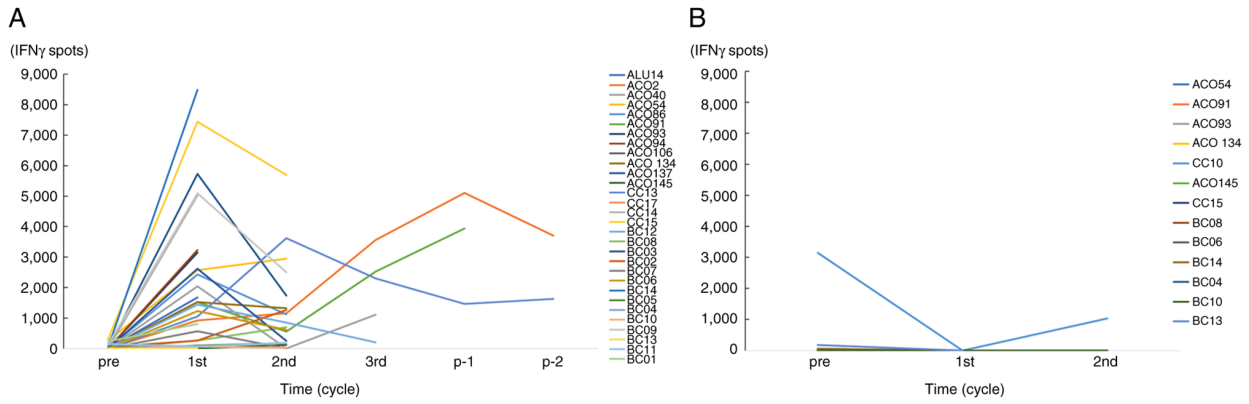

Figure 3. (A) Immune kinetics of CTL activity (IFNγ spots) against the vaccinated peptides in the 30 patients tested. (B) CTL activity against non-vaccinated peptides in the 13 patients tested at pre-vaccination, the end of the 1st cycle (2-3 months later), the end of the 2nd cycle (7-9 months later) and the end of the 3rd cycle (15-17 months later). CTL, cytotoxic T lymphocyte; IFN, interferon.

and all 43 patients whose samples were available, respectively. Moreover, the PPV induced robust (>50,000 FIU) humoral immunity for 41 of the 43 tested cancer patients without an active tumor even after the follow-up period (Fig. 2A-C). IgG boosting at the end of the 1st cycle and the 2nd cycle was observed in three of the seven recurrent patients and in three of the four recurrent patients tested, respectively. These results suggest that post-vaccination IgG boosting was a prognostic marker in PPV patients, which is consistent with the reported observations (13,14,17).

CTL responses. The CTL activity against the vaccinated peptides at the end of the 1st cycle was boosted in 23 of the 30 tested patients. However, these elevated CTL activities declined at the end of the 2nd cycle in most of the tested cases (Fig. 3A). The CTL activity against non-vaccinated peptides in the 13 patients tested was no boosted at the 2nd cycle (Fig. 3B).

IgG response to a lymphocyte-specific protein tyrosine kinase at positions 486-494 (Lck-486 peptide). We investigated the IgG response to the Lck-486 peptide, which is capable of inducing HLA-A24-restricted CTL activity; we did so because this peptide was vaccinated for the majority of the present HLA-A24 patients (23 of 30). We speculated that Lck-486 peptide could thus be a suiTable representative for the evaluation of the kinetics of each peptide. This investigation was also conducted based on our previous study of a monoclonal anti-Lck-486 IgG (IgG 2b) peptide capable of inhibiting tumor growth *in vivo* with a suppression of tumor-infiltrating T regulatory cells in a murine model (18).

Among the 44 patients, 30 were HLA-A24+ and the remaining 14 were HLA-A24- (Table I). Twenty-three of the 30 HLA-A24+ patients received a vaccination with the Lck-486 peptide; the remaining seven did not. Robust IgG boosting (>50,000 FIU) against the Lck-486 peptide was observed in all 22 HLA-A24+ patients tested, and the boosting levels were maintained even after vaccine termination (Fig. 4A). The patients' median IgG levels were significantly higher than their pre-vaccination levels ($P<0.0001$) (Fig. 4B). In contrast, IgG boosting against the Lck-486 peptide was not observed in any of the seven HLA-A24+ patients without Lck-486 vaccination, whereas it occurred in two of the 14 HLA-A24- patients without Lck-486 vaccination (Fig. 4C).

We also assessed the clinical benefits of the vaccinations, as our earlier study revealed that the patients who received the Lck-486 vaccination had significantly longer survival compared to patients with advanced cancer (19). Three of the 23 HLA-A24+ patients (13%) developed cancer recurrence after receiving the Lck-486 peptide vaccination (among the 23 patients, the recurrence rate was 13%), whereas two of the HLA-A24+ patients (29%) developed cancer recurrence after Lck-486 peptide vaccination. The RFS values were 11.3, 14.1 and 20.5 months respectively in the former three patients, and 6.7 and 11.0 months in the latter two patients. The OS values were 66.7, 43.4 and 46.7 months in the former three patients, and 24.8 and 20.6 in the latter two, respectively. All measurements of clinical events (recurrence rate, RFS, and OS) were favorable in the patients who received the Lck-486 peptide.

Pre-vaccination inflammatory signatures. We reported that pre-vaccination inflammatory signatures hampered the clinical benefits of PPV for patients with advanced cancer (13-17). Herein, we investigated the following pre-vaccination inflammatory signatures: 59.9% as the median percentage of neutrophils; 1.9 as the neutrophil-lymphocyte ratio; 4,800 as the white blood cell number; 31.2% as the percentage of lymphocytes; and $429x10^4$ as the red blood cell number. The median CRP level in the 17 tested patients without an active tumor was 0.09.

Discussion

The results of this study demonstrated that the PPV induced robust (>50,000 FIU) humoral immunity for 41 of the 43 tested cancer patients without an active tumor even after the follow-up period. In contrast, CTL activity was observed in 23 of 30 non-recurrent patients at the end of the 1st PPV cycle and in four of six recurrent patients at the end of the 2nd cycle, followed by a rapid decline. These results suggest that the CTL induction rate in the non-recurrent cases was not very different from that of the recurrent cases. The mechanisms involved in this decline of CTL activity are presently unclear. A kinetic study of T-cell

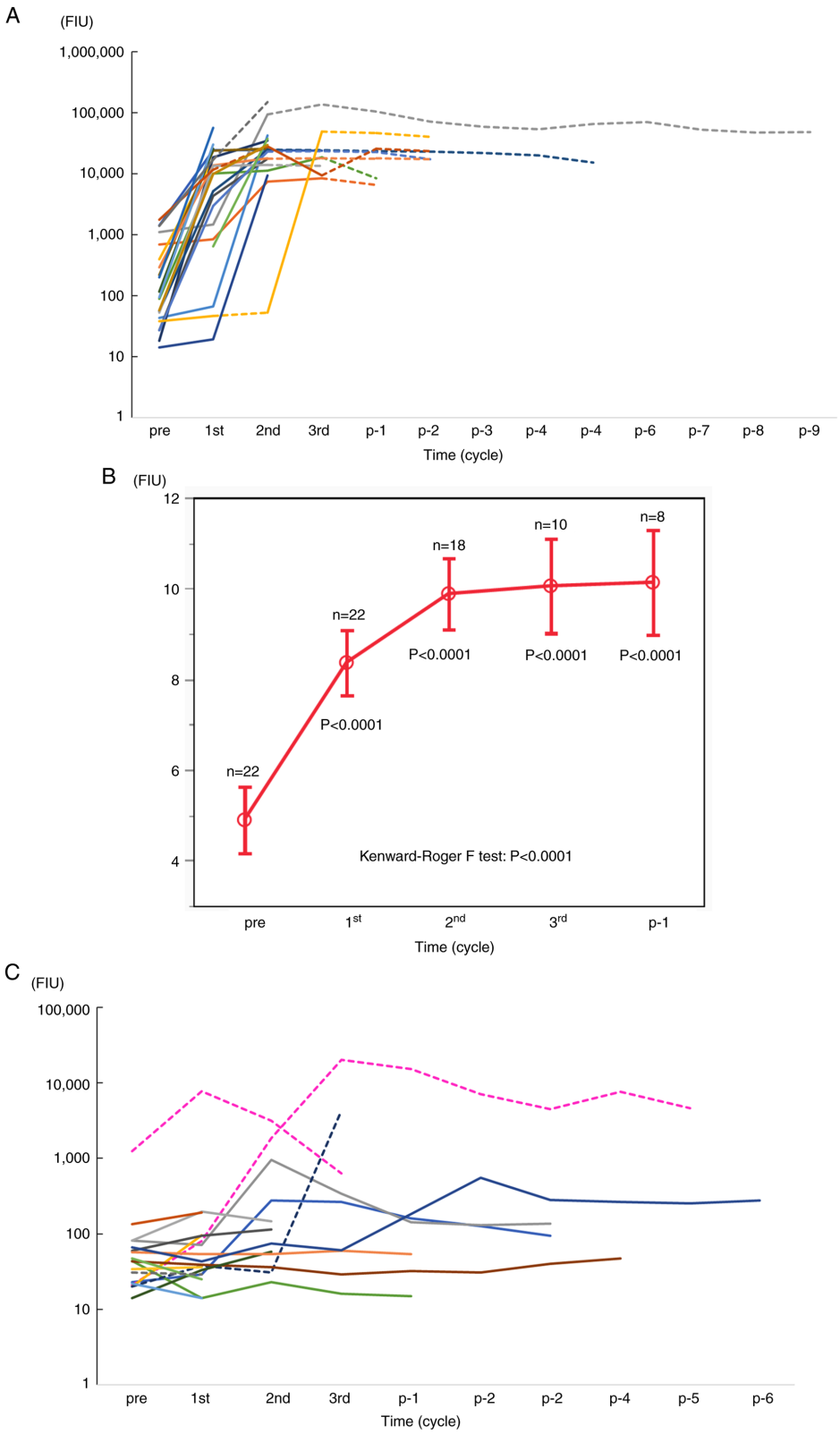

Figure 4. (A) Kinetics of IgG levels in response to the vaccinated Lck-486 peptide in 22 patients. (B) Median IgG level values in response to the vaccinated Lck-486 peptide in the 22 patients tested by using mixed model analysis and Kenward-Roger F test. (C) IgG levels in response to the non-vaccinated Lck-486 peptide in the 21 patients tested (14 HLA-A24+ and seven HLA-A24- patients) at pre-vaccination, the end of the 1st cycle (2-3 months later), the end of the 2nd cycle (7-9 months later), the end of the 3rd cycle (15-17 months later) and thereafter every cycle (cycle duration range, 3-34 months) during the follow-up. IgG, immunoglobulin G; HLA, human leukocyte antigen; FIU, fluorescence intensity unit.

subsets including regulatory T cells could be critical to solve this issue. The involvement of myeloid-derived suppressor cells in PPV-induced CTL suppression should also be fully examined, since our earlier investigation demonstrated that myeloid-derived suppressor cells were closely involved in the decline of CTL activity (13,14,17).

Another of our studies showed that the administration of monoclonal anti-Lck-486 IgG inhibited tumor growth and suppressed tumor-infiltrating T regulatory cells in a murine model, i.e., female BALB/c mice in which can bind to Lck-486 peptide (18). Lck antigen was reported as a key molecule for T-regulatory cell activity (20,21). In the present series of cancer patients, potent IgG boosting to Lck-486 peptide occurred in all 22 of the HLA-A24+ patients who received this peptide, and the boosting levels were maintained even after vaccine termination. These results indicate that a robust IgG response against the Lck-486 peptide may play a role in the prevention of recurrence among HLA-A24+ patients. However, this possibility must be confirmed by investigations of the administration of either PPV-induced anti-Lck-486 IgG or a monoclonal Lck-486 antibody to cancer patients.

The pre-vaccination inflammatory signatures in the present patients without active tumors were almost within the normal ranges. In contrast, we have repeatedly observed that these signatures were higher than the normal ranges in patients with advanced-stage cancer and were thus prognostic biomarkers for the cancer vaccine (13,14). Together our past and present findings demonstrate that the higher immune induction provided by a cancer vaccine is a crucial issue for recurrence prevention.

The effects of the PPV on the patients' clinical outcomes (RFS and OS) were examined. The reported OS rates of lung adenocarcinoma patients are ~80% for the stage I patients, 50% for stage II, 25% for stage III, and 8% for stage IV. The respective OS rates of the colon cancer patients are 90, 80, 77 and 22%, and those of the breast cancer patients were 92, 93, 77 and 39%; these data were obtained from a Pfizer Japan source, i.e., https://ganclass.jp/qa/link/qa_family_link02.php. However, it could be difficult to expect the same post-vaccination recurrence rates for the patients in the present small-scale study since the origin, histology, and cancer stages differed among the 44 enrolled patients (Table II).

An exact molecular basis of anti-tumor humoral immunity is not yet fully understood although the possible positive roles are partly reported as cited in the original manuscript (8-10). Several review articles might facilitate its deeper understanding. Dunn *et al*, well summarized the history of cancer immunosurveillance controversy from a view of elimination, equilibrium, and escape of tumor cells (22). Thomas *et al*, reviewed the perspectives of humoral and cellular immune responses based on NY-ESO-1 vaccines (23). Zitvogel *et al*, put forward a hypothesis that gut microbial proteins might be sufficiently similar to human tumor antigens and are thus capable of eliciting tumor-specific T lymphocytes and antibodies that can recognize future tumor cells via 'antigenic mimicry' (24). Discovery by Sivan *et al* reported that commensal Bifidobacterium promoted anti-tumor immunity and facilitate anti- PD-L1 efficacy, which in turn might provide us future direction of cancer immunity (25).

In conclusion, our analyses revealed that the PPV induced robust humoral immunity in the majority of cancer patients without active tumors.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SS, KI and SY conceived and designed the study. SS and KI collected and assembled data. SS, SY, UT, KY and KI were involved in the data analysis and interpretation. All authors wrote the manuscript. All authors have read and approved the final manuscript. SS and KI confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All protocols were first approved by the ethical committee of Kurume University and by the regional ethical committee (Fukuoka Clinical Research Board; approval no. 718004) and then registered in the UMIN Clinical Trials Registry of the Japanese government. All of the study protocols were in accordance with the Declaration of Helsinki and the International Conference on the Harmonization of Good Clinical Practice guidelines and were conducted in an outpatient setting. Before their inclusion in the study, all participants gave written informed consent to participate in the clinical trial and to have their data used for research and publication purposes.

Patient consent for publication

Not applicable.

Competing interests

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